Treatment of fungal disease in the setting of neutropenia

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Invasive fungal infections are important causes of morbidity and attributable mortality in neutropenic patients with hematological malignancies, myelodysplasia, and aplastic anemia. Successful risk-based strategies can be implemented for prophylaxis, empirical therapy, and preemptive therapy for the prevention and early treatment of invasive fungal infections in neutropenic hosts. The use of echinocandins for invasive candidiasis and voriconazole for invasive aspergillosis has significantly improved outcome. Recent studies demonstrate, however, that resistant fungal pathogens may emerge during the course of these antifungal interventions. Although triazole-resistant *Candida* spp. have been well described as causes of breakthrough candidemia, other organisms now pose a similar threat. Such organisms include echinocandin-resistant *Candida glabrata* and *Candida parapsilosis* species complex. The Mucorales, *Fusarium* spp., and *Scedosporium* spp. may emerge in the setting of voriconazole prophylaxis. The challenges of these emerging pathogens underscore the need for the development of new antifungal agents and strategies.

Introduction

Invasive fungal infections (IFIs) are important causes of morbidity and attributable mortality in neutropenic patients with hematological malignancies, myelodysplasia, and aplastic anemia.[1] The risk for IFIs in patients with hematological malignancies varies as a function of underlying neoplastic process and degree of immunosuppression. Understanding the epidemiology of these mycoses, risks for the development of infection, and approaches to diagnosis greatly facilitates early initiation of pathogen-specific therapy.[2]

Neutropenia as a key risk factor for development of IFIs in patients with hematological malignancies

In the classic description of the inverse relation between risk of infection and degree of neutropenia, Sipsas et al underscored the role of profound neutropenia (absolute neutrophil count < 100) in leukemia patients for increasing the risk for infection.[2] Neutropenia may develop as the result of chemotherapy, radiation, or BM failure (myelodysplasia and aplastic anemia) or by the replacement of hematopoietic cells in the BM by malignant cells.

Neutropenia is a key risk factor for the development of IFIs. In a seminal study of patients receiving treatment for acute leukemia Gerson et al demonstrated that the risk of invasive aspergillosis is directly related to the duration of neutropenia in patients with acute leukemia. After 14 days of neutropenia, the risk of aspergillosis increased in direct relation to the duration of neutropenia. Neutropenia is also a surrogate marker for other risk factors for development of IFIs. For example, mucositis associated with intensive chemotherapy increases the risk for translocation of *Candida* spp. across the alimentary tract.

Chemotherapy-induced neutropenia is also associated with lymphopenia and immune dysregulation. Lymphopenia in hematological malignancies increases the risk for fungal infections associated with impaired cell-mediated immunity. Fludarabine and corticosteroids markedly increase the risk of infection caused by *Pneumocystis jirovecii* and *Cryptococcus neoformans*. Fludarabine is lymphocytotoxic against predominantly $CD4^+$ lymphocytes. Corticosteroids also markedly alter cytokine and chemokine expression, as well as the distribution, trafficking, and functions of lymphocytes, neutrophils, and monocytes.

Corticosteroids also impair oxidative function and hyphal damage capacity of neutrophils and impair phagocytosis of macrophages. The risk of IFIs caused by filamentous fungi such as *Aspergillus* spp. and the *Mucorales* is significantly increased in those patients receiving a prednisone equivalent of 0.5 mg/kg for longer than 30 days. The increased risk of development of IFIs is added to the risk already conferred by IFIs.

Specific IFIs associated with neutropenia

Candidiasis

The alimentary tract is the principal portal of entry in patients with acute leukemia for *Candida* spp., which are a component of the endogenous microbiome that invade the bloodstream through disruptions in anatomical barriers. Infections may appear as oropharyngeal candidiasis, esophageal candidiasis, candidemia, acute disseminated candidiasis, or chronic disseminated candidiasis (hepatosplenic candidiasis).

Oropharyngeal and esophageal candidiasis. Therapy for oropharyngeal candidiasis initially includes clotrimazole troches or fluconazole. Esophageal candidiasis typically presents as odynophagia and dysphagia. The differential diagnosis includes herpes simplex virus, CMV (principally in hematopoietic stem cell transplantation [HSCT] recipients), and bacteria. Fluconazole is used as initial therapy for esophageal candidiasis and an echinocandin is used in refractory cases. *Candida glabrata* and *Candida krusei* may emerge resistant to fluconazole and cause recurrent symptoms of odynophagia in the setting of triazole prophylaxis.

Candidemia. With the advent of triazole and echinocandin prophylaxis and therapy, there has been a major shift in the causes of candidemia from *C albicans* toward non-*C albicans Candida* spp.

in neutropenic patients. A recent prospective, multicenter study specifically designed to investigate the epidemiology, risk factors, and outcome of candidemia among hospitalized patients with hematological malignancies found that most infections (87.5%) were caused by non-*C albicans* species, with *C parapsilosis*, being the most common.[3] Independent risk factors for the development of candidemia were the presence of central venous catheters, hypogammaglobulinemia, and high APACHE II score. The 28-day crude mortality was 45%. Among patients with candidemia, an elevated APACHE II score was an independent risk factor for death, whereas recovery from neutropenia was independently associated with improved survival. Early detection of invasive candidiasis has been enhanced with advanced blood culture detection systems and the availability of assays for detection of (1→3)-β-D-glucan.

Treatment. Among the different *Candida* species, *Candida glabrata* may emerge as breakthrough infection with resistance to all triazoles.[4] Therapy should be guided by in vitro antifungal susceptibility, where possible for treatment of candidemia caused by *C glabrata*. *Candida krusei* is always resistant to fluconazole. *Candida parapsilosis* is mostly associated with vascular catheters and may emerge during the course of echinocandin therapy. Among all *Candida* spp., *Candida tropicalis* is an important cause of acute disseminated candidiasis, which has a severe course with cutaneous lesions, visceral dissemination, myalgias, renal failure, and hemodynamic collapse. Among neutropenic patients with hematological malignancies, recent studies indicate that removal of central vascular catheters does not improve outcome.[5,6] This finding is consistent with the most likely portal of entry being the alimentary tract. If a multilumen catheter is not immediately removed, antifungal therapy should be administered parenterally through all lumens.

Chronic disseminated candidiasis. Candidemia in neutropenic patients may be complicated by chronic disseminated candidiasis of liver, spleen, kidney, and eyes. Therefore, ophthalmologic examination and CT scan of the abdomen are recommended upon recovery from neutropenia. Chronic disseminated candidiasis may persistent with new fever after recovery from neutropenia. After resolution of neutropenia, elevated alkaline phosphatase and the development of numerous target lesions in the liver and spleen develop. An open liver biopsy is advisable but may not be feasible. Antifungal therapy with fluconazole or echinocandin is initiated with anticipation of treatment for several months until resolution of lesions. The presence of persistent lesions does not preclude further chemotherapy.

Treatment. Because most patients with hematological malignancies are receiving triazole (fluconazole, voriconazole, or posaconazole) prophylaxis, an echinocandin (anidulafungin, caspofungin, or micafungin) is recommended as the initial therapy of invasive candidiasis in neutropenic patients with hematologic malignancies.[4] The rapid fungicidal activity against *Candida* spp. may also be beneficial in more rapidly eradicating organisms from infected tissues and the bloodstream. For non-neutropenic stable patients with uncomplicated candidemia, an initial course of echinocandin followed by fluconazole is reasonable if the organism proves to be *C albicans*.

Aspergillosis

Profound and persistent neutropenia, repeated cycles of prolonged neutropenia, concomitant corticosteroid therapy, and GVHD increase the risk of development of invasive sinopulmonary aspergillosis (ISPA).[7] Other risk factors include lymphopenia and respiratory viral infections.

Early ISPA may initially present only as fever. More advanced infection presents as sinus pain or congestion, cough, pleuritic chest pain, and hemoptysis. Diagnostic imaging of sinus aspergillosis may reveal sinus opacification with possible erosion of the nasal septum, osseous erosion into the orbit from the ethmoid sinuses, or extension into hard palate or orbit from the maxillary sinus. Diagnostic imaging of invasive pulmonary aspergillosis (IPA) reveals nodules, halo sign, bronchopneumonia, lobar consolidation, wedge-shaped segmental pneumonia, and cavitary lesions.

Early diagnosis of aspergillosis is important for improved outcome. Recovery of organism from bronchoalveolar lavage (BAL), percutaneous needle aspirate, and biopsies in sinopulmonary lesions is advised but may have limited sensitivity.

Aspergillus fumigatus followed by *Aspergillus flavus* are the most common species causing invasive aspergillosis. *Aspergillus terreus* is observed with increasing frequency at several hematological malignancies centers and is notable for being resistant to amphotericin B.

Detection of galactomannan in serum and BAL fluid by double-sandwich ELISA improves early detection of aspergillosis and complements CT scans. Serial quantitation of galactomannan antigenemia also predicts response to antifungal therapy. Serum (1→3)-β-D-glucan may also detect invasive aspergillosis and other invasive mold infections. PCR-based detection of *Aspergillus* DNA in BAL fluid may be useful for the diagnosis of IPA.

Treatment. Voriconazole is the preferred agent for initial therapy of ISPA and disseminated aspergillosis.[8] For patients for whom voriconazole is contraindicated, liposomal amphotericin B is used instead. Posaconazole is approved for use in the prevention of invasive aspergillosis in patients with acute leukemia and in HSCT recipients and may be helpful as alternative therapy in patients intolerant to voriconazole. Liposomal amphotericin B or ABLC are also indicated as salvage therapy for patients who are refractory or intolerant to voriconazole, particularly where there is a suspicion for concomitant invasive sinopulmonary mucormycosis.

All 3 available echinocandins (caspofungin, micafungin, and anidulafungin) have in vitro, in vivo, and clinical activity against *Aspergillus* spp. Caspofungin is licensed for salvage therapy of invasive aspergillosis. As strategies for salvage therapy, however, lipid formulations of amphotericin are the next logical step for patients who are intolerant of or refractory to conventional antifungal therapy. Neutropenic patients with invasive pulmonary aspergillosis have an estimated 10% to 15% coinfection with another mold, the most common of which are the Mucorales. Because these organisms are difficult to diagnose, a progressive pulmonary infiltrate in the setting of proven or probable invasive aspergillosis may be caused by mucormycosis for which a lipid formulation of amphotericin B would be most effective.

Combination therapy with an anti-*Aspergillus* triazole and an echinocandin may provide optimal medical intervention in the management of IPA. The rationale is that echinocandins target the FKS protein involved in biosynthesis of (1→3)-β-D-glucan in the cell wall, whereas triazoles target synthesis of ergosterol in the fungal cell membrane. The aggregate data from laboratory animal

studies, retrospective case controlled studies, and a recently completed prospective randomized controlled trial support the use of voriconazole and echinocandin in the treatment of invasive aspergillosis.

Patients who recover from an episode of ISPA are at risk for relapse of infection during subsequent immunosuppresssion. Secondary prophylaxis is indicated in those patients who undergo additional cycles of cytotoxic chemotherapy or require HSCT.

Mucormycosis

The agents of mucormycosis (zygomycosis) include the following members of the order Mucorales: *Rhizopus* spp., *Mucor* spp., *Lichtheimia* (formerly *Absidia*) *corymbifera*, and *Cunninghamella bertholettiae*.[9,10] Risk factors for mucormycosis among patients with neutropenic patients with hematological malignancies include prolonged neutropenia, corticosteroids, diabetic mellitus, and iron overload.[11]

Mucormycosis in patients with hematological malignancies typically manifests as pulmonary, sinus, sinoorbital, rhinocerebral, or cutaneous disease.[12] Patients with pulmonary mucormycosis may present with cough, hemoptysis, pleuritic pain, and single or multiple pulmonary nodules, which also may demonstrate a reverse halo sign. In rhinocerebral disease, fever, facial pain, and headache are common symptoms. Contiguous extension may lead to orbital involvement with proptosis and extraocular muscle paresis, involvement of hard palate, and extension into the brain. Invasion of the veins draining the ethmoid sinuses and orbits may lead to cavernous sinus thrombosis. An eschar over the palate or nasal turbinates is suggestive of mucormycosis, but other filamentous fungi can produce similar findings. Isolated primary cutaneous disease may follow minor trauma. The lesions of cutaneous mucormycosis may develop a thickened firm eschar overlying deep soft tissue infarction that extends well beyond the diameter of the cutaneous lesion.

Treatment. There are 4 cornerstones of therapy for mucormycosis: (1) early diagnosis, (2) lipid formulation of amphotericin B or conventional deoxycholate amphotericin B, (3) surgical debridement of infected tissue, and (4) reversal of immunosuppression, which includes correction of hyperglycemia in diabetic patients, recovery from neutropenia, and withdrawal or discontinuation of corticosteroid therapy. Granulocyte transfusions from donors with G-CSF–mobilized neutrophils may be helpful as adjunctive therapy until recovery from neutropenia.

The role of posaconazole in management of mucormycosis is controversial. The MICs for *Rhizopus oryzae*, the most common species casing mucormycosis, approximate those of itraconazole. These elevated MICs of posaconazole against *Rhizopus oryzae* correlate with animal studies of disseminated mucormycosis from several different laboratories, which found that posaconazole was no more active than normal saline despite serum concentrations exceeding the MICs of the infecting organism. In comparison, the MICs of posaconazole against *Mucor* spp. are substantially lower. These lower MICs against *Mucor* spp. correlate with a therapeutic response of posaconazole comparable to that of amphotericin B in the treatment of experimental disseminated mucormycosis caused by *Mucor* spp. Recent experimental studies suggest some response to pulmonary mucormycosis; however, these studies were not conducted in persistently neutropenic hosts, where the greatest challenge to treatment exists. Other laboratory studies indicate enhanced antifungal activity in combination therapies with amphotericin B and one or more of the following agents: posaconazole, echinocandins, and deferasirox.[13] Clinical data are needed for each of these combinations before they can be advocated as standard treatment of mucormycosis. Although salvage studies indicate that some patients may have a beneficial response to posaconazole, these cases are confounded by surgical resection, reversal of hyperglycemia, recovery from neutropenia, withdrawal of corticosteroids, and administration of concomitant amphotericin B. Impaired bioavailability of the oral formulation further limits the role of posaconazole in neutropenic patients. Although there are some patients who may respond to posaconazole, it is not a first-line alternative to the 4 cornerstones of early diagnosis, amphotericin B, surgical resection, and reversal of host impairments.

Fusarium *infections*

Fusarium spp. that infect neutropenic patients include *Fusarium solani* species complex and *Fusarium oxysporum* species complex. *Fusarium* spp. in patients with neutropenic patients with hematological malignancies cause sinopulmonary and disseminated infection. Prolonged neutropenia is the most common risk factor for invasive *Fusarium* infection. The portal of entry is most frequently the sinopulmonary tract, but may also be periungual and soft tissue infection. Fungemia with positive blood cultures occurs in approximately one-half of cases during neutropenia. Multiple hematogenously disseminated cutaneous lesions are common and usually reveal the organism in biopsy. Other sites of infection in the process of dissemination include CNS, bone, joints, eyes, and liver.

Initial localized manifestations of periungual infection in neutropenic patients include onychomycosis, paronychiae, and cellulitis. Early identification of localized skin disease and debridement may be lifesaving.

Treatment. As *Fusarium* species have variable *in vitro* susceptibility to amphotericin B and to voriconazole, initial therapy consists of both amphotericin B and voriconazole for spectrum (not synergy) while awaiting susceptibility results. Identification of species alone is not sufficiently predictive of antifungal susceptibility. Although interpretive breakpoints have not been established, readings of $> 4\mu g/mL$ usually signify lack of response ("resistance") to the antifungal agent. When susceptibility profiles become available, one can adjust therapy accordingly.

Survival from disseminated fusariosis is critically dependent on resolution of neutropenia. Granulocyte transfusions have been lifesaving in selected patients until recovery from neutropenia. Recurrences of disseminated fusariosis may develop during subsequent episodes of neutropenia.

Scedosporium *infections*

Although, changes in nomenclature have occurred through advances in molecular taxonomy of the genus *Scedosporium*, there are 2 principal pathogenic species that infect neutropenic patients: *Scedosporium apiospermum* and *Scedosporium prolificans*. In neutropenic patients, *Scedosporium* spp. cause sino-pulmonary disease, and dissemination to the CNS infection. *Scedosporium* spp cause infections in neutropenic patients who are cytologically and histologically indistinguishable from those of *Aspergillus* spp and *Fusarium* spp.

Treatment. As *S apiospermum* is often resistant to amphotericin B but susceptible to voriconazole and posaconazole, establishing a

microbiological diagnosis is important.[14] *Scedosporium prolificans*, by comparison, causes a similar spectrum of disease as that of *Aspergillus* but is resistant to all systemically available antifungal agents. Reversal of immunosuppression and surgical resection are the keys to the management of infections caused by *S prolificans*.

Infections caused by dematiaceous molds

Dematiaceous molds are distinguished as dark-walled filamentous fungi that contain melanin in their cell walls, which confers a black, brown, or olive-green pigment in culture. Infections caused by dematiaceous molds are sometimes termed phaeohyphomycosis. Among neutropenic patients with hematological malignancies, dematiaceous molds cause sinusitis, pneumonia, CNS infection, fungemia, soft tissue infection, and disseminated disease.

Dematiaceous molds have a strong predilection to cause CNS infection. Among the most common organisms are *Alternaria* spp., *Bipolaris* spp., *Ochroconis gallopava*, *Cladophialophora* (*Xylohypha* or *Cladosporium*) *bantiana*, *Exophiala* (*Wangiella*) *dermatitidis*, and *Exserohilum rostratum*. The recent outbreak of fungal meningitis caused by *Exserohilum rostratum* in the United States in association with exposure to contaminated methylprednisolone solution demonstrates the debilitating morbidity caused by these organisms.

Treatment. Treatment consists of systemic antifungal therapy and surgical excision of localized disease when feasible. Based upon susceptibility profiles and clinical reports, voriconazole is the primary agent for therapy. Amphotericin B and posaconazole may be alternatives. However, because antifungal susceptibility profiles vary according to species, guidance by an expert in infectious diseases and medical mycology is encouraged.

Trichosporonosis

Trichosporon spp. may emerge as breakthrough infections in neutropenic patients receiving amphotericin B. Trichosporonosis in profoundly neutropenic patients typically manifests with refractory fungemia, funguria, cutaneous lesions, renal failure, pulmonary lesions, and chorioretinitis. Disseminated trichosporonosis may yield a false-positive cryptococcal latex antigen test because of cross-reactivity with the glucuronoxylomannan capsular polysaccharide of *C neoformans*.

In vitro and experimental infections indicate that most *Trichosporon* spp. are inhibited, but not killed, by achievable serum levels of conventional amphotericin B. Fluconazole and voriconazole have superior activity in experimental infections and are the preferred antifungal agents. Antifungal triazoles have been successfully used in treatment of disseminated trichosporonosis in neutropenic patients whose disseminated trichosporonosis developed while receiving amphotericin B.

Malassezia *infections*

Malassezia furfur fungemia is often associated with lipid-containing parenteral nutrition administered through a central venous catheter in immunocompromised patients or premature infants. Clinical manifestations include persistent fungemia and pulmonary infiltrates. Blood culture recovery is enhanced by the addition of olive oil or other long-chain fatty acids to the culture plates. Discontinuation of lipid infusions and removal of the central catheter are essential. Because *M furfur* is resistant to amphotericin B therapy, fluconazole therapy is the drug of choice. Among neutropenic patients and patients treated with corticosteroids, a folliculitis resembling disseminated candidiasis may occur. This localized process does not imply disseminated infection.

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