Prevention, Diagnosis, and Treatment of Invasive Fungal Infections in Patients with Cancer and Neutropenia

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Key Words
Neutropenia, fever, Candida, Aspergillus, fungal infection

Abstract
Invasive fungal infections are a major cause of morbidity and mortality in patients with prolonged neutropenia and in allogeneic hematopoietic stem cell transplant recipients. The degree and duration of neutropenia influence the risk of opportunistic fungal infections. Because Candida and Aspergillus species are the major causes of invasive fungal infections in neutropenic patients, the fungal section of the NCCN guidelines focus on these two pathogens. Effective prevention and therapy of invasive fungal pathogens is a priority in highly immunocompromised patients with cancer. Three strategies in preventing and treating patients at high risk for fungal infection will be considered: (1) prophylaxis; (2) empirical therapy; and (3) treatment for probable or proven fungal infection. In addition to more effective antifungal agents, growing interest has been noted in novel non-culture detection methods to facilitate early diagnosis of invasive fungal infections. (JNCCN 2004;2:455–469)

Invasive fungal infections are a major cause of morbidity and mortality in patients with prolonged neutropenia and in allogeneic hematopoietic stem cell transplant recipients (HSCT). The deficits in host defense that render patients susceptible to fungal infections are complex, but can be broadly divided into the following categories: (1) neutropenia; (2) qualitative deficits in phagocyte function; (3) deficits in mucosal immunity; and (4) deficits in adaptive (cell-mediated and humoral) immunity.

Because Candida and Aspergillus species are the major causes of invasive fungal infections in neutropenic patients, the fungal section of the NCCN guidelines focuses on these two pathogens. Effective prevention and therapy of invasive fungal pathogens is a priority in highly immunocompromised patients with cancer. Three strategies in preventing and treating patients at high risk for fungal infection will be considered: (1) prophylaxis, (2) empirical therapy, and (3) treatment for probable or proven fungal infection. These strategies correspond to different levels of risk of fungal infection. Although these terms are useful operational definitions, the distinction between these modes is often not easily made in clinical practice. In addition to more effective antifungal agents, growing interest has been seen in novel non-culture detection methods to facilitate early diagnosis of invasive fungal infections. The current NCCN guidelines have been significantly modified compared with last year, reflecting important new data from clinical trials. Table 1 summarizes the major antifungal agents used in patients with prolonged neutropenia and HSCT recipients.

Invasive Candidiasis
The opportunistic yeasts cause a spectrum of clinical disease that ranges from superficial and mucosal infections such as mucosal candidiasis to disseminated disease involving visceral sites. Candida species are endogenous flora that gain access to the bloodstream through a breach in an anatomic barrier. The bowel is the principal portal of entry in patients with acute leukemia receiving highly mucotoxic regimens. Candida species are the fourth most common nosocomial blood culture isolates in the United States. The current NCCN guidelines have been significantly modified compared with last year, reflecting important new data from clinical trials. Table 1 summarizes the major antifungal agents used in patients with prolonged neutropenia and HSCT recipients.
Table 1 Antifungal Agents

<table>
<thead>
<tr>
<th>Antifungal Agents</th>
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<tr>
<td><strong>Azoles</strong></td>
<td></td>
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<tr>
<td>Fluconazole</td>
<td>Acceptable alternative to amphotericin B for candidemia at dose of 400 to 800 mg/d; broad range of MICs to <em>C. glabrata</em>; <em>C. krusei</em> is resistant; prophylaxis in high-risk patients (e.g., acute leukemia during neutropenia, hematopoietic transplantation); maintenance therapy for cryptococcal meningitis; inactive against filamentous fungi.</td>
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<tr>
<td>Itraconazole</td>
<td>Active against <em>Candida</em> sp., <em>Aspergillus</em> sp., dimorphic fungi, dark-walled molds. Cyclodextrin formulation has ↑bioavailability compared with capsules and can be administered parenterally. Itraconazole solution approved for empirical therapy for neutropenic fever.</td>
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<tr>
<td>2nd Generation Azoles</td>
<td>2nd generation antifungal triazoles (voriconazole, posaconazole, and ravuconazole) have broad spectrum of activity, including <em>Candida</em> sp. (including most, but not all, fluconazole-resistant isolates), <em>Aspergillus</em> sp., dimorphic fungi, <em>C. neoformans</em>, <em>Trichosporon</em> sp., <em>Fusarium</em> sp., <em>Scedosporium</em> sp., and dark-walled molds.</td>
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<tr>
<td>Voriconazole</td>
<td>New standard of care as initial therapy for invasive aspergillosis; treatment of other filamentous fungi resistant to amphotericin B (<em>Fusarium</em> sp., <em>Scedosporium</em> sp., and dark-walled molds); poor activity against zygomycetes; acceptable alternative to amphotericin B formulations as empirical therapy for neutropenic fever.</td>
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<tr>
<td>Posaconazole†</td>
<td>Similar spectrum of activity to voriconazole, but active against zygomycetes; growing clinical database from compassionate use protocols for treatment of <em>Aspergillus</em> sp. and other refractory filamentous fungi (<em>Fusarium</em> sp., <em>Scedosporium</em> sp., and dark-walled molds).</td>
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<tr>
<td>Ravuconazole†</td>
<td>Phase II study in progress.</td>
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<tr>
<td>Polyenes</td>
<td></td>
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<tr>
<td>Nystatin</td>
<td>Topical agent useful for mucosal candidiasis; parenteral liposomal nystatin is experimental.</td>
</tr>
<tr>
<td>Amphotericin B desoxycholate (Amb-D)</td>
<td>Broad spectrum of antifungal activity, but with significant infusion-related adverse events and nephrotoxicity</td>
</tr>
<tr>
<td>Lipid formulations of amphotericin B</td>
<td>Equal to or superior efficacy and ↓toxicity compared with Amb-D; ↑↑pharmacy acquisition cost.</td>
</tr>
<tr>
<td>Liposomal amphotericin B (LAMB)</td>
<td>↓proven breakthrough fungal infections and ↓infusion- and nephrotoxicity vs. Amb-D as empirical therapy for persistent neutropenic fever; ↓infusion- nephrotoxicity vs. amphotericin B lipid complex as empirical therapy.</td>
</tr>
<tr>
<td>Amphotericin B lipid complex (ABLC)</td>
<td>Extensive compassionate use database for patients with refractory invasive fungal infections or intolerance to Amb-D; successfully used in hepatosplenic candidiasis in pediatric patients; ↑↑levels in reticuloendothelial system.</td>
</tr>
<tr>
<td>Amphotericin B colloidal dispersion</td>
<td>Similar efficacy vs. Amb-D as therapy for invasive aspergillosis; ↓nephrotoxicity, but ↑infusion toxicity vs. Amb-D.</td>
</tr>
<tr>
<td>5-flucytosine (5-FC)</td>
<td>Randomized studies support combination Amb-D and 5-flucytosine for cryptococcal meningitis; pyrimidine analogue with dose- and duration-dependent myelotoxicity and gastrointestinal toxicity; monitoring of serum levels and adjustment of dosing for azotemia required.</td>
</tr>
<tr>
<td>Echinocandins</td>
<td>Class of antifungal peptides that inhibit synthesis of glucan, a fungal cell wall constituent; potently cidal against <em>Candida</em> sp., including fluconazole-resistant; fungistatic against <em>Aspergillus</em> sp., principally acting at growing hyphal tips; infrequent infusion-related events and not nephrotoxic.</td>
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<tr>
<td>Caspofungin</td>
<td>Compassionate use study of patients with refractory invasive aspergillosis or intolerance to licensed antifungal agents showed 41% successful responses (superior to carefully-matched historical controls) led to approval for this indication; favorable rate of successful outcome and ↓toxicity vs. Amb-D for invasive candidiasis; comparable efficacy and ↓toxicity vs. LAMB as empirical therapy for neutropenic fever.</td>
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<tr>
<td>Micafungin†</td>
<td>Trend toward ↓invasive aspergillosis and reduced frequency of empirical antifungal therapy compared with fluconazole in HSCT recipients</td>
</tr>
<tr>
<td>Anidulafungin†</td>
<td>Similar efficacy to fluconazole in AIDS-associated <em>Candida</em> esophagitis; phase III candidemia trial in progress.</td>
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*Non-licensed compounds*
States. The crude mortality rate varies in different series, but is generally between 30% to 60%.5

In a European surveillance study of candidemia in cancer patients, the overall 30-day mortality was 39%, with increased mortality occurring in older patients, in patients with poorly controlled malignancy, and in cases in which Candida (Torulopsis) glabrata was isolated.4 However, blood stream infection by non-C. albicans species was associated with neutropenia in solid tumor patients and acute leukemia and antifungal prophylaxis in hematology patients. Among hematology patients, additional factors associated with mortality were allogeneic bone marrow transplantation, septic shock, and lack of antifungal prophylaxis. In a retrospective study of 476 cases of candidemia at M. D. Anderson Cancer Center, the mortality rate was 52%. Neutropenia, a high APACHE score, and disseminated candidiasis were associated with poorer outcomes.7

Chronic disseminated candidiasis (also termed hepatosplenic candidiasis) is a complication of highly mucotoxic chemotherapy, such as anthracycline-containing regimens for acute leukemia. During neutropenia, the liver and spleen as well as kidneys, lungs, skin, bone, and other sites, become seeded by Candida in the blood stream (which may be undetected by blood culture). The only symptom may be persistent fever after neutrophil recovery. Usually after resolution of neutropenia, numerous target lesions in the liver and spleen become apparent by radiologic imaging, such as computed tomography (CT) scan, ultrasonography, or magnetic resonance imaging. Serial ultrasound analysis in patients in whom a high clinical suspicion exists may further enhance the likelihood of detecting new or evolving lesions.9 A liver biopsy is required for a definitive diagnosis, but because the lesions are discrete, a blind percutaneous biopsy may be falsely negative. An open or laparoscopic-guided liver biopsy should be considered if a percutaneous biopsy is non-diagnostic. Alternatively, a trial of systemic antifungal therapy (amphotericin B formulation, fluconazole, or caspofungin) may be considered without a definitive tissue diagnosis; resolution of fever and improvement radiographically in response to antifungal therapy would be considered presumptive evidence of candidiasis. Chronic disseminated candidiasis per se is not a contraindication for subsequent cytotoxic chemotherapy or hematopoietic transplantation.10,11 Patients in whom fever and lesions have resolved with antifungal therapy can undergo further episodes of neutropenia without progression of the fungal infection if antifungal therapy is reinitiated during the neutropenic periods.11

Therapy for Invasive Candidiasis

All candidemic patients should be treated with systemic antifungal therapy. The NCCN panel's recommendations on therapy for invasive candidiasis are in general agreement with recently published guidelines from the Infectious Diseases Society of America.12 Fluconazole has been shown to be a highly acceptable alternative to conventional amphotericin B in non-neutropenic patients with principally catheter-associated candidemia.13,14 High-dose fluconazole (800 mg daily) was recently compared with fluconazole (800 mg daily) plus conventional amphotericin B in non-neutropenic patients with primarily catheter-associated candidemia in a randomized, blinded trial.15 Failure of blood culture clearance was more common in fluconazole versus combination recipients (17% and 6%, respectively), and the combination regimen trended toward more rapid blood culture clearance. However, overall survival was similar (despite fluconazole alone recipients having a poorer physiologic score at randomization), and the combination arm had more frequent infusion-related nephrotoxicity. This study was not designed to address whether fluconazole plus amphotericin B was more effective than amphotericin B alone. Among neutropenic patients, fluconazole was as effective as amphotericin B in a matched cohort16 and in a randomized prospective study.17 To our knowledge, randomized trials evaluating lipid formulations of amphotericin B as initial therapy of invasive candidiasis have not been published.

Recently, a phase III, randomized, prospective, double-blinded study compared the echinocandin caspofungin with conventional amphotericin B in adults with invasive candidiasis.18 A total of 239 patients were enrolled. In the modified intent-to-treat analysis, the favorable response rates were 73.4% and 61.7% in the caspofungin and amphotericin B arms, respectively (P = NS). Among patients who received at least 5 days of study drug, which was a prespecified criterion for the “evaluable patients analysis,” caspofungin was statistically superior to amphotericin B (81% vs. 65% successful outcome, respectively; P < .05). In candidemic patients, the time to sterilization of blood was similar between the two arms, but caspofungin showed less toxicity. The small number of enrolled neutropenic patients precluded a comparison between study arms with adequate power. The overall
survival was similar between the two groups. This study strongly supports caspofungin as an option for initial therapy for invasive candidiasis in adults.

Based on these studies, the NCCN panel recommends either caspofungin or fluconazole (400 to 800 mg daily in patients with normal renal function) as initial therapy for candidiasis. Because caspofungin is fungicidal against virtually all clinical isolates of Candida species, including azole-resistant strains, the NCCN panel specifically recommends caspofungin as therapy for candidemia in the following settings: clinical instability, isolation of C. glabrata or C. krusei, or breakthrough candidemia in patients receiving azole prophylaxis. A phase III randomized study comparing voriconazole with conventional amphotericin B in patients with invasive candidiasis has completed enrollment, but the results have not yet been presented publicly.

Amphotericin B formulations are not recommended as initial therapy because of increased toxicity and lack of demonstration of benefit over less toxic alternatives. Amphotericin B lipid complex was safe and effective in adult and pediatric patients with disseminated candidiasis refractory to standard therapy and in patients intolerant to standard agents.19–21 In specific rare cases of complicated candidiasis, an amphotericin B formulation (often paired with 5-flucytosine) should be considered as initial therapy for invasive candidiasis, such as endocarditis, meningitis, or retinitis with macular involvement. An infectious diseases consultation is strongly advised.

Early catheter removal may reduce the likelihood of late complications by eliminating a potential nidus of ongoing candidemia and should therefore be considered in all patients with candidemia. Removal of intravenous catheters in candidemic patients has been shown to reduce the time to sterilization of the blood in non-neutropenic patients in which the catheter was the likely portal of entry.14,22 In patients who receive chemotherapy with significant mucotoxicity, candidemia was likely to arise from defects in the gut mucosa rather than the catheter.1,12,24 In a recent review of studies of candidemia, Nucci and Anaissie25 noted the lack of association between early central venous catheter removal and improved survival, and questioned the routine practice of catheter removal in all candidemic patients. If the catheter is not removed as part of the initial management of candidemia, we advise that it be removed in the setting of lack of resolution of fever within 2 to 3 days or persistent candidemia after 2 days of appropriate antifungal therapy.

Invasive Aspergillosis
Filamentous fungi (molds) are ubiquitous soil inhabitants whose conidia we inhale on a regular basis. Following inhalation, the respiratory mucosa and alveolar macrophages constitute the first line of host defense against conidia. At the hyphal stage, neutrophils are most important in controlling infection. Thus, prolonged neutropenia is a critical risk factor for invasive aspergillosis.26 Repeated cycles of prolonged neutropenia and concomitant corticosteroid therapy further increase the risk of filamentous fungal infection. Most filamentous fungal infections are caused by Aspergillus species. In addition, the frequency of rare but emerging pathogenic fungi commonly resistant to amphotericin B (Scedosporium species, Fusarium species, and dark-walled molds) has significantly increased over the past several years among patients with hematologic malignancies and in HSCT recipients.27

Prolonged and persistent neutropenia is a critical risk factor for aspergillosis.26 More recent studies have reported the predominance of aspergillosis cases occurring in the post-engraftment rather than the neutropenic period in allogeneic HSCT recipients, with immunosuppressive therapy for graft-versus-host disease (GVHD) being a principal risk factor.28–34 There are three likely reasons for the increased proportion of invasive filamentous fungal infections in the post-engraftment period: (1) shortening of the duration of neutropenia as a result of infusion of larger numbers of myeloid progenitors and treatment with colony stimulating factors; (2) increased proportion of unrelated donors and HLA-mismatched transplants, which predispose to GVHD; and (3) increased proportion of patients surviving beyond the early transplant period.

Aspergillosis can involve virtually any organ in the immunocompromised host, but sinopulmonary disease is the most common. In addition to air, hospital water systems may be a source of nosocomial aspergillosis.35 Invasive aspergillosis in the neutropenic host may present as fever, sinus pain, or congestion, cough, pleuritic chest pain, and hemoptysis. Erosion through a large central blood vessel wall can lead to massive pulmonary hemorrhage. The radiographic
appearance of pulmonary aspergillosis includes bronchopneumonia, lobar consolidation, segmental pneumonia, nodular lesions resembling septic emboli, and cavitary lesions. The central nervous system is a common target for hematogenous aspergillosis. Gastrointestinal aspergillosis usually coexists with pulmonary disease, but in rare instances, a sole organ is involved. Other sites of disseminated aspergillosis include the skin, heart, eye, bone, kidney, liver, and thyroid.

**Diagnosis**

Early diagnosis of aspergillosis in highly immunocompromised patients remains difficult. Blood cultures are rarely positive; sputum and bronchoalveolar cultures have approximately 50% sensitivity in focal pulmonary lesions, and definitive diagnosis often requires an invasive procedure and is usually only made when the disease is advanced. In a patient with neutropenia and a pulmonary infiltrate, isolation of an Aspergillus species from a sputum or bronchoalveolar lavage specimen should be presumed to represent invasive disease.36

Chest CT scans facilitate detection of pulmonary aspergillosis in patients with persistent neutropenic fever leading to earlier initiation of therapy, which in turn may be associated with an improved outcome.37 A CT scan may show peripheral or subpleural nodules inapparent on plain chest radiographs. The “halo sign” is a characteristic chest CT feature of angioinvasive organisms.38 The hazy alveolar infiltrates appear to correspond to regions of ischemia and are highly suggestive of invasive aspergillosis.39

A sensitive double-sandwich enzyme-linked immunosorbent assay (ELISA) for detection of the fungal cell wall constituent galactomannan has been developed.39 Maertens et al.40 obtained serial serum galactomannan levels from neutropenic and HSCT patients at high risk for aspergillosis. The positive and negative predictive values for predicting invasive aspergillosis 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.41

Herbrecht et al.42 evaluated the galactomannan antigenemia assay in 4 groups of patients: neutropenic fever of unknown etiology, suspected pulmonary infection, suspected extrapulmonary aspergillosis, and surveillance in HSCT recipients. Among cases of neutropenic fever (n = 261), only 1 possible case of invasive aspergillosis occurred. The positive predictive value of the antigenemia assay was 7.1% (1 true positive, 13 false positives) and the negative predictive value was 100% (247 true negatives and no false negatives). Patients received prophylactic or empiric antifungal agents according to the judgment of the treating physician.

The galactomannan assay was evaluated using 1,890 blood samples from 170 patients at high risk for invasive mold infection from 3 major cancer centers in North America. Using a lower cut-off (0.5 units) than in the European studies, this study found that the galactomannan assay identified 25 of 31 patients with invasive aspergillosis (81% sensitivity), and had a specificity of 89%.43 The FDA recently approved the Platelia Aspergillus enzyme immunoassay (Bio-Rad Laboratories, Redmond, WA).

These prospective studies showed significant differences in the sensitivity and positive predictive value of the antigenemia assay. In the study by Herbrecht et al.,44 the very low incidence of invasive aspergillosis in patients with neutropenic fever would be expected to reduce the positive predictive value of the assay. In addition, the sensitivity of the assay may be reduced by concomitant use of antifungal agents with activity against molds. Serial galactomannan sampling will increase sensitivity. False-positive results may be more common in children and allogeneic HSCT recipients42 and in persons receiving concomitant piperacillin-tazobactam.45 The variable sensitivity between studies even in cases of definite aspergillosis highlights the limitation of this assay as the sole diagnostic tool for detecting early Aspergillus infection.

The NCCN panel recommends a chest CT scan in patients with prolonged neutropenia (≥10 days) and persistent or recurrent fever of unknown origin and unresponsive to empirical antibacterial agents. A chest CT scan may be considered earlier in patients with multiple prior cycles of potently cytotoxic chemotherapy and in those receiving systemic corticosteroid therapy. In patients at high risk for invasive mold infection with a pulmonary infiltrate, a positive galactomannan assay establishes the diagnosis of “probable
aspergillosis," and in general obviates an invasive diagnostic procedure. Insufficient data are available to recommend routine serial galactomannan monitoring in patients with persistent neutropenic fever without physical examination findings suggestive of fungal infection or a lesion on chest CT scan.

Therapy
Important new developments in the antifungal armamentarium have occurred. Lipid formulations of amphotericin B have allowed for greater amounts of drug delivery with reduced toxicity. Amphotericin B colloidal dispersion had similar efficacy and survival, reduced nephrotoxicity, and increased infusional toxicity compared with conventional amphotericin B in a randomized study of patients with invasive aspergillosis. Liposomal amphotericin B and amphotericin B lipid complex have been evaluated in open-label non-randomized studies in invasive aspergillosis. These lipid formulations are safer than conventional amphotericin B, but such studies do not permit definitive conclusions as to whether they are more efficacious.

Voriconazole, posaconazole (SCH 56592), and ravuconazole (BMS 207147) are second-generation triazoles with a broad spectrum of activity against opportunistic yeasts and molds. Currently, only voriconazole is licensed. Voriconazole was compared with conventional amphotericin B (1.0 to 1.5 mg/kg daily) as initial therapy in an open-label, randomized trial of patients with invasive aspergillosis. Voriconazole was more effective than amphotericin B (51% vs. 32% of subjects had a complete or partial response) and was associated with improved survival at 12 weeks (71% vs. 58%, respectively). Among neutropenic patients, the success rate in the voriconazole arm was 51%, which was superior to the amphotericin B arm. 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. In both the randomized and non-comparative studies, the poorest prognosis was seen in extrapulmonary aspergillosis and in allogeneic HSCT recipients. In a retrospective analysis of 86 patients with central nervous system (CNS) aspergillosis treated with voriconazole either as primary or salvage therapy, 34% had a complete or partial response. This success rate compares very favorably to previous series in which the frequency of successful responses to amphotericin B was almost nil. Voriconazole appears to have comparable safety and efficacy in children with invasive mold infections compared with adults. Based on the strength of this database, the NCCN panel recommends voriconazole as first-line therapy for invasive aspergillosis.

Aspergillus fumigatus and Aspergillus flavus are the most common species causing invasive disease in neutropenic patients and after HSCT. Aspergillus terreus is seen with increasing frequency at several cancer centers and is notable for being resistant to amphotericin B. Treatment of A. terreus with voriconazole was associated with improved survival compared with amphotericin B.

Caspofungin has been evaluated as salvage therapy in patients with invasive aspergillosis refractory to standard antifungal therapy and in patients intolerant of standard therapy. The frequency of a successful outcome ranged between 40% to 45%, which compares favorably with carefully matched historical controls. However, echinocandins have not been evaluated as initial therapy for invasive aspergillosis. Significant interest has been noted in combination antifungal therapy pairing an echinocandin with either an amphotericin B preparation or a second-generationazole with activity against Aspergillus species. The rationale is that echinocandins target a unique site (the B-glucan constituent of the fungal cell wall) distinct from that of amphotericin B and azoles, which target the fungal cell membrane. Studies in vitro have shown neutral to synergistic activity (but no antagonism) involving the combination of an echinocandin with anazole or amphotericin B preparation, and combination therapy has been effective in animal models of aspergillosis. The published clinical experience involving combination regimens is limited to small series from single centers. Randomized studies are required to define the role of combination therapy as primary therapy for invasive aspergillosis.

Several centers reasonably use combination regimens as salvage therapy for refractory aspergillosis. The combination of caspofungin and liposomal amphotericin B as salvage therapy led to a favorable outcome in approximately 40% to 60% patients with either proven or possible invasive aspergillosis. In 47 patients with invasive aspergillosis refractory to amphotericin B preparations, the combination of...
voriconazole and caspofungin was associated with increased survival compared with voriconazole alone (Kieren Marr, personal communication). Although these results are promising compared with the historic success rate of salvage regimens for invasive aspergillosis, these studies are retrospective. Therefore, other host- and infection-related factors may have influenced the outcome. In cases of invasive aspergillosis refractory to voriconazole, salvage therapy with caspofungin plus a lipid formulation of amphotericin B (≥ 5 mg/kg/d) is reasonable.

Patients who recover from an episode of invasive aspergillosis are at risk for recurrence of infection during subsequent immunosuppression. In a multicenter European series of 48 patients with aspergillosis who subsequently underwent HSCT (77% allogeneic), 12 of 41 (29%) receiving secondary prophylaxis had recurrence of infection, compared with 4 of 7 (57%) who did not.61 Fourteen of 16 (88%) patients with relapsed infection died. Smaller series showed that systemic antifungal therapy (with or without surgical resection) of primary fungal infection followed by secondary prophylaxis suppressed reactivation in the majority of patients undergoing additional cycles of cytotoxic chemotherapy or HSCT.

Surgical excision of locally invasive disease, such as sinusitis, primary cutaneous lesions, intravitreal disease, or bone lesions should be performed when feasible. In neutropenic patients and in allogeneic HSCT recipients, combined surgery and systemic antifungal therapy should be considered in cases of apparent localized disease because of the risk of subclinical dissemination.

**Antifungal Prophylaxis**

The rationale for prophylaxis is to prevent fungal infections in a targeted group of high-risk patients. In HSCT recipients, two double-blinded, placebo-controlled trials have shown that prophylactic fluconazole controlled yeast colonization and reduced the rate of mucosal candidiasis and invasive Candida infections.62,63 The use of empirical amphotericin B for prolonged neutropenic fever also was delayed. A reduction in mortality was noted in the study by Slavin et al.,61 in which most of the patients were allograft recipients. This effect of fluconazole was found to confer significant long-term improvement in survival, possibly by reducing Candida antigen-induced gut GVHD.64

In a meta-analysis, antifungal prophylaxis with either azoles or low-dose amphotericin B reduced the frequency of superficial and invasive fungal infection and fungal infection-related mortality in HSCT recipients and in non-transplant patients with acute leukemia and prolonged neutropenia.65 Viscoli et al.66 noted an association between antifungal prophylaxis and an increased risk of bacteremia based on a retrospective analysis of clinical trials. This possible association merits evaluation in a prospective study.

Fluconazole prophylaxis reduced fungal colonization, invasive infection, and fungal infection-related mortality in non-transplant patients with leukemia and in autologous transplant recipients in a placebo-controlled trial.67 The benefit of fluconazole prophylaxis was greatest in autologous transplant recipients not receiving colony growth factor support and in patients receiving mucotoxic regimens consisting of cytarabine plus anthracyclines. This finding is consistent with the bowel being a principal portal of entry for Candida bloodstream infections. Other studies of non-transplant patients with acute leukemia showed no significant benefit of fluconazole.68,69 Fluconazole prophylaxis in this population is associated with colonization by azole-resistant Candida strains, which may be less intrinsically virulent than azole-sensitive C. albicans based on the low frequency of candidemia, invasive candidiasis, and attributable mortality.70 Fluconazole is not active against filamentous fungi.

The erratic bioavailability of itraconazole capsules limits its usefulness as prophylaxis in neutropenic patients, particularly those receiving mucotoxic regimens. The cyclodextrin solution formulation of itraconazole is a more viable option as prophylaxis in patients with prolonged neutropenia because intravenous administration leads to therapeutic levels by 3 days,71 and oral absorption is significantly improved over the capsule form. In a double-blind, placebo-controlled study of 405 patients with hematologic malignancy and prolonged neutropenia, prophylactic oral solution of itraconazole (2.5 mg/kg twice a day) initiated at the time of chemotherapy resulted in fewer suspected and proven fungal infections compared with the control group (24% vs. 33%, respectively; P < .05).72

A lower incidence of candidemia (1 vs. 8 cases) and a reduction in use of empirical amphotericin B was also noted in the itraconazole group. Four cases of aspergillosis occurred in the itraconazole arm and 1
case in the placebo arm; the overall low incidence of mold infection precluded a comparative analysis. Additional studies have shown that the oral cyclodextrin formulation of itraconazole is, in general, safe and effective as prophylaxis during prolonged neutropenia as long as adequate serum levels are maintained.\textsuperscript{73-77}

Winston et al.\textsuperscript{78} compared itraconazole solution with fluconazole as prophylaxis in allogeneic HSCT recipients in an open-label, multicenter, randomized trial. Antifungal prophylaxis was administered from day 1 until day 100 after transplant. Proven invasive fungal infections (mostly Candida and Aspergillus species) occurred in 6 of 71 itraconazole recipients (9%) and in 17 of 67 fluconazole recipients (25%) during the first 180 days of transplantation ($P = .01$). Although the researchers noted a trend toward reduction in fungal infection-related mortality in itraconazole recipients, overall 180-day mortality was similar.

In another randomized study, itraconazole (solution) or fluconazole prophylaxis was administered to allogeneic HSCT recipients ($n = 304$) for the first 180 days of transplant and until 4 weeks after therapy for GVHD was stopped.\textsuperscript{79} Fewer invasive mold infections were seen in itraconazole (5%) versus fluconazole (12%) recipients, and the rates of invasive candidiasis were similar (3% and 2%, respectively). Hepatic toxicity and discontinuation because of gastrointestinal intolerance were more common in itraconazole recipients. No difference in survival or fungal-infection-free survival occurred. Itraconazole, led to an increase in cyclophosphamide metabolites, which in turn correlated with hyperbilirubinemia and nephrotoxicity during the early transplant period.\textsuperscript{80} 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. It also highlights the need for well-designed randomized trials that evaluate safety and efficacy to guide decisions about use of antifungal agents in specific settings.

Low-dose amphotericin B has been used as prophylaxis in patients receiving chemotherapy for acute leukemia. Conventional amphotericin B has not been shown to be more effective than azoles; however, it does have significantly greater nephrotoxicity,\textsuperscript{81} and in our opinion should not be used as primary prophylaxis. Low-dose amphotericin B lipid complex and liposomal amphotericin B appear to have similar efficacy and were tolerated well as prophylaxis in patients with acute myeloid leukemia and myelodysplastic syndrome undergoing induction chemotherapy.\textsuperscript{82} Prophylaxis with aerosolized amphotericin B (conventional or lipid formulations) merits further evaluation in clinical trials.

The NCCN panel recommends either fluconazole or itraconazole (solution) as prophylaxis in allogeneic HSCT recipients. Prophylaxis should be administered until at least day 100 after transplantation. Prophylaxis continuation should be considered in patients with GVHD requiring corticosteroid therapy. Fluconazole or itraconazole should be considered in patients without transplantation patients with acute leukemia and in autologous HSCT recipients receiving mucotoxic regimens; prophylaxis should be administered until neutrophil recovery. Itraconazole, voriconazole, and, to a lesser degree fluconazole are potent inhibitors of specific cytochrome p450 isoenzymes, requiring close monitoring for drug-drug interactions and appropriate dosing modifications of agents metabolized via this pathway. Itraconazole is contraindicated in persons with significant cardiac dysfunction based on its negative inotropic properties. Intravenous (but not oral) itraconazole should be avoided in patients with preexisting azotemia based on the potential for the cyclodextrin vehicle to accumulate systemically and worsen kidney function. A multicenter randomized trial comparing fluconazole with voriconazole as prophylaxis in allogeneic HSCT recipients has begun; at this point, no data are available to support voriconazole as prophylaxis. If an amphotericin B product is used as primary prophylaxis, a lipid formulation is preferred over conventional amphotericin B because of reduced toxicity. The echinocandin, micafungin, appears to be highly promising as prophylaxis in HSCT recipients,\textsuperscript{83} but has not yet been approved by the FDA. Secondary prophylaxis with an appropriate antifungal agent is advised in patients with prior chronic disseminated candidiasis\textsuperscript{84} or invasive filamentous fungal infection\textsuperscript{4} during subsequent cycles of cytotoxic chemotherapy or HSCT.

**Protected Environments**

The Centers for Disease Control (CDC) have proposed detailed guidelines related to infection control procedures to minimize opportunistic infections after...
HSCT. The guidelines related to invasive mold infections can reasonably be extrapolated to other patients with cancer at high risk for mold infection (such as those with prolonged neutropenia). Although well-designed clinical trials have not validated the use of high-efficiency particulate air (HEPA) filtration, we agree with the CDC recommendation that HEPA filters be used in rooms of allogeneic HSCT recipients. The principal benefit of HEPA filtration is probably related to prevention of mold infections. In a retrospective analysis, HEPA filters were protective in highly immunocompromised patients with hematologic malignancies in the setting of an outbreak of aspergillosis. The value of laminar air flow in preventing infections is unclear and is not generally recommended. Routine air sampling to quantify fungal spore concentrations is also not advised.

Hospital policies vary with regard to patients at high risk for mold infections using masks when outside of a protected environment. The value of using masks is unproven. N95 respirators are likely to provide the best protection; however, fit-testing and training are required for optimal benefit. They are also uncomfortable and may not be tolerated by patients for prolonged periods. Routine surgical masks may not provide any protection from inhalation of fungal spores. Guidelines related to minimizing patient exposure to fungal spores during hospital construction have been previously described in authoritative reviews.

**Empirical Antifungal Therapy in Persistent Neutropenic Fever**

The rationale for empirical antifungal therapy for persistent febrile neutropenia is that clinical examination and collection of cultures are not sufficiently sensitive for early detection of fungal infections. Before standard implementation of empirical antifungal therapy, there was a correlation between prolonged neutropenic fever and mortality in patients with cancer, and fungal infection was frequently found at autopsy. Two randomized prospective studies showed that empiric amphotericin B was associated with a trend toward fewer serious fungal infections in antibiotic-treated neutropenic patients with persistent fever. Because fungal infections are uncommonly encountered in the first 7 days of neutropenic fever, empirical antifungal therapy is typically begun between days 4 to 7 of neutropenic fever. We suggest that empiric antifungal therapy be continued for the duration of neutropenia.

In a randomized study of patients with neutropenic fever unresponsive to standard antibacterial agents, liposomal amphotericin B (LAMB) was associated with fewer proven breakthrough fungal infections and less infusion-related and renal toxicity compared with conventional desoxycholate amphotericin B. Using decision analysis models in which both drug cost and risk of nephrotoxicity were considered, break-even points for the cost of LAMB were derived. This analysis is valuable in highlighting overall cost of care rather than solely pharmacy acquisition prices. In another randomized study of empiric antifungal therapy for neutropenic fever, LAMB reduced infusion-related toxicity and nephrotoxicity compared with amphotericin B lipid complex.

Intravenous followed by oral itraconazole solution (cyclodextrin formulation) was as effective as, but less toxic than conventional amphotericin B as empirical therapy for neutropenic fever in an open, randomized study, leading to FDA approval of itraconazole solution for this indication. Prior use of prophylactic fluconazole was similar in both groups. This is an important consideration given the potential for cross-resistance of fungal pathogens to different classes of azoles. Fluconazole also has been used successfully as empirical therapy for neutropenic fever. However, its lack of activity against molds makes fluconazole unsuitable as empirical antifungal therapy in patients at high risk for mold infection.

Newer generation azoles and echinocandins are attractive candidates for antifungal prophylaxis and empirical therapy for neutropenic fever. Voriconazole was compared with LAMB in a non-blinded, randomized study of empirical antifungal therapy in patients with persistent neutropenic fever (n = 837 patients, 72% with hematologic malignancies) unresponsive to antibacterial agents. Treatment success was stringently defined and required fulfillment of all criteria grouped into a composite outcome. Based on the composite analysis, the overall success rates were 26% with voriconazole and 31% with LAMB. Empirical voriconazole was associated with fewer breakthrough fungal infections (1.9% vs. 5.0%), with the greatest protective benefit occurring in the protocol-defined high-risk patients (relapsed acute leukemia and allogeneic HSCT).
Although patients were stratified according risk of fungal infection at the time of enrollment and the study was prospectively powered to evaluate differences in breakthrough fungal infections, this endpoint was not by itself a protocol-defined determinant for successful outcome. Infusion-related and nephrotoxicity were more common in the LAMB arm, whereas transient visual changes and visual hallucinations were more common in the voriconazole arm. Because of the lack of proof of non-inferiority of voriconazole compared with LAMB based on pre-specified endpoints for a successful outcome, voriconazole was not approved by the FDA for use as empirical therapy. Voriconazole has poor in vitro activity against zygomycetes (agents of mucormycosis), and recent case reports note breakthrough zygomycosis in allogeneic HSCT recipients receiving voriconazole as prophylaxis and empirical antifungal therapy.95

Caspofungin was recently compared with LAMB as empiric therapy for persistent neutropenic fever in a randomized double-blind study.96 The overall success rate, as defined by a pre-specified composite analysis, was 34% in both arms. The frequency rates of breakthrough fungal infections were similar in caspofungin (5.2%) and LAMB (4.5%) recipients. Drug-related toxicities and premature withdrawals because of drug-related adverse events were significantly lower in caspofungin recipients. A trend to improved 7-day post-therapy survival was seen in caspofungin as compared with LAMB recipients (92.6% vs. 89.2%, respectively; \( P = .051 \)). In patients with a baseline fungal infection, mortality was 11% in caspofungin and 44% in LAMB recipients, respectively (\( P < .01 \)). The results of this study have been published in abstract form only.

The selection of an empirical antifungal agent should be tailored to the individual patient and should broadly consider risk of breakthrough fungal infection, toxicity, and a pharmacoeconomic analysis as opposed to solely the pharmacy acquisition costs. The NCCN panel considers the following agents to be acceptable as empirical therapy for neutropenic fever: amphotericin B (conventional and lipid formulations); fluconazole; itraconazole (solution); voriconazole; and caspofungin. Of the amphotericin B formulations, the committee believes LAMB to be preferable based on reduced nephrotoxicity. The NCCN panel would consider caspofungin to be preferable to amphotericin B formulations as empirical antifungal therapy.

**Immune Augmentation**

**Colony-Stimulating Factors**

A cornerstone in controlling invasive fungal infections relates to immune reconstitution. In neutropenic patients, rapid recovery from neutropenia is of key importance in resolving an established infection, particularly invasive fungal infections. Whenever feasible, discontinuation of immunosuppressive medications (such as corticosteroids) is advised in the setting of serious fungal infections.

Primary administration of colony-stimulating factors (CSF) has reduced the incidence of febrile neutropenia by approximately 50% in randomized trials in adults in whom the incidence of febrile neutropenia was greater than 40% in the control group. In patients with acute myelogenous leukemia, CSFs have not produced a modest decrease in the duration of neutropenia, which in some studies has translated into a reduction in the duration of fever, use of antibiotics, and hospitalization.97,98 This benefit has mainly been shown in patients 55 years of age or older and after consolidation chemotherapy. With the exception of one placebo-controlled study in which granulocyte-macrophage (GM)-CSF was associated with a lower frequency of fatal fungal infections and early mortality in acute myelogenous leukemia,99 CSFs have not produced a survival advantage.

The American Society of Clinical Oncology (ASCO) has recommended that prophylactic CSFs (G-CSF and GM-CSF) be used only in populations in which the frequency of febrile neutropenia is likely to exceed 40%.100 The ASCO guidelines also suggested that certain patients receiving a relatively non-myelo-
In our opinion, patients with prior serious or life-threatening infection such as invasive fungal infection should also be considered for CSF treatment during subsequent chemotherapy.

The rationale for CSFs for established infections (as opposed to prophylaxis) stems from both the quantitative and qualitative effects of these agents on phagocytic cells. In neutropenic patients with life-threatening infections, survival is strongly influenced by the rapidity of neutrophil recovery. Randomized trials have not shown a benefit for CSFs as adjunct therapy for uncomplicated neutropenic fever. Although the benefit of a CSF for established infections is unproven, it may be considered in the setting of profound neutropenia (ANC < 100/mcl) and in serious infections, including invasive fungal infection (Category 2B).

Granulocyte Transfusions

The rationale for granulocyte transfusions is to provide supportive therapy for the neutropenic patient with a life-threatening infection by augmenting the number of circulating neutrophils until myeloid regeneration occurs. Today, the impetus to reexamine the role of granulocyte transfusions stems largely from improvements in donor mobilization methods. Price et al. conducted a phase I to II study of granulocyte transfusions derived from unrelated, non-HLA-matched community donors, after G-CSF and dexamethasone mobilization. Chills, fever, and oxygen desaturation of 3% or more occurred in association with 7% of transfusions, but did not limit therapy. Eight of 11 patients with bacterial infections or candidemia survived, but all 8 patients with invasive mold infection died. This study showed the safety and feasibility of using community donors for granulocytapheresis donations.

In the absence of modern, prospective, randomized studies, the NCCN panel recommends that granulocyte transfusions should be reserved for patients with prolonged neutropenia and life-threatening infections refractory to conventional therapy (Category 2B). Filamentous fungi are likely to constitute the majority of such refractory infections. Given the potential toxicity and unproven benefit of granulocyte transfusions, it is also acceptable to not use granulocyte transfusions as adjunctive therapy. Currently, there is no justification (outside of a clinical trial) to use granulocyte transfusions either as prophylaxis or in cases of documented infections that are likely to respond to conventional therapy. In some highly alloimmunized patients, transfused granulocytes are rapidly consumed and are likely to have more toxicity than benefit. In allogeneic transplants in which the donor and recipient are cytomegalovirus (CMV) seronegative, using CMV seronegative granulocyte donors is advised.

Other Immune Augmentation Strategies

Other immune augmentation strategies have been used in refractory invasive fungal infections, which include infusions of donor lymphocytes in allogeneic HSCT recipients and use of recombinant interferon-gamma (IFN-γ). Data are limited to case studies, and the NCCN panel is therefore unable to make any recommendations about these adjunctive therapies.

Conclusions

Invasive fungal infections are a major cause of morbidity and mortality in patients with prolonged neutropenia and in allogeneic HSCT recipients. Seminal advances in the antifungal armamentarium have been made, leading to new standards of care. In addition, newer non-culture–based diagnostic assays permit earlier diagnosis of invasive aspergillosis and may obviate an invasive procedure in specific settings. We have stressed an evidence-based approach to guide the use of these new treatment and diagnostic modalities. New areas of research will focus on modeling combination antifungal agents, drug discovery (facilitated by identification of promising drug targets using A. fumigatus genomics data), and immune augmentation.

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