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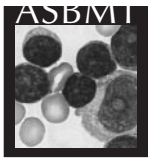

Fungal infections after bone marrow transplant

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ABSTRACT

With improved control of cytomegalovirus infection, invasive fungal infections have become the leading cause of infectious mortality after bone marrow transplantation (BMT). A number of changes in transplant practices have led to changes in patterns of fungal infections: neutropenic episodes have been shortened through the use of hematopoietic growth factors and peripheral blood as a source of stem cells. More potent immunosuppressive regimens, including T-cell depletion techniques, have encouraged the use of alternate donor sources with greater numbers of transplant recipients experiencing more prolonged and more profound immunodeficiency following engraftment. The advent of new antifungal agents has led to a decline in *Candida* infections, but has encouraged the emergence of other less susceptible fungal pathogens. The development of molecular techniques to distinguish different fungal strains has led to identification of nosocomial transmission as an unexpected means for the spread of fungal infections in BMT units. These shifts in fungal infection patterns emphasize the need for infection control monitoring. The development of more accurate diagnostic tools and the incorporation of new antifungal agents into practice are needed to further improve outcomes.

KEY WORDS

Hematopoietic cell transplant • Fungal infections • Antifungal agents

INTRODUCTION

Invasive fungal infections are major causes of morbidity and mortality in the bone marrow transplant (BMT) setting. Improved understanding of changes in the spectrum of pathogens and risk factors for infection, along with recognition of nosocomial transmission as a major mode of infection, and the introduction of new therapeutic agents have necessitated changes in the way the clinician approaches this problem.

THE BIOLOGY OF FUNGAL MICROORGANISMS

Fungal organisms are eukaryotic with rigid cell walls containing chitin, β-glucan, and mannoproteins. They may be unicellular or multicellular and can be multinucleate. They are grouped by morphology as either yeasts or filamentous molds. Some organisms may have both morphologies. Reproduction occurs by the formation of spores through mitosis; some also reproduce sexually. The fungal cell wall takes up the methenamine silver stain, a useful technique to identify the organisms in tissue sections. Many fungi, except for *Candida*, do not take up the Gram stain very well and may be missed if appropriate fungal stains are not used. Within the cell wall is a sterol-containing cytoplasmic membrane. The major fungal sterol is ergosterol, whereas cholesterol is the major sterol of human and other mammalian cell membranes. The cytoplasmic membrane is a major target for antifungal agents currently in use, including azoles, allylamines, and polyenes.

Fungal organisms are ubiquitous in nature. Although there are an estimated 250,000 fungal species, fewer than 150 have been described as human pathogens. Yeasts are the most common fungal organisms that colonize and infect humans. Of the hundreds of yeast species, however, only a few (such as the genus *Candida*) cause human or animal disease. The genus *Candida* has approximately 200 species. *Candida* species are found on many plants. Some *Candida* species are also part of the normal flora within the gastrointestinal tract of humans and other mammals. Approximately 80% of normal healthy individuals carry one or more *Candida* species. These are only a minor constituent of the gut flora, however. *Candida albicans* represents one-half to two-thirds of human yeast isolates; a dozen other species are also occasionally present.

Nearly 20 *Candida* species have been described as human pathogens. Virulence factors associated with pathogenicity include rapid germination capacity, protease production, adherence factors, complement protein-binding receptors, phenotypic switching, and hydrophobicity. Virulence factors have been reviewed elsewhere in detail [1–3].

Molds ordinarily are not found in or on the human body. Typically, the portal of entry is a break in the epidermis or inspiration into the nasal passages or respiratory tract. The genus *Aspergillus* is the most common human pathogen of the various molds. The most prevalent species are *Aspergillus fumigatus*, *A flavus*, and *A niger*. Other species, including *Fusarium*, *Penicillium* species, *Alternaria*, *Trichosporon* and members of the *Mucorales* order, are also increasingly being recognized as opportunistic human pathogens. The dermatophytes, including *Trychophyton* and *Microsporum*, organisms that have the capacity to digest keratin, normally reside within soil habitats that can occasionally infect keratinized tissues (hair, nails, and skin) but only rarely invade tissue percutaneously.

Infection of humans by most fungal species is usually an accidental occurrence; the human host is not a significant reservoir. Thus, most fungal infections are not contracted by person-to-person contact, but rather acquired through exposure to a source in nature. In contrast, some fungi reside in or on the human body (e.g., *Candida*). Recovery of such organisms, therefore, has no clinical meaning other than in the setting of immune compromise, where such organisms may become opportunistic by invading host barriers, entering normally sterile tissue, and causing tissue damage.

Invasive infection occurs through an interplay of a number of factors: host acquisition of the potential pathogen, successful pathogen competition with microbial competitors to grow in sufficient numbers to overcome phagocytes, the inherent virulence properties of the pathogenic organism to invade and cause tissue damage, and a compromise in the integrity of the host's normal defenses. For invasive infection to take place, generally speaking, an alteration in one or more of the above factors must take place. For example, an exposure to substantial numbers of certain exogenous organisms with appropriate virulence properties might be sufficient to cause an infection in an individual without immune compromise; inhalation of *Histoplasma* or *Coccidioides* organisms by healthy individuals in certain geographic locales would be examples of this. In contrast, mucosal infection by commensal *Candida* organisms only occurs if the competing endogenous bacterial flora is suppressed by antibiotics; a compromise in host defenses, such as mucosal damage along with neutropenia, may be additionally required before a mucosal infection can lead to a systemic infection.

Fungal organisms can be divided into three major categories according to their infection patterns [4]. The first category, including fungi such as *Coccidioides imitis*, *Blastomyces dermatidis*, and *Sporotrix schenckii* cause infections in individuals who do not appear to have an immune compromise. Infections by these organisms do not generally occur more readily in immunocompromised patients. The second category of fungal organisms cause infections to a greater degree in patients whose immunity is compromised. Examples of such organisms include *Cryptococcus neoformans* and *Histoplasma capsulatum*. The third category of organisms appears to be most predominantly associated with the treatment of an underlying disease rather than the disease itself. *Candida* and *Aspergillus* organisms exemplify this group of organisms. Neither *Candida* nor *Aspergillus* infections were noted in leukemia patients or in patients with other malignancies until the advent of chemotherapy [5]. It is not the disease itself which predisposes the patient to infection by these opportunistic organisms, but the alteration of host defenses by treatment (e.g., mucositis and neutropenia caused by chemotherapy, and fungal overgrowth in the gut facilitated by antibiotic suppression of bacteria).

RISK FACTORS

Disease treatment rather than the underlying disease condition itself has historically been the most prominent factor associated with predisposition for the common invasive fungal infections. Indeed, as noted above, the emergence of opportunistic and fungal infections occurred only after the introduction of cytotoxic therapeutic agents in the 1950s [5]. Before that, fungal infections in cancer patients were rare. The contribution of leukopenia, damage to the mucosa, and concomitant bacterial infection were suggested in that initial report as factors associated with the occurrence of invasive fungal infection.

Historically, in both oncology practice as well as the management of the BMT recipient, neutropenia has been recognized as the major risk factor for all infectious complications, including fungal infections. Both depth and duration of neutropenia influence the risk for infection. Fungal infections are rarely the cause of fever during the first week of neutropenia. During the second and subsequent weeks of neutropenia, the risk of febrile fungal infections increases incrementally [6]. Prolonged neutropenia today remains a major risk factor in the BMT setting. This is especially problematic in patients who fail to engraft or in patients in whom the stem cell graft contains low numbers of progenitors.

Today, with an emphasis on optimizing the progenitor cell content of the stem cell graft, prolonged neutropenia is less frequent than in former years. Thus, the risk for fungal infection before engraftment is lower. This is illustrated by two recent reports in which neutropenia was noted to be present in only a minority of patients with invasive aspergillosis; the vast majority of infections occurred after engraftment [7,8].

Damage to the mucosa allows opportunistic organisms with appropriate virulence factors to make the transition from colonization to tissue invasion. The portal of entry for *Candida*, a commensal organism normally residing in the lumen of the gastrointestinal tract, is the gastrointestinal tract. In contrast, *Aspergillus*, an airborne organism, enters via the nasal passages and respiratory tract. For *Candida*, the first step leading to infection is a proliferation of organisms, made possible by antibiotic suppression of bacterial flora. For *Aspergillus*, the first step is entry into the host. For both, the crucial next prerequisite step is getting past the mucosal barrier. This usually occurs only if there is damage to the mucosal barrier to permit tissue invasion. In one treatment center, the rates of invasive fungal infection in sequential cohorts of leukemic patients receiving different chemotherapy regimens, each producing comparable neutropenic intervals, were noted to be markedly different [9]. The infection rate correlated with mucosal injury as assessed by absorption of D-xylose [9,10].

Mucositis, neutropenia, and antibiotic use are the major risk factors prior to engraftment. Following engraftment there is a second risk period for invasive fungal infection, which, as noted above, has now emerged as the most important risk period in allogeneic transplant recipients. The use of corticosteroids and the occurrence of acute graft-vs.-host disease (GVHD) are the major predisposing factors [11–13]. Both GVHD and corticosteroid use are associated with a suppression of cell-mediated immunity. Corticosteroids also impair effective phagocytosis, and GVHD is associated with an impaired reticuloendothelial system, both representing important host defenses that are also adversely affected.

The risk for infection correlates with both the dose of steroids and duration of use. After matched unrelated donor or mismatched family donor transplants, the risk for late infection is substantial even in the absence of GVHD or steroid use [14]. Although not formally assessed, this is presumably due to inadequate T-cell immune responses.

The use of T-cell depletion was expected to lead to lower rates of fungal infection due to less GVHD and a resulting decrease in corticosteroid use. In contrast, however, rates of invasive fungal infection were found to be higher in several series [11,15]. The greater risk for fungal, cytomegalovirus (CMV), and Epstein-Barr virus infectious complications observed after pan–T-cell depleted allografts emphasizes the need to preserve certain protective subpopulations of lymphocytes and phagocytic precursors in the graft.

Prior *Aspergillus* infection has been noted in numerous studies to be a risk factor for reactivation after repeated chemotherapy courses [16] and subsequent BMT. Indeed, the risk has been perceived to be so high for transplant-related mortality that many centers in the past have regarded this as a contraindication to transplantation [17–19].

Other risk factors have been described by various investigators. They include older age, type of transplant (alternate donor vs. sibling-matched donor vs. autologous source in descending order of risk), use of total-body irradiation, low stem cell dose, CMV seropositivity, and prior splenectomy [8,11–14,20–23].

THE CHANGING EPIDEMIOLOGY OF FUNGAL PATHOGENS

Candida albicans has historically been the leading fungal pathogen in BMT patients. In recent years, however, a wide range of nonalbicans *Candida* species have emerged as important pathogens, now accounting for one-half of all *Candida* infections [24]. Other organisms such as *Fusarium*, *Trychosporon* species, and *Alternaria* are among the myriad non-*Candida* organisms that have also emerged as pathogens in the BMT patient population [25]. Their ascendancy has been linked to a variety of health care measures [24,26,27] (Table 1). Various innovations in transplant practices have led to shifts in pathogens (Table 2).

Table 1. *The link between emerging fungal pathogens and certain health care practices*

Fungal pathogen	Health care practice implicated
C tropicalis	Cytoreductive agents that cause mucositis
C krusei	Fluconazole use
C glabrata	Fluconazole use
C lusitania	Polyene use
C parapsilosi	Contamination of intravenous solutions and devices
Aspergillus	Fireproofing material, hospital construction

Investigation of outbreaks of *Candida* infections by recombinant DNA technology in several BMT centers have led to the identification of a common source in some instances [28–34]. Indeed, surveillance studies in critical care units and BMT centers have shown contamination of environmental surfaces and health care workers' hands by the same strain of organism. Although endogenous organisms remain the major source for *Candida* infections, increasingly important are exogenous organisms acquired from the health care practice environment. Any outbreak should be investigated with molecular techniques to identify and correct at-risk practices [35–36].

High efficiency particulate air (HEPA) filters (or laminar airflow) have reduced the risk for nosocomial acquisition of *Aspergillus* spores [8,37–39]. Aspergillosis control, however, remains as challenging today as a decade ago. Many transplant patients enter the hospital already colonized by *Aspergillus*. Moreover, as we increasingly shift care to the outpatient setting, the patient spends less time in the hospital and more time in outpatient clinics and offsite residential areas, none of which have specialized air filtration systems. The impact of these shifts in practice patterns on infection rates and the pathogen spectrum remains to be seen.

Table 2. *Innovations in transplant practices that have led to shifts in fungal infection patterns*

Innovation	Effect on host	Fungal sequela
Hematopoietic growth factors	Shorter time to neutrophil recovery	Decrease in infection rate before engraftment
Use of peripheral blood as source of stem cells	Shorter time to neutrophil recovery	Decrease in infection rate before engraftment
T-cell depletion	Slower recovery of cell-mediated immunity	Increase in postengraftment infection rates
More potent immunosuppressive regimens	Slower recovery of cell-mediated immunity	Increase in postengraftment infection rates
Alternate donor transplants	Slower recovery of cell-mediated immunity	Increase in postengraftment infection rates
Fluconazole		Reduction in *C albicans* infection rate
		Increase in other fungal infections
		Emergence of drug resistance (still uncommon)
Outpatient transplant activity	Greater exposure to exogenous fungal organisms	(? may lead to higher infection rates)

Table 3. *Fungal targets for antifungal therapeutics*

Fungal targets	Antifungal agents
Cell wall	Echinocandins Pradimicins Nikkomycins
Cell membrane	Polyenes (amphotericin B, nystatin) Azoles (fluconazole, itraconazole) Allylamines
DNA and RNA synthesis	Flucytosine

MANIFESTATIONS AND DIAGNOSIS

The most common manifestation of *Candida* infection is unexplained fever. Endophthalmitis, rare before engraftment, is a more common sign of candidemia in the postengraftment period. The retinal lesions may be subtle and should be investigated by indirect ophthalmoscopy. Macronodular skin lesions, polyarthralgias or polymyalgias (due to tissue invasion of joints and muscle), and azotemia (due to an invasion of renal tubules) are occasional manifestations of *Candida* fungemia. Persistent fever at engraftment and an elevation of serum alkaline phosphatase may signal the presence of hepatosplenic candidiasis [40].

Pulmonary disease and, less commonly, sinusitis are manifestations of aspergillosis. The propensity of *Aspergillus* hyphae to invade blood vessels and cause pulmonary infarction produces the signs of pleuritic chest pain, hemoptysis, localized wheezing, pleural friction rub, sinus tenderness, nasal discharge, epistaxis, nasal eschar, and rales. Any of these signs or symptoms in the setting of persistent fever should lead to the suspicion of aspergillosis [41,42]. Pulmonary infiltrates tend to be nodular and peripherally located. Chest and sinus computerized tomography scans can detect disease earlier than plain radiographs [43–45].

Primary cutaneous aspergillosis can occur at the exit site or tunnel of a Hickman intravenous catheter. In one report, outbreak of *Aspergillus* catheter infections during a period of hospital renovation was caused by aerosolized spores being introduced into the surgical wound [46]. Organisms were recovered from air samples in operating rooms where infected catheters were placed, but were not recovered from other operating rooms.

Brain abscesses in the BMT patient are commonly due to fungi. In one center, 92% of all cases of brain abscesses were caused by fungi [47]. *Aspergillus* accounted for 58% of cases and *Candida* for 33%. Bacteria were involved in fewer than 10% of cases. *Aspergillus* brain abscesses usually occurred concomitantly with pulmonary disease (87% of cases). *Candida* brain abscesses were associated with fungemia or neutropenia.

Surveillance cultures have limited utility [48–51]. They are costly and time consuming. Although their negative predictive values are high, positive predictive values are low. Because of these shortcomings, surveillance cultures have largely been abandoned. With the emergence of antifungal resistance, however, and a desire to target only individuals at risk for antifungal therapy, one group has proposed the use of fungal surveillance cultures to identify those at risk and to exclude those not at risk (those with negative surveillance cultures) to avoid unnecessary antifungal prophylaxis [52]. For those with positive surveillance cultures, preemptive antifungal prophylaxis could be used to prevent invasive infection. This would have the desirable benefit of providing early therapy for those at risk while minimizing the hazard for emergence of antimicrobial resistance, a result of widespread indiscriminate antimicrobial usage. Such an approach has not been formally tested prospectively.

Because of the subtlety of clinical signs and symptoms and the unreliability of blood cultures, a variety of new techniques have been investigated to improve the early documentation of infection. Antigen assays for *Cryptococcus* and *Histoplasma* antigens are currently available in the United States and can be quite useful in detecting infections by both organisms. Unfortunately, rapid diagnostic tests for the most common opportunistic infections in BMT patients, *Candida* and *Aspergillus* infections, are not currently available. For these two pathogens, assays for fungal antigens, antibodies, and metabolites have generally failed to discriminate colonization from infection or, where promising [53], are unavailable commercially. Early work using polymerase chain reaction (PCR) probes sensitive to *Candida* DNA are promising [54–56]. Both antigen detection and PCR assays for *Aspergillus* are under development [57–63]. Whether these will prove useful can only be determined through wider testing of specificity and sensitivity, and commercial development.

ANTIFUNGAL AGENTS

There are several classes of antifungal agents. These include polyenes, nucleoside analogs, azoles, echinocandins, pradamicins, allylamines, and nikkomycins. These have recently been comprehensively reviewed [64]. The fungal targets for various agents are listed in Table 3.

Agents in current use

Amphotericin B, a polyene antifungal, is a lipophilic rod-shaped molecule that acts on the cytoplasmic membrane by binding to sterols such as ergosterol to increase membrane permeability. Loss of intracellular potassium and other molecules then ensues which leads to cell death. Resistance is uncommon, but can occur by alteration of the membrane sterol content [65–67]. Certain fungal species, most notably *C lusitaniae*, are frequently resistant to amphotericin B [68].

Unfortunately, amphotericin B has a very narrow therapeutic ratio. Infusional toxicities are quite frequent and can be quite severe. They include fever, shaking chills, and, occasionally, respiratory distress, hypoxia, and hypotension. Nephrotoxicity can also be quite problematic and can, on occasion, necessitate hemodialysis [69]. Wasting of potassium and magnesium, anemia, hepatotoxicity and other side effects can also occur. Most toxicities are reversible with cessation of therapy. There are no acceptable pharmacologic assays to monitor amphotericin B therapy. Generally, doses of 0.3–0.6 mg $\cdot$ kg^{-1} $\cdot$ day^{-1} are used for *Candida* infections. Higher doses such as 0.75–1.0 mg $\cdot$ kg^{-1} $\cdot$ day^{-1} are generally used for *Aspergillus* infections. Dose adjustments must be made if toxicities occur.

To improve the therapeutic ratio, lipid formulations of amphotericin B have been developed. Three products are

now licensed in the United States—amphotericin B in lipid complex (ABLC), liposomal amphotericin B, and amphotericin B in colloidal dispersion (ABCD). The pharmacologic properties of these agents have been reviewed [70–74]. A variety of studies have demonstrated that these agents can be given in up to 10 times higher doses than the parent compound with a lesser degree of nephrotoxicity. Infusional side effects still occur; there appear to be some differences in the rates of these infusional toxicities with the three agents, but a direct comparison has not been performed. These agents have been successful in treating patients with invasive fungal infections in which amphotericin B has failed or caused intolerance [75,76]. However, prospective randomized studies of the lipid formulations of amphotericin B have failed to produce consistent and substantial improvements in efficacy when compared with amphotericin B as primary therapy for invasive fungal infections [77,78]. The improved therapeutic ratio is thought to be due to higher concentrations of the drug reaching target tissues, such as lung, liver, and spleen, with lower concentrations of the drug in the kidney (the site of major toxicity). The mechanisms by which the lipid formulations of amphotericin B actually deliver the active drug to the fungal cell membrane has not yet been elucidated [70].

Delivery of amphotericin B to the nasal passages and respiratory tract by a nasal spray or aerosolization is intended to deliver high concentrations to suppress growth on mucosal surfaces [79–81]. Although appealing, one prospective randomized trial of aerosolized amphotericin B failed to demonstrate a reduced incidence of pulmonary aspergillosis [82].

Flucytosine is a pyrimidine analog that is deaminated to 5-fluorouracil within the fungus and then converted to 5-fluorodeoxyuridylic acid monophosphate, which in turn inhibits thymidylate synthetase and thereby interferes with DNA synthesis. Drug resistance develops by loss or mutation of any of the enzymes that activate the drug and can occur readily if flucytosine is used alone; for that reason, it is generally used in combination with amphotericin B [67]. A combination of amphotericin B and flucytosine has been widely adopted as a standard treatment for cryptococcal meningitis. This combination treatment has also been advocated for aspergillosis and refractory systemic *Candida* infections, but there are no controlled trials for these latter two situations. Toxicities that can occur include both mucositis and myelosuppression (toxicities frequently observed with 5-fluorouracil), obvious disadvantages since both can exacerbate the deficiencies in host defenses that predisposed the patient to infection in the first place. Toxicity appears to be associated with excessive serum levels. Blood levels between 50 and 100 μL/mL are appropriate therapeutic targets.

Azole antifungals, which include miconazole, ketaconazole, itraconazole, and fluconazole, inhibit 14-alpha-demethylation of lanosterol by binding to fungal cytochrome P450 enzymes. Ergosterol in the cytoplasmic membrane is reduced, leading to increased permeability and inhibition of cell growth.

Toxicities of azoles tend to be less frequent and milder than polyene toxicities. Hepatoxocity can occur and infrequently can be serious. Fluconazole is available in both oral and intravenous formulations. Its bioavailability is excellent (exceeding 90%) with little variability by ingestion of food or intragastric pH. Peak concentrations occur 1–2 hours after ingestion. Excretion is mostly via the kidneys. Adult doses of 100 mg/day are used to treat mucosal infections, and 400 mg/day for systemic infections. Doses up to 1200 mg/day are well tolerated. Fluconazole is very active against most *Candida* species and has been shown to be as effective as amphotericin B for systemic *Candida* infections [83,84]. Fluconazole also has excellent activity against *Cryptococcus* and *Coccidiodes*, and less activity against several other fungal species [64].

Itraconazole is currently available only as an oral agent. Bioavailability of itraconazole is variable in BMT patients [85–87]. A new oral cyclodextrin formulation of itraconazole improves the bioavailability [88,89]. Preliminary data in one center demonstrated the achievement of therapeutic plasma concentrations early after transplant [90]. Some centers have encountered patient tolerance difficulty early in the transplant treatment course. More information is clearly needed.

Resistance to azoles has been uncommon, but it has been increasing in recent years. There are several different mechanisms of development of azole resistance. For fluconazole, three general mechanisms have been described. One mechanism is an alteration in the target enzyme, 14-alpha-demethylase, making it less susceptible to inhibition by azoles. A second mechanism is a reduction in drug accumulation either by reduced uptake or increased efflux. A third mechanism is a deficiency of delta 5, 6 desaturase. This deficiency leads to production of 14-methylfecosterol, which permits retention of viability even when 14-alpha-demethylase is inhibited. Some fungal species are natively resistant to fluconazole. *C krusei* is such an organism. Studies indicate that there are qualitative differences between the 14-alpha-demethylase of *C albicans* and *C krusei* that lead to a low susceptibility to inhibition by fluconazole [91]. In other organisms (e.g., *C glabrata*), mutations may occur during a course of treatment, leading to the emergence of drug resistance in initially susceptible organisms. Reduced drug accumulation was noted in one study to account for itraconazole resistance to *C krusei* [92].

The emergence of fluconazole-resistant organisms as pathogens was initially seen in patients with HIV infection receiving chronic suppressive therapy. Most of these organisms were mucosal pathogens and infrequently caused systemic infection. However, in recent years, systemic *Candida* infections have been noted in leukemia and BMT patients who had been receiving the agent for a short period of time [93,94]. With the increasing use of fluconazole and the recent recognition of the potential for nosocomial transmission of organisms such as those noted above, vigilance must be maintained and may necessitate restrictions on use. The emergence of drug resistance may have profound effects on antifungal therapeutic strategies [95,96].

Agents under development

Both the positive attributes (excellent tolerance) and the shortcomings (limited antifungal spectrum in the case of fluconazole and variable bioavailability and frequent drug interactions in the case of itraconazole) have encouraged the development of other azole compounds with broader spectrum and more desirable pharmacokinetic properties. Several are currently in development. Two triazoles are currently in clinical trials. Both have excellent bioavailability, oral and

Table 4. *Goals of antifungal prophylaxis*

Reduce acquisition of pathogen
Environmental decontamination
Reduction of nosocomial transmission
Suppression of colonizing pathogens
Pharmacologic agents
Enhance host resistance
Hematopoietic growth factors
Granulocyte transfusions
Stem cell infusions
Mucosal stem cell growth factors

intravenous formulations, and are active against the major fungal pathogens. Voriconazole has broad spectrum antifungal activity not only against *C krusei*, *C glabrata*, and *Aspergillus* species—organisms not covered by fluconazole—but also against a variety of other emerging pathogens [97–100]. Animal models have shown *in vivo* efficacy against such pathogens, including fluconazole-resistant *C albicans* isolates [101]. Several clinical trials have demonstrated excellent patient tolerance as well as clinically relevant drug activity against *Aspergillus* [101–104]. Temporary visual side effects have been noted in some patients.

SCH56592 is another triazole with broad spectrum activity (including fluconazole-resistant *C albicans* strains) with activity against *Aspergillus* and other filamentous fungi [105–110]. Its mechanism of action is similar to that of other azoles. There are some drug interactions (such as phenytoin and rifabutin) and some variability of absorption with eating. Some transient hepatotoxicity has been noted in clinical trials. In general, however, it has been well tolerated. Ongoing clinical trials are under way.

The echinocandin group of molecules are amphophilic lipopeptides. They act by inhibiting fungal β[1,3] glucan synthetase, an enzyme at the cell membrane. This inhibition eventually results in lysis of the fungal organism by interfering with synthesis of chitin, an important cell wall constituent. Certain members of this family show synergy with amphotericin B and lack of cross resistance with the azoles. A broad spectrum of antifungal activity has been noted [111].

The pradimicins and benanomycins have benzonaphthacene quinone frames that act by complexing with the mannoproteins in the cell wall that lead to leakage of intracellular molecules and cell death. These have been shown to have a broad spectrum of antifungal activity in preclinical trials [112].

The allylamines (terbinafine) are excellent agents especially active against dermatophytes and are used topically. Allylamines have demonstrated *in vitro* activity against a broad range of organisms, but preclinical *in vivo* models have failed to show consistent activity.

Liposomal nystatin, a lipid formulation of this widely used oral and topical polyene antifungal, has been shown to have broad spectrum activity against systemic pathogens and good tolerance in preclinical models [113]. Clinical trials are currently under way [114].

The nikkomycins are pyrimidine nucleosides that are structurally similar to precursor substrates for chitin and act as competitive inhibitors of the fungal chitin synthase enzymes [115]. Activity against several important fungal pathogens has been noted [116]. Synergy with several other classes of antifungal agents gives promise that this might be particularly useful in combination therapy.

STRATEGIES TO CONTROL INFECTION

Prophylaxis

Strategies to reduce infection include measures to reduce host acquisition of the fungal organisms, use of agents to suppress organism growth, and efforts to bolster host defenses (Table 4). Clearly, infection control measures to reduce nosocomial transmission are of paramount importance. Air filters are crucial for prevention of patient exposure to exogenous organisms, such as aspergillosis [8,37–39]. Hand washing and exercise of universal precautions are the key to minimizing the spread of endogenous organisms, such as *Candida*, from patient to patient [28–34]. As noted earlier, investigation of any cluster of infections should be carried out by an infection control team in cooperation with the transplant clinicians [35,36]. This investigation may require molecular typing techniques to distinguish strains, in order to determine if nosocomial transmission may be playing a role. The transplant clinician must be vigilant for changes in transplant practice associated with infection clusters (Tables 1 and 2) [24,26,27].

Agents such as nystatin, clotrimazole, and ketoconazole have been used to suppress colonization and thereby reduce infections. These have had only marginal success. Intravenous miconazole, an imidazole, was found in a randomized trial to reduce the risk for fungal infections [117], but its narrow spectrum of activity and toxicity profile limited its widespread use. Fluconazole, a triazole, has been shown in randomized trials to reduce the incidence of fungemia [118,119], deaths attributable to fungal infection, and in one study overall mortality [119]. In a multivariate analysis of factors associated with outcome after unrelated donor transplants, fluconazole use was found to be an independent factor associated with survival [120]. Several other studies have shown fluconazole to be superior to oral amphotericin B, nystatin, or clotrimozole [121,122].

Unfortunately, fluconazole's spectrum of antifungal activity is limited. Its most notable shortcoming is its lack of activity against *Aspergillus*. *C krusei* is natively resistant to fluconazole, and sporadic breakthrough infections have been occasionally noted. In several centers, outbreaks of *C krusei* occurred [123,124]. Some strains of *C glabrata* are resistant. Sporadic infections by *C glabrata* and an outbreak in BMT patients receiving fluconazole have been reported [125]. Two centers have described breakthrough *C parapsilosis* infections in BMT centers using fluconazole [126,127]. This was unexpected since *C parapsilosis* is ordinarily susceptible to fluconazole.

One randomized trial of itraconazole prophylaxis using the capsule formulation in patients with hematologic malignancies failed to show any significant benefit [128]. Studies evaluating the cyclodextran formulation have yet to be reported.

Amphotericin B given prophylactically to patients with prior aspergillus infection has been found to be effective in patients undergoing bone marrow transplantation

[129–132]. Prior aspergillosis was once grounds for exclusion from consideration for bone marrow transplantation; today most centers proceed with transplant under antifungal prophylaxis [133]. Resection of a localized lesion is recommended when possible [133–135].

Because of toxicity with full-dose amphotericin B, prophylaxis with low doses of amphotericin B has been tested: nephrotoxicity is less, but infusional toxicities remain. Two historical comparisons, one during pre-engraftment the other postengraftment, have suggested a benefit [136,137]. In the latter study, a greater incidence of GVHD was noted. Cyclosporin levels were lower in patients receiving amphotericin B. In a randomized study [138], amphotericin B given at a dose of 0.1 mg · kg^{-1} · day^{-1} was well-tolerated without significant nephrotoxicity. Overall, the rate of fungal infection in the amphotericin B group was lower than that in the placebo group. However, there was a very low rate of *Aspergillus* infection (there was only one infection by *Aspergillus* in the group that had received amphotericin). The utility of this approach in groups at higher risk remains uncertain, however. In another randomized trial in autograft recipients [139], the rate of fungal infection in the control group was only 1%, too low to assess the efficacy of the study regimen.

These data suggest that low dose amphotericin B can be given with little nephrotoxicity. Unfortunately, infusional toxicities remain problematic. The randomized trials have not demonstrated a consistent benefit, and most infections prevented in the sole randomized trial showing a benefit [138] could have been prevented by fluconazole. Breakthrough *Aspergillus* infections in patients receiving higher doses of amphotericin B (0.5 mg · kg^{-1} · day^{-1}), and the emergence of polyene resistance in centers where amphotericin B and nystatin use is widespread [140] make this approach questionable as to whether it offers any real benefit against *Aspergillus* and other mold infections.

In order to capitalize on the lower risk for nephrotoxicity of the lipid formulations of amphotericin B, a randomized trial was conducted evaluating liposomal amphotericin B in BMT patients [141]. Although there was a lower rate of colonization in patients receiving liposomal amphotericin B, there was no significant reduction of invasive infections. Unfortunately, the sample size was small and a benefit might have been missed.

Although conceptually appealing, efforts to bolster host defenses have been largely limited. Several investigational measures will be discussed below.

Treatment

Amphotericin B has been the treatment of choice for invasive fungal infection for many years. Doses of 0.3–0.7 mg · kg^{-1} · day^{-1} are generally given for *Candida* infections; doses of 0.7 to 1.0 mg · kg^{-1} · day^{-1} for invasive aspergillus and other mold infections. The use of combination therapy with flucytosine is supported by some animal models and clinical evidence for cryptococcal disease. An *in vivo* murine model has also suggested synergy between amphotericin B with rifampin or flucytosine for aspergillosis [142]. In a review of more than 2,000 cases of aspergillus infections, patients receiving amphotericin B plus either rifampin or flucytosine had slightly greater response rates [134]. Preclinical testing of combinations of polyenes and azoles have indicated synergism in some cases and antagonism in others. Thus, their use in combination should be avoided outside of a clinical trial.

There are a number of drawbacks to amphotericin B treatment. The failure rate is high: 73–85% [11,118,143] in *Candida* infections and 75–95% [11,12,144] in *Aspergillus* infections.

There are several reasons for the high failure rate for treatment. First and foremost is delay in starting treatment. Manifestations of infection are frequently subtle and often overlooked until the burden of organisms is substantial and the prospects for eradication are poor even in the best of circumstances. The burden of *Candida* organisms influences the outcome: for example, in one report there was a 39% mortality from *Candida* fungemia alone in contrast to 90% when fungemia was accompanied by tissue invasion [11]. In another study, mortality from non-*Candida* infections was 0% in patients with only isolated fungemia, in contrast to 41% in patients with single organ or site infection, and 83% in patients with disseminated infection [144].

Amphotericin B's narrow therapeutic ratio is especially problematic in the allogeneic BMT patient where maintenance of immunosuppressive treatment is important for control of GVHD. Because of the frequent occurrence of nephrotoxicity with either cyclosporine or amphotericin B individually and an almost certain compromise in renal function when both are used concomitantly [69,145], the clinician is often faced with the difficult dilemma of choosing between dose reduction of the immunosuppressive regimen or reducing the dose of the antifungal therapy. Either action may increase the hazard for a life threatening consequence.

The ability of the host to assist in clearance of fungal pathogens is also paramount for recovery. Certainly the inferior capacity to mount an immune response is contributory to the lower rate of survival from non-*Candida* invasive infection in allograft patients (15%) compared to autograft patients (35%) [25]. Similarly, an important predictor of outcome is the physiologic status of the patients (as measured by the Acute Physiology, Age, Chronic Health Evaluation [APACHE] II score) at the onset of infection [146]. Without restoration of the host's defenses, some have argued with good cause, few patients will survive an invasive fungal infection [147].

Other obstacles to successful treatment include limited spectrum of activity of antifungal agents. For example, fluconazole has poor activity against *C krusei*, many strains of *C glabrata*, and *Aspergillus* species. Amphotericin B has limited activity against *Fusarium* and *C lusitania*.

Bioavailability is problematic for several agents. Although itraconazole has excellent activity against *Aspergillus* species, its bioavailability is erratic, as noted earlier. Monitoring of blood levels is important since its effectiveness correlates with plasma levels [86]. The use of antacids or H2 blockers should be avoided since gastric acidity is necessary for absorption of itraconazole. In addition, rifampin should be avoided since it may lead to low itraconazole plasma concentrations.

Less toxic alternatives to amphotericin B have now been shown to be effective for the treatment of invasive *Candida* infection. In comparative trials in both neutropenic and non-neutropenic patients, fluconazole was found to be as

Table 5. *Treatment strategies under development*

Strategy	Reference number
Molecular tools to identify fungal strains causing outbreaks	28–36
Rapid diagnostics for *Candida*	53–56
Rapid diagnostics for *Aspergillus*	57,63
Fluconazole as treatment of candidosis	83,84
Lipid formulations of amphotericin B	70–78,141 148,149,152,154
Cyclodextrin formulations of itraconazole	88–90
Aerosolized amphotericin B	79–82
Wider spectrum triazoles	97–110
Enhanced granulocyte transfusions	159–161
Gamma interferon	162

efficacious as amphotericin B in the treatment of hematogenous Candida infection but with less toxicity [83,84]. That survival was not improved in these trials, since toxicity did not limit the use of antifungal therapy, is disappointing. In another randomized trial, ABLC was also found to be as effective as amphotericin B but less toxic [77]. The lipid formulations of amphotericin B appear to be quite promising in the treatment of aspergillus and other mold infections, as noted earlier. Several historical case control studies have indicated at least comparable survival with less nephrotoxicity [75,76,148,149]. A recent randomized trial comparing ABCD with amphotericin B showed equivalent efficacy with a decrease in nephrotoxocity [78].

Removal of central venous catheters in *Candida* fungemia has been debated. Several studies support both sides of the issue as to whether catheters should be routinely removed in all patients with fungemia. However, it is clear that any patient not responding promptly to antifungal therapy with persistent positive cultures should have the catheter removed.

The role of surgical resection of infarcted tissue in aspergillosis has similarly been debated. Certainly, there are selected patients in which resection can improve prospects for long-term control [134]. Patients with *Aspergillus* sinusitis may similarly benefit from drainage and irrigation of the infected sinus and debridement of soft tissue or bony infarction [134].

Empiric therapy

Because of the difficulty in accurately documenting the early course of invasive fungal infections, and the poor results when treatment initiation is delayed, the use of empiric antifungal therapy has been embraced, as has the use of empiric antibacterial therapy for neutropenic fever. Two studies have supported empiric fungal therapy in oncology practice for treating persistent fever during neutropenia 4–7 days after initiation of antibacterial therapy [150,151].

The shortcoming of this strategy is the toxicity caused by amphotericin B. Certainly, fluconazole could be used in this fashion instead of prophylactically, but with the gaps in its coverage, important fungal pathogens would not be treated. The variability and delay in achieving therapeutic concentrations of itraconazole make that agent unsatisfactory.

The lipid formulations of amphotericin B do offer an acceptable alternative to the parent compound as demonstrated by several Phase II and prospective randomized trials [145,148,149,152–154]. These studies show that considerably less nephrotoxicity is observed when one of the lipid formulations is substituted for amphotericin B; efficacy is comparable. The major shortcoming of wholesale substitution of one of the lipid formulations for the parent compound of amphotericin B is the extraordinary difference in cost. Several studies, however, emphasize the especially high risk for nephrotoxicity [155,156] and hemodialysis [69,157] in the BMT recipient. Certainly, hemodialysis and extra time spent in the hospital are costly and can easily offset any savings gained by avoidance of a costly pharmaceutical. Careful cost effective analyses are needed to help guide clinical decisions as to which patients and at what time substitution of the lipid formulation of amphotericin B is appropriate.

Several antifungal strategies are under development (Table 5). Current and future studies will determine what role these will have in the BMT patient.

STRATEGIES TO BOLSTER HOST DEFENSES

Improvement of host defenses is among the least developed strategies for fungal infection control and yet, ultimately, the most vital determinant of infection control. Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been quite successful in speeding neutrophil recovery in nontransplant chemotherapy-induced myelosuppression and in the transplant setting. There was considerable hope that their use would lead to a reduction in invasive fungal infection. Unfortunately, the effect of these molecules on bacterial and fungal infection rates has been quite disappointing [158]. Indeed, none of 19 randomized clinical trials of these molecules in BMT recipients noted a reduction in fungal infection. Further, there is no evidence that amphotericin B use for suspected infection was altered. That those trials were not designed to address this question could be argued, and certainly that was the case. However, if there is a benefit, it probably must be small.

Granulocyte transfusions in some cases have been useful as adjuncts to antifungal agents. Pretreatment of donors by colony stimulating factors (G-CSF, GM-CSF, or macrophage-CSF) before leukapheresis yields markedly greater numbers of neutrophils. Such "enhanced" granulocyte transfusions can be used as prophylaxis or as treatment [159–161]. Alternatively, G-CSF or GM-CSF can be given to the patient as an adjunct to antifungal therapy. GM-CSF is particularly attractive since *in vitro* assays suggest that it improves the functional activity of monocytes and macrophages in addition to its stimulatory effects on neutrophils. As adjuncts to antifungal therapy for treatment of infection, the results to date have similarly been disappointing [158].

Gamma interferon improves the functionality of neutrophils against aspergillus in patients with chronic granulomatous disease [162]. This cytokine may be useful as an adjunct to antifungal therapy or as prophylaxis.

Restoration of mucosal integrity after chemotherapy-induced injury and prevention of severe mucosal injury are desirable goals. Several approaches have been evaluated in

preclinical models. Keratinocyte growth factor (KGF) has been noted to affect proliferative activity in mucosal epithelial cells in cell lines and animal models [163–165]. In an animal model of radiation and chemotherapy-induced gastrointestinal mucositis and mortality, KGF was found to have protective effects with decreases in weight loss and mortality, and a marked increase in the intestinal proliferative crypt activity during the healing phase. In another animal model, KGF was found to prevent GVHD while preserving graft-vs.-leukemia after experimental allogeneic BMT [166]. This was thought to be through preservation of gut mucosal integrity reducing translocation of lipopolysaccharide from the gastrointestinal lumen into the circulation and, thereby, reduction of other proinflammatory cytokines that incited GVHD. KGF is currently undergoing clinical trials to assess tolerability and protective effects on the mucosal barrier function.

Transforming growth factor (TGF)-β has been shown to inhibit the cycling of human buccal mucosal epithelial cells. Pretreatment of cells with TGF-β prevents them from toxicity mediated by a variety of chemotherapy agents [167]. Unfortunately, this is currently not under clinical development. Interleukin-11, a molecule now Food and Drug Administration–approved for speeding thrombopoietic recovery after chemotherapy-induced thrombocytopenia, also has restorative effects on the mucosa. It has been noted to inhibit apoptosis and stimulate repair of intestinal crypt cells following cytoreductive therapy in preclinical models. These effects have resulted in reduced mucositis from chemoradiation injury *in vitro* and in animal models [168–170]. Whether this will have beneficial effects clinically remains to be seen.

Several trials with myeloid growth factors applied topically to the mucosa have been disappointing. One study of topical oral G-CSF, however, had a borderline significant benefit [171].

CONCLUSION

Fungal infections pose an important challenge for the BMT clinician. The major risk for fungal infections has shifted from preengraftment to the postengraftment period, with GVHD and the use of systemic corticosteroids emerging as the major host risk factors for infection. The clinician now faces a larger array of fungal pathogens. Although less toxic alternatives to amphotericin B have been evaluated as treatment in randomized trials, outcomes have still not improved. Clearly there is a need for more accurate diagnostic tests to permit initiation of treatment earlier in the course of infection, when the burden of organisms is more manageable. Until such time, clinicians should strongly consider preventive measures. Reduction in the acquisition of organisms by HEPA filters, the avoidance of invasive procedures, and vigorous infection control maneuvers to prevent nosocomial transmission from patient to patient must be the mainstays of any approach to control these infections. Suppression of colonizing organisms by pharmacologic agents reduces the threat of systemic infection from certain fungal species but not others. New formulations of agents such as itraconazole are being evaluated to improve bioavailability and thus enhance their prospects as a prophylactic agents. Amphotericin B lipid formulations of offer a less toxic means of administering high doses of amphotericin B. New triazoles with a wider spectrum of action and excellent bioavailability are entering clinical trials. A variety of other classes of antifungal molecules are being evaluated. Strategies to bolster host defenses by hematopoietic growth factors, cytokines with immunomodulatory or mucosal effects, and primed granulocyte transfusions all hold promise to reduce the host vulnerability to these infections. Combining these different modalities offers hope for improved control of fungal infections.

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