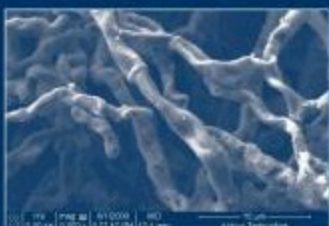
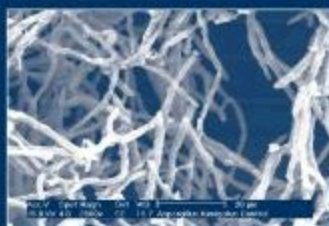
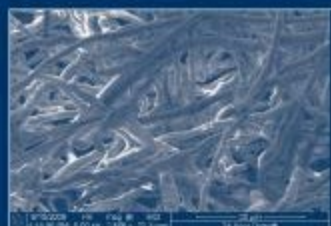
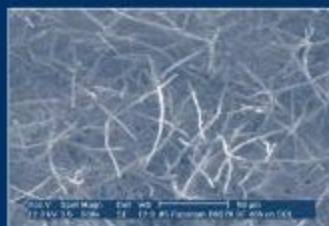
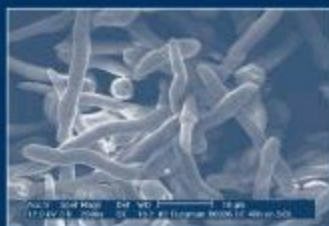


Antifungal Therapy



Edited by
Mahmoud A. Ghannoum
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Preface

This book is designed to provide a comprehensive but insightful examination of antifungal therapy in the changing clinic millennium of modern medicine. It is clear that as medicine advances to treat and cure severe underlying diseases, the collateral consequences of this management can be immunosuppression and opportunistic fungal infections. Furthermore, there are a series of primary fungal infections such as dermatophytosis and endemic mycoses which continue to plague normal hosts. Furthermore, the pandemic of HIV which has impacted the entire world laid in its immunosuppressive path the rise of invasive mycoses. It is clear that most clinicians who care for the seriously sick will be faced at times with the appearance of a fungal infection and a need to manage its disease. There are many aspects of invasive mycoses including genetic susceptibility, risk factor predictions, diagnosis, epidemiology and outcome of underlying diseases which require a present and future knowledge base for medical practice. In this book, we have attempted to focus the presentation on the management aspect of fungal disease. With the rising number of fungal infections world wide and the development and clinical use of a variety of antifungal agents, it is quite clear that the statement: "Amphotericin B is the gold standard for the invasive mycoses" is no longer true. We have safer and effective alternative drugs to use. It is our mission in this book to provide clinicians with a foundation and insights into current antifungal management.

There are four sections in this book. First, we approach some general antifungal agent issues from the history of antifungal, fungal epidemiology, antifungal agent preclinical development to drug resistance. Second, we examine in depth the antifungal classes of drugs, Third, there is an attempt to provide clinical management issues and strategies around specific fungal infections in which the clinician may face frequently or rarely depending on the patient population in their practice. In these sections there are insights provided into dosing, choice of drugs, concerns about complications and outcome which are both evidence-based but mixed with personal opinions and experiences. Fungal infections are treated "one patient at a time" and there is no "cookbook recipe" that fits all patients all the time. In fact, the underlying disease simply gets in the way too often or our evidence-based material is either weak or non-existent. Finally, we conclude with management of several risk groups or unique patient populations or infection sites and their fungal infections. It is not an exhaustive list but provides illustrative exposure to these patients but also lays the ground work/foundation for the principles of managing other risk groups which occur today or may occur tomorrow.

Fungal diseases have risen to prominence over the last 50 years. They have paralleled the technological advances in the care of serious medical diseases. Fungi, as eukaryotic organisms, play an interesting role in the human condition. They have been harnessed to help make our bread and beverages. In fact, we eat some of them and during the traffic of life, we are constantly exposed to millions of them. During health they are rarely a problem for us and after death they degrade us. Many of our critical exposures for health and fungi come between these stations of life. It is in this arena as a "human petri dish" that fungal disease raises its ugly consequences. It is the hope of these authors that this book reveals the tools, strategies, and insights to manage these irritating, costly and life-threatening infections. At times, it may seem the patient is defenseless against these marauders but in fact, present antifungal therapy is very good and applied early and correctly can make a difference in patient outcome. This success story is told in the following pages.

*Mahmoud A. Ghannoum
John R. Perfect*

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1 History of Antifungals

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INTRODUCTION

Over the past decades, the incidence and diversity of fungal infections has grown in association with an increasing number of immunocompromised patients. The human immunodeficiency virus (HIV) epidemic, technological improvements in the fields of solid organ transplantation medicine, stem cell transplantation, neonatology, coupled with the advent of new immunosuppressive drugs have collectively attributed to an increase in the incidence of systemic fungal infections including those caused by *Candida*, *Aspergillus*, *Cryptococcus*, *Coccidioides*, *Pneumocystis*, and *Zygomycetes* species. More recently, other species have begun to rival *Candida albicans* as major causative agents of fungal disease. For example, fluconazole-resistant non-*albicans* *Candida* species such as *C. glabrata* are now more prevalent in some hospitals (1,2). Although *Aspergillus* species remain the most common causes of mold infections in humans, other molds such as *Scedosporium*, *Fusarium*, *Rhizopus*, and *Mucor* species are now increasingly responsible for superficial and systemic mycoses (3,4).

Healthcare professionals must carefully consider the expanded role of medically important fungi. In order to provide optimal treatment of fungal infections in immunocompromised patient populations, the development of new antifungal agents must keep pace with changes in the etiology and the resistance patterns of fungal pathogens. Additionally, novel therapeutic modalities with targeting host defenses, fungal biofilm physiology, and immunomodulations are emerging.

EARLY TREATMENTS

Antifungal therapies evolved slowly during the early years of the past century. Potassium iodide was the standard treatment for cutaneous fungal infections including actinomycosis, blastomycosis, sporotrichosis, and tinea from the beginning of the 20th century until after the Second World War (5). First derived from sea algae, potassium iodide was considered to exert a direct antifungal effect, although the complete mechanism of action remains unclear (6–8). Contemporarily, radiation was used to treat severe tinea capitis infections, often with significant complications including skin cancer and brain tumors (9).

In the 1940s, Mayer et al. (10) demonstrated that sulfonamide drugs, such as sulfadiazine, exhibited both fungistatic and fungicidal activities against *Histoplasma capsulatum* (11). This discovery led to the formation and the use of sulfonamide derivatives for the treatment of blastomycosis, nocardiosis, and cryptococcosis (12–14).

Griseofulvin, a compound derived from *Penicillium griseofulvum*, has been widely used to treat superficial fungal infections since its isolation in 1939 (15). In 1958, Gentles (16) reported the successful treatment of ringworm in guinea pigs using oral griseofulvin.

These successful attempts to develop novel and effective antifungal drugs encouraged the further study and discovery of new agents. This chapter reviews the history of antifungal drugs.

POLYENES

In 1946, polyene antifungals (Fig. 1) were developed from the fermentation of *Streptomyces* (17). Polyene antifungals are effective against organisms with sterol-containing cell membranes (e.g., yeast, algae, and protozoa) (18). These drugs disrupt the fungal cell membrane by binding to

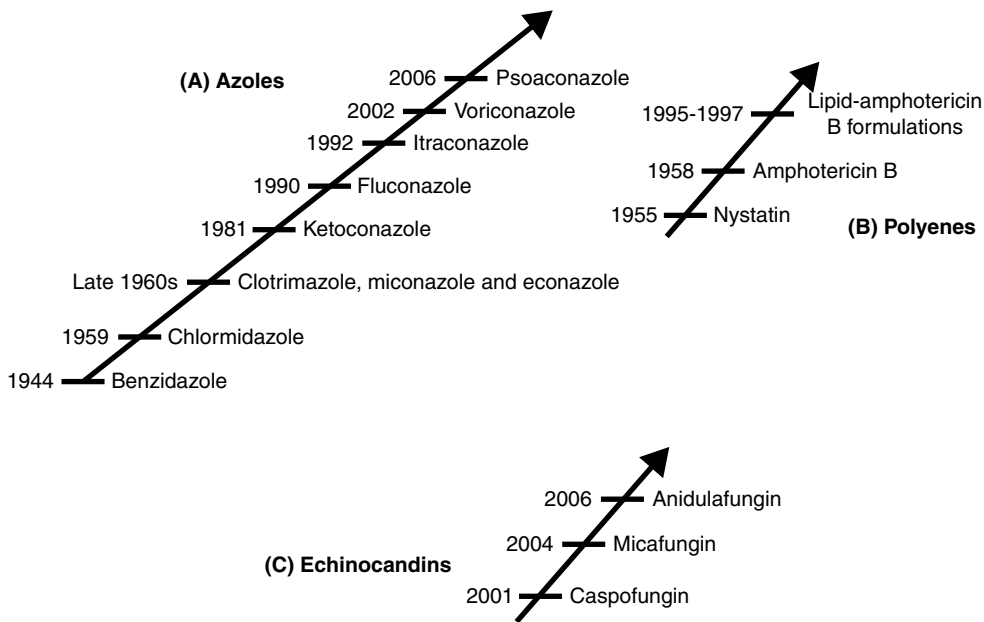


Figure 1 Historical development of the antifungal agents: (A) azoles, (B) polyenes, and (C) echinocandins.

ergosterol, the main cell membrane sterol moiety. As a result, holes form in the membrane allowing leakage of essential cytoplasmic materials, such as potassium, leading to cell death. From the 1950s until the advent of effective azole compounds in the 1960s, polyene antifungal agents remained standard therapy for systemic fungal infections (19).

Nystatin

In 1949, while conducting research at the Division of Laboratories and Research of the New York State Department of Health, Elizabeth Lee Hazen and Rachel Fuller Brown discovered nystatin, a polyene derived from *Streptomyces noursei* (20–22). In 1955, Sloane (23) reported topical nystatin to be particularly effective for treatment of noninvasive moniliasis (candidiasis), a frequent complication observed in children enrolled in early chemotherapeutic leukemia trials underway during this period (24). Nystatin exhibited good activity against *Candida* and modest activity against *Aspergillus* species. In aqueous solutions, nystatin forms aggregates that are toxic to mammalian cells both in vitro and in vivo. The insolubility and toxicity precluded its use as an intravenous therapy for systemic mycoses.

Recently, a more soluble liposomal nystatin formulation (Nyotran[®]) with reduced toxicity was developed (25). The liposomal formulation consists of a freeze-dried, solid dispersion of nystatin mixed with a dispersing agent such as a poloxamer or polysorbate (26,27). The dispersing agent prevents aggregate formation in solution, increasing the drug's solubility and decreasing toxicity while maintaining efficacy (27,28). Liposomal nystatin has good activity in vitro against a variety of *Candida* species including some amphotericin B-resistant isolates (28). Recent studies by Oakley et al. (29) showed that Nyotran[®] was more effective than liposomal amphotericin against *Aspergillus* species. Although the liposomal form of nystatin was less toxic than conventional nystatin, unacceptable infusion-related toxicity unfortunately caused a halt in the development of this drug (30–32).

Amphotericin B

Amphotericin B deoxycholate has been widely considered the most effective antifungal drug for the past four decades. Amphotericin B is a fungicidal antibiotic, and like other members of the polyene class is effective against organisms with sterol-containing cell membranes (19). Amphotericin B was extracted from *Streptomyces nodosus*, a filamentous bacterium, at the Squibb Institute for Medical Research in 1955 and until recently served as the standard treatment for

invasive fungal infections (33). At the time of its discovery, amphotericin B provided activity against invasive *Aspergillus* superior to that of previously available antifungal agents (33,34). Amphotericin B continues to be effective and widely used in the treatment of fluconazole-resistant fungal infections (19,30). Like other polyenes, amphotericin B exhibits dose-dependent toxicities including renal impairment and hypokalemia (19,30,34,35). Renal toxicity associated with polyene antibiotics is believed to be mediated by the drug interaction with cholesterol within the mammalian cell membrane, resulting in pore formation, abnormal electrolyte flux, decrease in adenosine triphosphate (ATP), and eventually a loss of cell viability (19).

In the early 1980s, several research groups developed new liposomal amphotericin B formulations. Graybill et al. (36) published the first extensive study investigating the treatment of murine cryptococcosis with liposome-associated amphotericin B. They realized lower tissue fungal burden of *Cryptococcus* in mice treated with the liposome-associated formulation than those treated with conventional amphotericin B. Based on this finding, these authors concluded that reduced toxicity of liposome-associated amphotericin B permitted much larger doses of drug to be given than was possible with amphotericin B deoxycholate (conventional amphotericin B formulation) (36,37).

In the past decade, three novel liposomal formulations of amphotericin B have been approved for use in the United States: amphotericin B colloidal dispersion (ABCD; Amphocil® or Amphotec®), amphotericin B lipid complex (ABLC; Abelcet®), and small unilamellar vesicle liposomal formulation (L-AmB; Ambisome®).

The development of lipid-based amphotericin B formulations afforded significant advantages in treatment of systemic fungal infections including decreased toxicity and improved tolerance (38–40).

AZOLE ANTIFUNGALS

Progress in the development of new antifungal agents lagged behind that of antibacterial antibiotics. The delay can be explained by two factors: (i) before the HIV/AIDS period, the occurrence of fungal infections was believed to be too low to warrant aggressive research by the pharmaceutical industry; and (ii) the apparent lack of a highly selective fungal target not present in other mammalian cells limited the number of potential pharmacologic mechanisms not associated with shared pathogen–host cell toxicity (19,41).

The discovery of the azole antifungal drugs (Fig. 1) was seminal in the history of antifungal development. Until the discovery of azoles, amphotericin B was the only available agent to treat disseminated fungal infections including invasive aspergillosis—although not without concerns regarding nephrotoxicity and administration.

Azoles inhibit the synthesis of ergosterol, the major sterol in the fungal cell membrane, via inhibition of the cytochrome P450 enzyme, lanosterol demethylase (41,42). This inhibition results in disruption of cell membrane integrity with eventual death.

Early Azoles

In 1944, Woolley (43) described the antifungal activity of the first azole, benzimidazole. Descriptions of the antifungal properties of substituted benzimidazole were followed by the discovery of chlormidazole (44–46).

In the late 1960s, clotrimazole was developed in Germany by Bayer AG (17). Miconazole and econazole were developed subsequently by Janssen Pharmaceutica, Antwerp, Belgium (46). The early imidazoles such as clotrimazole, miconazole, and tioconazole showed good topical antifungal activity, but were of limited value for treating systemic infections.

Second Generation Azoles

In 1981, the Food and Drug Administration (FDA) approved the systemic use of ketoconazole, an imidazole derivative synthesized and developed by Janssen Pharmaceutica, Antwerp, Belgium (45). Ketoconazole was also available commercially as an anti-dandruff shampoo, branded (Nizoral®) by the same company. For almost a decade, ketoconazole was regarded as the standard oral agent for treatment of fungal infections including chronic mucocutaneous candidiasis (47). Mendes et al. (48) considered azole derivatives as the drugs of choice for the treatment of eumycetomas.

In 1978, Pfizer developed fluconazole, a drug suitable for oral and intravenous treatment of superficial and systemic fungal infections (47–49). Fluconazole was shown to have a good safety profile and was approved for the treatment of oropharyngeal, esophageal, vaginal, peritoneal, and genito-urinary candidal infections, disseminated candidiasis, and cryptococcal meningitis. Unlike ketoconazole, fluconazole is highly water soluble and can be administered parenterally. Recently, the utility of fluconazole has been limited by the emergence of resistant organisms, such as *C. krusei* and *C. glabrata*, against which fluconazole has poor activity (50,51).

In 1992, the FDA approved itraconazole, a broad spectrum triazole antifungal agent developed by Janssen Pharmaceutica (Sporanox[®]). Itraconazole was shown to be less toxic than previous azoles, with a spectrum of activity broader than that of ketoconazole (50,51). Consequently, itraconazole has replaced ketoconazole as the treatment of choice for invasive aspergillosis (50).

Although the discovery of fluconazole and itraconazole represented a major advancement in the management of systemic fungal infections, these triazole antifungal agents have some important limitations (52,53). Fluconazole activity has a narrow spectrum, targeting mainly yeast (*Cryptococcus neoformans*, *C. albicans*) and dimorphic fungi, with no activity against molds (54,55). In comparison, itraconazole has a broader spectrum that includes activity against *Aspergillus* species and some yeast strains that are intrinsically resistant to fluconazole, such as *C. krusei* and *C. glabrata* (54,55).

Third Generation Azoles

Voriconazole, a derivative of fluconazole, is a synthetic third-generation triazole developed in the late 1980s by Pfizer Pharmaceuticals, Antwerp, Belgium (56,57) and approved by FDA in May 2002. Voriconazole is more active than fluconazole and itraconazole against *Candida* species (58). The activity of voriconazole against filamentous fungi, particularly *Aspergillus*, was found to be superior to that of amphotericin B (55,56). Voriconazole is now considered the gold standard for the treatment of aspergillosis (59–61).

Posaconazole, a hydroxylated analogue of itraconazole, was developed by the Schering-Plough Research Institute and approved for use in 2006 (62). Posaconazole is effective against opportunistic and endemic fungi such as *Aspergillus* spp., *Zygomycetes* and *Candida* species (62,63). Posaconazole has been shown to be superior to amphotericin B, fluconazole, and itraconazole against most common fungal pathogens at in vitro and animal studies (64). It is approved for prophylaxis of invasive fungal infections (aspergillosis and candidiasis) in immunocompromised patients and for the treatment of oropharyngeal candidiasis.

The story of the azoles is still evolving. New triazoles are being developed and will likely reach the market. Compounds under development include ravuconazole, isavuconazole, and albaconazole. Initially developed by Bristol-Myers Squibb, Antwerp, Belgium, the development of ravuconazole is now being pursued by Eisai Co., Ltd. Ravuconazole has a broader antifungal spectrum than fluconazole and itraconazole against strains of *C. krusei* and *C. neoformans* (65), and albaconazole is being developed by Stiefel Pharmaceutical for the treatment of dermatophyte infections. Isavuconazole (Basilea Pharmaceutica, Antwerp, Belgium) is undergoing phase II/III clinical trials for the treatment of invasive fungal infection and candidiasis.

ECHINOCANDIN ANTIFUNGALS

The advent of echinocandins (Fig. 2), the newest class of antifungal agents, was heralded by the development and approval of caspofungin acetate (Cancidas; Merck & Co., Inc.) for the treatment of candidiasis in 2002 (66). The echinocandins are a group of large, semisynthetic, cyclic lipopeptides discovered in the 1970s (Fig. 2). Large molecular weight may explain their poor absorption through the digestive tract. Therefore, all three commercially available echinocandin compounds—caspofungin acetate, micafungin, and anidulafungin—are used only intravenously (66,67). Echinocandins inhibit synthesis of 1,3- β -D-glucan, an essential component of the fungal cell wall (68). The synthesis of caspofungin acetate based on pneumocandin B₀ requires chemical modification at two sites of the peptide core, reduction of a primary amide to an amine, and condensation of the hemiaminal moiety with ethylenediamine (68).

Caspofungin acetate (Cancidas[®]) is fungicidal against yeasts and dimorphic fungi such as *C. albicans*, including triazole-resistant isolates, and fungistatic against *Aspergillus* species (69). *Aspergillus fumigatus* is unable to sustain polarized growth in the presence of multiple doses of

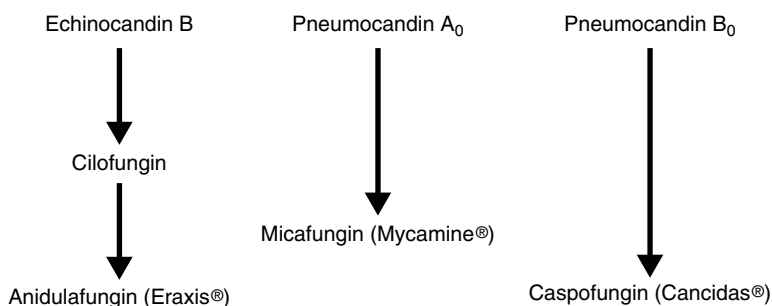


Figure 2 Echinocandin history, discovered in the 1970s as fermentation products that inhibit fungal cell wall synthesis.

caspofungin, leading to significant fungal cell death in tissues (65,66). Isham and Ghannoum (70) recently concluded that voriconazole demonstrated greater in vitro inhibitory activity than caspofungin against the non-*albicans* isolates.

Micafungin (Mycamine[®], Pfizer Co., Ltd., Antwerp, Belgium) and anidulafungin (Eraxis[®], Versicor, Inc., Fremont, CA) were approved for use in 2006. Micafungin was first isolated from the culture broth of *Coleophoma empedri* (71). It is a novel water-soluble lipopeptide derived by semisynthetic modification of FR901379, a naturally occurring cyclic hexapeptide with a fatty acryl side chain, and is similar in structure to echinocandins and pneumocandins (71,72). Micafungin may prove useful in the treatment of infections due to azole-resistant *Candida* (73).

Anidulafungin is a derivative of a naturally occurring candin, echinocandin B, produced by *Aspergillus nidulans* or *A. rugulosus* (74). Cilofungin was the first semisynthetic derivative of echinocandin B to be evaluated in clinical trials; however, the trials were discontinued due to associated nephrotoxicity. Further structure modification of cilofungin led to the synthesis of anidulafungin (74).

Aminocandin (Novexel Pharmaceuticals, Inc., Antwerp, Belgium) is a new member of the echinocandin class of antifungal compounds that may be useful for the treatment of a broad spectrum of systemic invasive fungal infections. Isham and Ghannoum showed that aminocandin demonstrated potent in vitro activity against *Aspergillus*, *Zygomycetes*, and fluconazole-resistant *Candida* (72–76).

The development of the new triazole and echinocandin antifungals provides clinicians new alternatives in the treatment of invasive and resistant systemic fungal infections.

ANTIFUNGAL THERAPY FOR BIOFILMS

Many fungi form biofilms (communities of fungal cells and cell components embedded within a matrix of extracellular materials) on a variety of implanted medical devices. Growth in biofilm increases the ability of the fungus to adhere to host tissue or prosthetic material and to resist antifungal drugs (77,78). For example, biofilm-associated *C. albicans* cells resist antifungals, such as amphotericin B, nystatin, chlorhexidine, and fluconazole, to which planktonically grown cells are sensitive at lower concentrations (78,79). Existence in the biofilm state may protect fungi from antifungal drugs by the following mechanisms: (i) decreased penetration of the antimicrobial agent into the biofilm, (ii) alteration of the chemical microenvironment within the biofilm leading to decreased or halted growth, and (iii) novel adaptation strategies in response to environmental stressors (80–82).

Several properties of the fungal biofilm have been shown to affect resistance to antifungal drugs. For example, drug-resistance mechanisms may depend on the stage of biofilm maturation. In the early-phase biofilm (6 hours maturation), resistance to azole drugs is affected by efflux pumps. At later stages of biofilm maturation (48 hours), azole resistance is achieved through alteration in cell wall sterol composition (83). Other factors within the biofilm, such as cell density, may increase resistance to antifungal agents such as fluconazole, ketoconazole, amphotericin B, and caspofungin (84).

As biofilms are highly resistant to conventional antifungal treatment strategies, much research is devoted to indentifying novel antibiofilm therapies. Combination therapy, such as the addition of rifampin and doxycycline to amphotericin B, resulted in synergism in vitro against the biofilms of non-*albicans* *Candida* (85).

Methods of preventing or eradicating biofilm formation on catheter surfaces are under investigation. For example, yeast alcohol dehydrogenase, through the conversion of glucose to ethanol, was found to restrict the ability of *C. albicans* to form biofilm on catheter surfaces in vitro (86). Additionally, a combination of minocycline-EDTA and 25% ethanol instilled into catheters as a "lock solution" was shown to be effective in salvaging catheters infected with *C. parapsilosis* and *S. aureus* biofilms (87).

Finally, the composition and surface properties of indwelling prosthetic medical materials are being studied with regard to their ability to influence biofilm adherence and growth—a potentially useful approach to reduce or manage biofilm formation on medical devices (88,89).

FUTURE AGENTS

Powerful historical precedents support the use of antibody-based therapies to treat infectious diseases (90,91). However, although still in very early stages of development, newer approaches to the treatment of fungal infections will likely include the consideration of the host immune system and the interplay of drugs and host immunomodulators (92).

Immunomodulator therapies can be categorized as either pathogen specific or pathogen nonspecific (92). Pathogen-specific immunomodulators include antibody reagents and vaccines, whereas cytokines, antimicrobial peptides, and probiotics are considered pathogen nonspecific immunomodulators (93). Studies have shown immune sera to be protective in animal models of systemic candidiasis (92–95). Combination therapies using antifungal antibiotics with immunomodulators to treat invasive fungal disease are currently under investigation (92,94). To be of any clinical benefit, these regimens must improve efficacy without producing unacceptable side effects (92–95).

The immunodominant fungal antigen heat shock protein 90 (HSP90), expressed on the cell surfaces of yeasts and certain malignant cells, has been investigated as a potential target for antibody therapy (96,97). Mycograb[®] (NeuTec Pharma, Antwerp, Belgium), a human recombinant monoclonal antibody against HSP90, was shown to have synergistic activity with amphotericin B in vitro against a broad spectrum of *Candida* species (98,99). Mycograb[®] consists of an antigen-binding variable domain of heavy and light chains linked together to create a recombinant protein that can be expressed in *Escherichia coli*. The antifungal activity of this drug can be demonstrated using assays, such as minimal inhibitory concentration testing, used to assess conventional antifungal drugs (99–101).

Other new antifungal agents under study include naturally derived molecules with antifungal properties, such as the antifungal protein (AFP) secreted by *Aspergillus giganteus*. AFP is a small (94 amino acids), positively charged amphipathic protein that exerts no cytotoxic or immunogenic effect on mammalian cells, but interferes with the physiological properties and synthesis of the fungal cell wall leading to fungal cell death (102,103).

Recently, Zumbuehl et al. (104) reported that a new dextran-based hydrogel containing amphotericin B prevented fungal infections for at least 53 days when implanted in mice.

The history of antifungal agents continues to evolve and no doubt will produce novel agents that, it is hoped, will target the organism as well as the host immunity.

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2 | Epidemiology of Fungal Infections: What, Where, and When

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INTRODUCTION

Invasive fungal infections are frequently reported in the literature. This is due not only to development of a clear case definition, improved diagnostic methods, and reporting, but also due to an ever-enlarging at-risk population (1). Surveillance data, though not perfect and likely still reflecting underdiagnosis and underreporting of these entities, indicate that over the past several decades, there has been an increasing incidence of invasive fungal infections due to *Candida*, *Aspergillus*, *Cryptococcus*, and *Zygomycetes* (2). Epidemiologic trends also suggest that although *Aspergillus* remains the most common mold associated with invasive disease, other filamentous hyphomycetes such as *Fusarium* and *Scedosporium* are becoming more common (3–5). This chapter reviews the epidemiology of the most common fungal infections including typical clinical manifestations associated with each fungal pathogen.

ASPERGILLOSIS

Aspergillus is a ubiquitous hyalohyphomycete (mold with nonpigmented, regularly septate hyphae) found in soil, dust, compost, rotted plants, and other organic debris including foods and spices (6,7). Over 200 species are known, though only a few have been reported as pathogenic to humans. The more commonly reported human pathogens include *Aspergillus fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*. Of these, *A. fumigatus* is the most common species to cause invasive disease, and *A. flavus* is the second most commonly reported. Though *A. terreus* is less common, it is resistant to amphotericin B and has historically been associated with an exceptionally high mortality (8–11). Infections with other *Aspergillus* species such as *A. clavatus* and *A. nidulans* are increasingly reported (7).

Aspergillus grows best at 37°C, forming hyaline hyphae with asexual reproduction by conidia that give each species a distinctive colony color. Conidia are easily aerosolized and, when small airborne conidia (2–3 μm for *A. fumigatus*) are inhaled, they can settle deep in the lung, where colonization and a variety of clinical syndromes may develop. The type of host plays a role in the clinical spectrum of disease, as the host's immune response and the ability of *Aspergillus* to invade and destroy tissue determine the clinical presentation. In patients with asthma, the inflammatory condition of allergic bronchopulmonary aspergillosis (ABPA) may develop. Allergic sinusitis is also a feature of *Aspergillus* that can set up a fungus ball or aspergilloma in lungs with preformed cavities. Those with underlying chronic lung disease can progress to chronic necrotizing aspergillosis or present with tracheobronchitis. In immunocompromised hosts, invasive disease may develop as invasive pulmonary aspergillosis, invasive sinusitis, or dissemination to extrapulmonary sites (7,12).

Allergic bronchopulmonary aspergillosis (ABPA) arises from a hypersensitivity reaction to *Aspergillus* antigens. Patients with asthma or cystic fibrosis may develop ABPA late in the course of their disease (13–15). In patients with cystic fibrosis (CF), one study has shown lung function to deteriorate over time in those CF patients with ABPA compared with CF controls (16). Similarly, patients with bronchiectasis and evidence of ABPA have been shown to have worse lung function when compared to those with bronchiectasis without ABPA (17). The diagnosis of ABPA is suspected on clinical findings and confirmed by radiologic and serologic results. Impaired mucous clearance, productive cough with mucous plugs or brown specks, mucoid impaction, and episodic bronchial obstruction are characteristics of ABPA. Those with chronic disease may present with bronchiectasis and fibrosis. Imaging with computed tomography (CT) may show pulmonary infiltrates or bronchiectasis, and laboratory findings, such as growth of

Aspergillus in culture or immunologic response with skin reactivity to *Aspergillus* antigens, support the diagnosis.

Allergic fungal sinusitis tends to arise in patients with atopy, history of allergic rhinitis/sinusitis, nasal polyps, and sometimes, asthma (7,18). Direct microscopy often reveals thick green mucus or mucopurulent secretions, crusting, or the presence of polyps. Histologic examination of tissue biopsy demonstrates thick allergic mucin, hyaline, septate hyphae without invasion of tissue, and a chronic inflammatory response. Growth in culture of the offending mold and high levels of IgE aid in the diagnosis of this entity.

Aspergilloma, or fungus ball, is the most common form of pulmonary involvement due to *Aspergillus* (7,12). It usually develops in a preformed pulmonary cavity (e.g., a consequence of prior tuberculosis or bronchiectasis) or in the paranasal sinuses and consists of masses of mycelia, inflammatory cells, debris, and mucus (19). The aspergilloma can remain asymptomatic for a prolonged period of time, though some patients with pulmonary aspergilloma may experience hemoptysis, ranging from mild to severe, secondary to bleeding from bronchial blood vessels. A fungus ball in the sinus cavity can likewise remain asymptomatic, evolve to cause allergic-type presentation, or invade the contiguous tissue. The latter may occur in patients who are immunosuppressed with hematologic malignancy, diabetes, chronic steroid use, solid organ transplant (SOT), and acquired immunodeficiency syndrome (AIDS) (20). Invasion of tissue and bone may progress to invasion of adjacent structures, such as the orbit or the brain. The clinical presentation is variable and requires a high index of suspicion, along with imaging, tissue histology, and culture to establish the diagnosis.

Endobronchial fungal infections are being increasingly described with the use of surveillance flexible bronchoscopy (21). Presentation can range from mild mucosal inflammation to central airway obstruction with invasive disease. In lung transplant recipients, ulcerative or pseudomembranous tracheobronchitis, including infection of the anastomotic site, has been described (22,23).

Chronic necrotizing pulmonary aspergillosis is due to locally destructive invasion of lung parenchyma by *Aspergillus* without distal invasion or dissemination to other organs (7,12). Patients usually have chronic underlying lung diseases, such as chronic obstructive pulmonary disease (COPD), and present with fever, cough productive of sputum, and weight loss over a period of several months. In immunocompromised patients (neutropenia, corticosteroid use, transplant recipients, hematologic malignancy, cytotoxic chemotherapy, AIDS), invasive pulmonary aspergillosis (IPA) may develop, presenting with fever, cough, which may be dry or productive, and dyspnea. Pleuritic chest pain and hemoptysis may be present, as can altered mental status and respiratory failure. IPA is characterized by being more invasive than chronic necrotizing aspergillosis as it often includes invasion of small vessels with hemorrhage and/or infarction and the possibility of dissemination (7,12,24). Radiologically, alveolar infiltrates, either bilateral or diffuse, nodules, cavitation, and pleural effusion can be present. Review of the baseline chest CT findings from 235 patients with IPA, who participated in the global multicenter trial comparing voriconazole with amphotericin B for treatment of invasive aspergillosis (IA) revealed that, at presentation, most patients (94%) had one or more macronodules (25). In patients with neutropenia and IPA, the CT scan may have a nodule surrounded by ground glass attenuation, the classic halo sign. As this occurs early, it allows the presumption of IPA diagnosis to be made prior to cavitation. However, this lesion is transitory and by the first week, three-fourths of the CT halo signs disappear. With recovery of the neutrophil count, an air crescent sign (representing early cavitation) may be seen, which is highly indicative of IPA (26).

Patients who are immunocompromised can have dissemination of *Aspergillus* to the central nervous system (CNS) (24,27,28). At-risk immunocompromised individuals are posttransplant and hematologic malignancy patients, but aspergillosis of the CNS has also been reported in AIDS, chronic asthma with steroid use, burn patients, patients with hepatic failure, and infections in the postoperative period. Cultures from non-CNS sites (most of which are from lung) are positive for *Aspergillus* in approximately half the patients with CNS aspergillosis (28). Pathology reports from a series of CNS aspergillosis cases diagnosed by autopsy described hemorrhagic necrosis, abscesses, large hemorrhages, bland nonhemorrhagic infarctions, myelitis, mycotic aneurysm, basilar meningitis, sino-orbital disease, carotid artery invasion and thrombosis, dural abscesses, as well as findings of minimal inflammation in CNS lesions (28). Imaging studies of

patients with cerebral aspergillosis reveal three general patterns: single or multiple infarcts, ring lesions (single or multiple) consistent with abscess formation after infarction, and dural or vascular infiltration arising from the paranasal sinuses or orbits. Other findings on imaging include mycotic aneurysm and contrast enhancement of affected parenchyma, as well as hemorrhagic transformation of infarcted areas (29).

The cumulative incidence of IA for two of the highest populations at risk, hematopoietic stem cell transplant (HSCT) and SOT recipients, has been reported from a recent multicenter study in the United States (30). In the HSCT population, *Aspergillus* now exceeds *Candida* as the most common invasive fungal pathogen and the cumulative incidence is higher at 12 months in patients with allogeneic unrelated donors (3.9%) than with allogeneic human leukocyte antigen (HLA)-mismatched (3.2%), allogeneic HLA-matched (2.3%), and autologous (0.5%) donors (30–32). The rates were similar for myeloablative and nonmyeloablative conditioning regimens. Mortality at three months was 53.8% for autologous transplants versus 84.6% for allogeneic transplants with unrelated donors. In the SOT population, the cumulative incidence of IA at 12 months was 2.4% for lung, 0.8% for heart, 0.3% for liver, and 0.1% for kidney transplant recipients. Mortality at three months ranged from 20% for lung recipients to 66.7% for heart and kidney recipients. As with the HSCT population, *A. fumigatus* was the species most commonly isolated (76.4%), followed by *A. flavus* (11.8%) and *A. terreus* (11.8%).

Studies have also evaluated risk factors for IA in transplant populations (33–35). Factors associated with development of early IA (≤ 40 days posttransplant) in the HSCT population included older age at transplant, underlying disease other than chronic myelogenous leukemia in the chronic phase (aplastic anemia, myelodysplastic syndrome, and multiple myeloma), the type of transplant (receipt of T-cell depleted or CD-34–selected stem cell products or cord blood), prolonged neutropenia, cytomegalovirus (CMV) disease, and receipt of corticosteroids for treatment of acute graft-versus-host disease (GVHD). Risk factors for IA following engraftment (days 41 to 180) in the HSCT population included older age at the time of transplant, receipt of T-cell depleted or CD-34–selected stem cell products, multiple myeloma as an underlying disease, delayed engraftment of T-lymphocytes, neutropenia, lymphopenia, grade II–IV GVHD, treatment with high dose steroids, CMV disease after day 40, and respiratory viral infections (especially parainfluenza 3) (33,36). In the very late period (> 6 months) post HSCT, risk factors for IA included neutropenia, clinically extensive chronic GVHD, CMV disease, and receipt of an unrelated or HLA-mismatched peripheral blood stem cell transplant. The outcome of IA was poor independent of the timing post transplant of IA; survival was approximately 30% at six months and 20% at 12 months after the diagnosis of the infection. Different risk factors have been identified for IA among the various SOT populations as well. In general, poor status prior to transplant, severe immunosuppression, colonization with *Aspergillus*, and complicated postoperative course are the common risk factors (37–44).

NON-ASPERGILLUS HYALOPHYPHOMYCETES

The hyalohyphomycete molds are a heterogeneous group; however, they do have in common septate, hyaline hyphae when visualized in tissue (6). It is important to remember that fungal hyphae of the various hyalohyphomycetes (including *Aspergillus*) as seen in direct specimen examination and tissue preparation are indistinguishable. Culture of infected tissue or body fluid is therefore required to definitively identify the invading pathogen. Over 30 non-*Aspergillus* hyalohyphomycetes have been implicated in human disease including, most commonly, species of *Acremonium*, *Fusarium*, *Paecilomyces*, and *Scedosporium* (Table 1) (45). Several of the non-*Aspergillus* hyalohyphomycetes are unique in their capability of producing adventitial forms that are able to sporulate in vivo, which permits release of propagules into the bloodstream and dissemination to other organs (46).

Acremonium

Acremonium is a mold found in soil, decaying vegetation, and food that can be pathogenic to plants, insects, and humans. Human infection has been reported with *Acremonium alabamense*, *A. falciforme*, *A. kiliense*, *A. roseogriseum*, *A. strictum*, *A. potronii*, *A. curvulum*, *A. artrogriseum*, and *A. recifei*.

Table 1 Currently Documented Agents of Hyalohyphomycoses^a

<i>Acremonium</i> spp	<i>Emmonsia Parva</i>	<i>Paecilomyces</i> spp	<i>Trichoderma</i> spp
<i>A. alabamense</i>	<i>Engyodontium album</i>	<i>P. lilacinus</i>	<i>T. harzianum</i>
<i>A. atrogriseum</i>	<i>Fusarium</i> spp	<i>P. variotti</i>	<i>T. longibrachiatum</i>
<i>A. curvulum</i>	<i>F. chlamydosporum</i>	<i>Penicillium</i> spp	<i>Tritirachium oryzae</i>
<i>A. falciforme</i>	<i>F. dimerum</i>	<i>P. chrysagenum</i>	<i>Verticillium serra</i>
<i>A. kilinse</i>	<i>F. incarnatum</i>	<i>P. citrinum</i>	<i>Volutella</i>
			<i>cinerescens</i>
<i>A. potronii</i>	<i>F. moniliforme</i>	<i>P. commune</i>	
<i>A. roseogriseum</i>	<i>F. napiforme</i>	<i>P. decumbens</i>	
<i>A. strictum</i>	<i>F. nivale</i>	<i>P. expansum</i>	
<i>Aphanoascus fulvescens</i>	<i>F. nygamai</i>	<i>P. marneffe</i> ^b	
<i>Arthrographis kalrae</i>	<i>F. oxysporum</i>	<i>Phaeoacremonium</i>	
		<i>parasiticum</i>	
<i>Beauveria</i> spp	<i>F. pallidoroserum</i>	<i>P. inflatipes</i>	
<i>B. alba</i>	<i>F. proliferatum</i>	<i>P. rubrigenum</i>	
<i>B. bassiana</i>	<i>F. solani</i>	<i>Phialemonium</i>	
		<i>obovatum</i>	
<i>Cephalophora irregularis</i>	<i>F. veriticillioides</i>	<i>Phialemonium</i>	
		<i>curvatum</i>	
<i>Chrysonilia sitophila</i>	<i>Gymnascella dankaliensis</i>	<i>Polycyctella hominis</i>	
<i>Chrysosporium</i> spp	<i>Lecytophora hoffmannii</i>	<i>Schizophyllum</i>	
		<i>commune</i>	
<i>C. pannicola</i>	<i>Lecytophora mutabilis</i>	<i>Scedosporium</i> spp	
<i>C. zonatum</i>	<i>Metarhizium anisopliae</i>	<i>S. apiospermum</i>	
<i>Coprinus cinereus</i>	<i>Myceliophthora</i>	<i>S. prolificans</i>	
	<i>thermophila</i>		
<i>Cylindrocarpon</i> spp	<i>Onychocola canadensis</i>	<i>Scopulariopsis</i> spp	
<i>C. destructans</i>	<i>Ovadendron</i>	<i>S. brevicaulis</i>	
	<i>sulphureoohraceum</i>		
<i>C. lichenicola</i>	<i>Neocosmospora vasinfecta</i>	<i>Scytalidium dimidiatum</i>	
<i>C. vaginae</i>			

^aList not inclusive.

^bMost authorities refer to disease as penicilliosis.

Source: Adapted from Ref. 43.

In immunocompetent individuals, *Acremonium* has been implicated in cases of keratitis and endophthalmitis either following trauma or laser in situ keratomileusis (Lasik) (47–50). It has also been reported as causing cutaneous and subcutaneous dermal infections, eumycetoma, onychomycosis, osteomyelitis, peritonitis in patients undergoing continuous ambulatory peritoneal dialysis (CAPD), prosthetic valve endocarditis, and CNS infection (51–55). In immunocompromised patients, dialysis fistula infections, peritonitis, pneumonia, cerebritis, and disseminated infection have been reported (4,6,46,56–61). Although rare, *Acremonium* eumycetoma has also been reported in SOT recipients (62). Given the presence of adventitial forms, *Acremonium* can disseminate through the bloodstream to distant sites (63–67). The portal of entry may be either the lungs or gastrointestinal tract skin, with dissemination at times producing endophthalmitis, meningitis, or fungemia with sepsis and end organ damage.

Fusarium

Members of the genus *Fusarium* are ubiquitous filamentous fungi commonly found as soil saprophytes and plant pathogens. Characterized by canoe-shaped macroconidia, *Fusarium solani*, *F. oxysporum*, and *F. moniliforme* are the species most frequently isolated. Human disease ranges from mycotoxicosis, caused by ingestion of fusarial toxins, to invasive infections, which may be superficial, localized, or disseminated (4,6,68).

In a multicenter study involving 9 centers, 61 bone marrow transplant (BMT) patients with fusariosis were reported. The overall incidence was 5.97 cases/1000 transplants (69). Fifty-four patients were allogeneic and 7 were autologous BMT recipients. Disseminated infection

with metastatic skin lesions was the most frequent presentation (75%) followed by fungemia alone (11%). Lung infiltrates were seen in 64% and sinusitis in 36% of the cases. Presenting symptoms included fever (92%) and papular or nodular skin lesions with or without central necrosis (69/70). At the time of diagnosis, 46% of the patients were neutropenic, and most had acute or chronic GVHD. There was a trimodal distribution of infection: an “early peak” was seen prior to engraftment (median posttransplant day 16), a “second peak” was seen late (median posttransplant day 64), and a “very late” third peak was observed after posttransplant day 360. Mortality was very high at 75% to 90%, and median survival after diagnosis was 13 days, with only 13% of patients alive at 90 days (70). Persistent neutropenia and corticosteroid treatment were significant prognostic factors (69,70).

A study of *Fusarium* infection conducted in Israel reported a slightly different clinical scenario, with 76% patients considered immunocompetent (71). These tended to be older patients who had ischemic heart disease, diabetes, peripheral vascular disease, and chronic renal failure as the underlying disease. Of the mycologic data available, 10 infections were with *Fusarium oxysporum*, 8 were *F. solani*, and 4 were *F. dimerum*. The proportion of disseminated and localized disease was about equal in immunocompetent and immunosuppressed patients, and as in the BMT population, skin ulcerations were a common clinical presentation. Risk factors for infection were hematologic malignancy, immunosuppression, burns, other disseminated infections, and chronic renal failure. Mortality was 11% during hospitalization, significantly lower than that reported in the BMT series.

Isolated outbreaks of *Fusarium* keratitis associated with contact lenses have been reported from several states in the United States (72). Most outbreaks have been traced to contaminated contact lens fluids (73). *Fusarium* has also been isolated from a hospital water reservoir during an outbreak of fusariosis (74). The epidemiologic investigation determined that aerosolization occurring during showers constituted the potential source of infection.

Paecilomyces

Paecilomyces species are isolated from soil and decaying plant matter and are often implicated in decay of food and cosmetics. The two most common species are *Paecilomyces lilacinus* and *P. variotii*, both rarely pathogenic for humans. In immunocompetent hosts, these organisms have been reported as the cause of keratitis after corneal implants, endophthalmitis, onychomycosis, skin infections, peritonitis in CAPD patients, pneumonitis, sinusitis, and endocarditis following valve replacement (75–77). A case of pulmonary fungus ball by *Paecilomyces* in an immunocompetent individual has also been reported (78). Immunosuppressed patients may also present with *Paecilomyces* infection. It has been reported as causing infection in patients with chronic granulomatous disease (CGD) including cellulitis, osteomyelitis, pneumonitis, and splenic abscess, and pneumonia and lung abscess in patients with hairy cell leukemia, CGD, and CF (78,79). Disseminated disease appears to occur predominantly in immunosuppressed hosts.

Paecilomyces variotii has been reported in a multiple myeloma patient who had undergone autologous HSCT six months prior to presentation. Fever was the predominant symptom, and *P. variotii* was isolated from line and peripheral blood cultures (80). *P. variotii* has also been recovered from the cerebral spinal fluid (CSF) of a patient with metastatic breast cancer and multiple enhancing brain lesions on magnetic resonance imaging (MRI) (81). CSF parameters were abnormal, and numerous fungal cells and septate hyphae were seen on mycological examination. Importantly, disseminated *P. variotii* infection has also been reported breaking through voriconazole prophylaxis in a neutropenic child with relapsed leukemia (82). The clinical presentation consisted of persistent fever with a pink macular and nodular rash on the child's forearms and face.

Paecilomyces lilacinus has been isolated from many sites of infection. In a large study from Spain, 119 cases were reported from 1964 to 2004 (83). Most cases of *P. lilacinus* were onychomycosis (51.3%) followed by cutaneous and subcutaneous infection (35.3%). For cutaneous infections, risk factors included SOT, BMT, surgery, primary immunodeficiency, and AIDS. Lesions presented as painful red nodules that sometimes progressed to excoriated nodules and draining pustules. Severe onychomycosis, as with *Fusarium* spp., may constitute a risk factor for invasive disease since the toenail may serve as a portal of entry and provide contiguous or

lymphangitic spread (84). Cases of oculomycosis presenting as scleritis, keratitis, and endophthalmitis have also been reported, with lens implantation, diabetes, prior scleritis, surgery, and immunosuppression constituting risk factors (83,85,86).

An outbreak of invasive *P. lilacinus* was reported in severely neutropenic patients due to contaminated skin lotion (87,88). Neutropenic patients in a laminar flow ward presented with cutaneous lesions that erupted either during the neutropenic period or shortly thereafter. Invasive disease occurred in 36% of patients treated with chemotherapy for leukemia or lymphoma and in 100% of BMT recipients. Moisturizing skin lotion was found to be contaminated with the organism. *P. lilacinus* causing cutaneous lesions have also been reported in SOT, steroid users, and patients with CGD (89). Lesions may be papular, pustular, nodular, or ulcerated, and located on any part of the skin.

Scedosporium

Species of the genus *Scedosporium* are frequently encountered in soil from rural areas, parks, potted plants, from compost, manure of cattle and fowl, polluted waters and sewage, and occasionally from hospital air during construction (4,6,56,90). Infections are caused by two species: *Scedosporium apiospermum*, the asexual form of *Pseudallescheria boydii*, and *S. prolificans* (previously *S. inflatum*). No known sexual state of *S. prolificans* is known, and it occasionally has been designated as a dematiaceous mold (91). The first case of *S. prolificans* (then *S. inflatum*) was reported in 1984 (92). Since that time, multiple cases have been reported in the literature, with fairly large case series from Spain, Australia, and the United States (93–96). In one series, approximately 66% of the patients with *S. prolificans* infection were receiving amphotericin B prior to the infection (97). In a series from a tertiary care cancer center, the incidence of *Scedosporium* infection increased from 0.82 cases per 100,000 patient-inpatient days (1993–1998) to 1.33 cases per 100,000 patient-inpatient days, with all cases of *S. prolificans* presenting as breakthrough infections after the year 2000 (98). The increase in *S. prolificans* infections may be linked to the increasing use of antifungal prophylaxis, which in turn may select for this opportunistic pathogen that is notoriously resistant to practically all antifungal agents.

It is important to note that both *S. apiospermum* and *S. prolificans* may simply colonize body sites without overt disease, or they may produce a variety of clinical syndromes in a wide range of hosts. For example, *S. apiospermum* has been isolated as a colonizer from the airways of CF patients, and *S. prolificans* has been reported to colonize airways and external auditory canals (94,95,99–103). Patients in these reports had the organism isolated from culture on multiple occasions; however, they did not appear to have clinical disease nor did they receive systemic antifungal therapy. On the other hand, both organisms may cause severe infection of the eye, lung, skin and soft tissues, bone, CNS, and bloodstream. In fact, disseminated infection is the most commonly reported presentation of *S. prolificans*, and blood cultures are frequently positive (in 75% to 100% of cases) in the setting of disseminated disease (91,93,94,98,104–109). The high rate of bloodstream infection may be one feature distinctive of infection with *S. prolificans* when compared with *S. apiospermum* infection. In one large review of transplant recipients with scedosporiosis, fungemia occurred in 40% of cases with *S. prolificans* infection versus 4.7% of cases with *S. apiospermum* infection (97). The overall mortality rate among transplant recipients with scedosporiosis was 58%. Among the HSCT recipients, overall mortality rate was 68% (77.8% for *S. prolificans* and 61.5% for *S. apiospermum*) whereas the mortality for SOT recipients was 54% (77.8% for *S. prolificans* and 54.5% for *S. apiospermum*).

The respiratory tract is a common site of *Scedosporium* infection. This may remain a localized process or be the portal of entry for hematogenous dissemination. Pulmonary scedosporiosis with *S. apiospermum* has been described in patients with CGD, chronic steroid use, hematologic malignancy, and after bone marrow and solid organ transplantation (33,97,110–117). Initial presentation includes fever, cough, sputum production, pleuritic chest pain, tachypnea, and malaise. Imaging of the lung may demonstrate bilateral infiltrates, nodules, abscess, fungus ball, cavitory lesions, effusion, or empyema. Infection of the respiratory tract with *S. prolificans* has been reported most commonly in immunosuppressed patients including patients with malignancy (usually hematologic), post SOT or HSCT, chronic immunosuppressive therapy or chronic corticosteroids, and AIDS (94,97,116). Pulmonary infection with *S. prolificans* is

indistinguishable from *S. apiospermum* based on clinical and radiographic presentation alone. Dissemination from the lungs to multiple organs, including brain and skin, has been described in both BMT and SOT recipients for both organisms (97,98,113,117). As previously noted, the incidence of dissemination appears to be lower for *S. apiospermum* compared with *S. prolificans* (97). The large majority of patients with disseminated *S. prolificans* infection have predisposing risk factors, such as immunosuppression, neutropenia, BMT, SOT, malignancy, and AIDS.

Scedosporium apiospermum and *S. prolificans* have also been implicated as the etiologic agent of keratomycosis, with and without overt ocular injury, as well as endophthalmitis (103,106,107,118–127). Patients with keratomycosis may experience eye pain, photophobia, foreign body sensation, conjunctival or corneal erythema, tearing, and changes in visual acuity. Cases have been reported in patients with a retained contact lens, and in patients who had experienced scleral necrosis after pterygium surgery with adjunctive beta-irradiation. The lesions have ranged from corneal abrasion to frank corneal ulceration or abscess and anterior chamber hypopyon. Cases of *Scedosporium* endophthalmitis have occurred in a different setting, many times as part of hematogenously disseminated disease in an immunosuppressed host. Endogenous endophthalmitis presents with eye pain, photosensitivity, and worsening visual acuity. Fundoscopic exam reveals exudates and hazy vitreous. Mortality is almost uniform in patients with *Scedosporium* endophthalmitis in the setting of disseminated disease with either *S. prolificans* or *S. apiospermum* (106,107,124). Some patients who have *S. apiospermum* endophthalmitis without extra-ocular sites of infection may survive after enucleation or evisceration of the eye (3 of 9 in one series) (123,124).

Scedosporium has been frequently implicated as the cause of CNS infection including meningoencephalitis, encephalitis, and cerebral abscesses. *S. apiospermum* infection of the CNS has been reported after near-drowning episodes, in patients with hematologic malignancy, after BMT, SOT, and penetrating trauma of the foot complicated by osteomyelitis (111,113,128–136). Central nervous system infection with *S. prolificans* has been reported in the setting of disseminated infection (93,97,108,137). For both organisms, patients may present with variable neurologic findings such as headache, confusion, disorientation, agitation, cognitive decline, progressive lethargy, hemiparesis, or tonic-clonic seizures. Although typically present, the absence of ring enhancement has been reported (128,132,134,135).

Scedosporium has been reported presenting as skin lesions following traumatic inoculation and sometimes in the setting of disseminated infection (97,113,134,138–141). Skin lesions may appear as skin nodules, or as erythematous to purple papulae or papulo-bullae, that may develop a necrotic center and that can have lymphangitic spread. Biopsy of skin nodules reveals an inflammatory granulomatous lesion with abscess, necrotic areas, large multinucleate giant cells, and vascular proliferation. A nodule may even contain a mycetoma, with branched septate fungal hyphae visualized under microscopic examination. Soft tissue infection, arthritis, and osteomyelitis due to *S. apiospermum* and its sexual form, *P. boydii*, as well as *S. prolificans* have also been reported (95,96,111,141–148). The most frequently reported predisposing event was trauma to the affected extremity. Initial presentations included laceration or cellulitis at the site, with progression to joint effusion with inflammation and tenderness, and low-grade fever.

PHAEOHYPHOMYCOSES

The dematiaceous fungi are a heterogeneous group of organisms with darkly pigmented hyphae, conidia, or both owing to dihydroxynaphthalene melanin in their cell walls. Melanin is thought to play a role in pathogenesis as it is a known virulence factor in fungi (149,150). Though these organisms are molds, several have a pleomorphic appearance, and a yeast or mold form can predominate during different phases of growth. This has led to confusion and frequently changing nomenclature (6). Dematiaceous molds most often implicated in human infections include species of *Alternaria*, *Bipolaris*, *Cladophialophora*, *Curvularia*, *Dactylaria*, *Exophiala*, and *Phialophora* (Table 2). Cutaneous and subcutaneous infections after penetrating injury, such as chromoblastomycosis and keratitis, are seen in immunocompetent patients, whereas disseminated disease, often referred to as phaeohyphomycosis, may occur in immunosuppressed individuals (91,151–159).

Table 2 Currently Reported Agents of Phaeohyphomycosis^a

<i>Alternaria</i> spp	<i>Exophiala</i> spp	<i>Piedraia hortae</i>
<i>A. alternata</i>	<i>Exophiala dermatitidis</i> (<i>Wangiella dermatitidis</i>)	<i>Phaeoannellomyces werneckii</i>
<i>Aureobasidium pullulans</i>	<i>Exophiala jeanselmei</i>	<i>Phaeoacremonium parasiticum</i>
<i>Bipolaris</i> spp	<i>Exserohilum</i> spp	<i>Phialemonium</i>
<i>B. spicifera</i>	<i>E. rostratum</i>	<i>Phialophora</i> spp
<i>B. hawaiiensis</i>	<i>E. longirostratum</i>	<i>P. richardsiae</i>
<i>Chaetomium</i> spp	<i>E. mcginnisii</i>	<i>P. verrucosa</i>
<i>Cladophialophora</i> spp	<i>Fonsecaea</i> spp	<i>Pseudoallescheria boydii</i>
<i>C. bantiana</i>	<i>F. compacta</i>	<i>Phoma</i>
<i>C. carrionii</i>	<i>F. pedrosoi</i>	<i>Ramichloridium mackenzii</i>
<i>Curvularia</i> spp	<i>Hormonema dermatoides</i>	<i>Scedosporium prolificans</i>
<i>C. clavata</i>	<i>Madurella</i> spp	<i>Scytalidium</i> spp
<i>C. lunata</i>	<i>M. grisea</i>	<i>Ulocladium</i>
<i>Dactylaria gallopava</i> (formerly <i>Ochroconis gallopavum</i>)	<i>M. mycetomatis</i>	<i>Wangiella (Exophiala) dermatitidis</i>

^aList not inclusive.

Alternaria

Exposure to *Alternaria* has been associated with both development and severity of asthma (160,161). Exposure may occur outdoors or in indoor environments, with biologically active moieties consisting of spores, fragments of spores, and dust particles. In one study, practically all (95–99%) of the dust samples collected in homes contained detectable levels of *Alternaria alternata* antigens, and active asthma was positively associated with the *A. alternata* antigen level in the home (160).

Alternaria keratitis has been reported, usually in association with foreign body removal, Lasik, or due to a keratoprosthesis (162–164). Alternariosis has also been reported in patients receiving SOTs (165–173). Interestingly, most of these patients had cutaneous manifestation of infection. Lesions were solitary or multiple and presented as papules, plaques, nodules, recurrent cellulitis with ulceration, and in one report, the cutaneous lesions presented in a sporotrichoid distribution. Invasive fungal infection due to *Alternaria*, including rhinosinusitis and rhinocerebral infection, has been reported in patients with hematologic malignancy and in BMT recipients. A patient with CGD was documented to have *Alternaria* causing dermal induration (174–178).

Bipolaris

Bipolaris spicifera and *B. hawaiiensis* have been reported in human infections including keratitis, endophthalmitis, and skin and soft tissue infections (179–181). Prior use of topical corticosteroids and antibiotics have been associated with the development of a corneal ulcer caused by *Bipolaris*, and cutaneous infections with *Bipolaris* have been reported in patients who either used topical steroids, had atopic or seborrheic dermatitis, or onychomycosis (182). Cutaneous *Bipolaris* has also been reported in a child with acute lymphoblastic leukemia (ALL) and neutropenia without preceding trauma (183). A tender erythematous patch with central punctate areas of hemorrhage appeared on the left cheek. A skin biopsy revealed epidermal necrosis and dematiaceous, septate hyphae in an edematous papillary dermis with infiltration of vessel wall. Culture revealed the pathogen to be *B. spicifera*.

Cladophialophora

Cladophialophora bantiana is notoriously associated with CNS infection in immunosuppressed and immunocompetent patients (184–186). In a large review of 101 cases of CNS phaeohyphomycosis, 48% were caused by *C. bantiana* (187). Though 37% of the infected patients had some degree of immune dysfunction, over half (52%) of the cases had no known underlying risk factors for infection. Of CNS infections, brain abscesses constituted the most common clinical presentation (87% of cases) with single lesions present in 71%, meningitis 9%, encephalitis 2%,

and myelitis 2%. For those in whom histopathology was available, fungal hyphae were noted in 86%; however, granulomatous inflammation was present in only 48% of cases. Overall mortality was 73%, with equivalent mortality between immunocompromised (71%) and immunocompetent individuals (74%). Recipients of SOTs appeared to have lower mortality at 64%, though in most cases, despite antifungal therapy and surgical intervention, the outcome was still fatal (185,187–190).

Extracerebral involvement with *Cladophialophora* has been reported in both immunocompromised and immunocompetent patients as well (191–193). Cutaneous lesions are the most common extracerebral manifestation. Chromoblastomycosis caused by *Cladophialophora carrionii* is an endemic cutaneous infection presenting with desquamating erythematous papules or squamous plaques (194). The extremities are mainly involved, and treatment is protracted and may not lead to resolution, leaving deformities and incapacitation.

Curvularia

Curvularia, such as *C. lunata* and *C. clavata*, has been implicated as the cause of infections in both immunocompetent and immunosuppressed hosts. Cases of keratitis, cutaneous and soft tissue infections, sinusitis with and without invasion of the brain, brain abscess, peritonitis, and saline breast implant contamination have all been reported (18,155,159,195–203). Keratitis due to *Curvularia* may be secondary to trauma or nontrauma, and it appears to vary with the season in the subtropical region, with a higher incidence in late summer and throughout autumn (155).

Dactylaria

Several reports of *Dactylaria gallopavum* (previously *Ochroconis gallopavum*), a neurotropic dematiaceous mold, have been described in SOT recipients (204–211). Isolated lung involvement has been described in three reports with the patients being asymptomatic, or having fever and cough as presenting symptoms (206,212,213). Radiographic studies showed a nodule or cavitary lesion. Cutaneous and joint involvement with *Dactylaria* has also been reported (208). In three other reports, patients had disseminated disease with lung and CNS involvement (207,213). Presenting symptoms in these cases included cough, pleuritic chest pain, and/or hemiparesis. Imaging of the lungs revealed infiltrate or cavitary lesions, while head CT revealed space-occupying lesions. *Dactylaria* may present as single or multiple cerebral abscesses, which on microscopic examination of tissue appear black, with extensive necrosis-containing neutrophils, multinucleated giant cells, and pigmented septate hyphae (204,205,207,209–211). Disseminated *Dactylaria* infection in other immunocompromised patients such as HIV have also been reported, with CNS involvement similar to that described in SOT patients (214).

Exophiala (Wangiella) dermatitidis* and *Exophiala jeanselmei

Exophiala is a dematiaceous mold that, in certain phases of its development, appears yeast-like, with black creamy colonies and unicellular forms that replicate by budding (215). Clinically, *Exophiala* can have a wide range of presentations. Infection of the CNS with *Exophiala (Wangiella) dermatitidis* has been reported (187,216–221). This may present as primary brain abscess, secondary cerebral infections from contiguous or hematogenous spread, and meningitis (217,221). Though the majority of cases are reported from East Asia, cases in the United States have also been described. *E. dermatitidis* has also been recovered from some European steam baths, where conditions are hot and moist (222).

Exophiala jeanselmei has been implicated in eumycotic mycetoma and in rare cases of chromoblastomycosis (223–226). Infection of the skin and subcutaneous tissue with *Exophiala* has been reported in SOT recipients and other immunosuppressed individuals (156–158,227). Trauma typically precedes cutaneous infection, which can present as a necrotic skin lesion with surrounding erythema, nodules, or subcutaneous cysts. Disseminated disease has also been reported involving blood, heart valves, lung, and the CNS (91).

Environmental contamination of products has been implicated in *E. dermatitidis* infections, including peritonitis in patients undergoing CAPD and meningitis from contaminated compounded injectable steroids (221,228,229). Similarly, *E. jeanselmei* fungemia has been associated with contaminated water products in immunocompromised patients (230). An outbreak of *E. jeanselmei* fungemia over a 10-month period was ultimately related to contaminated deionized

water from a hospital pharmacy. Over half the patients presenting with fungemia had malignancy, mostly hematologic, while the other patients had AIDS, agranulocytosis, and systemic lupus erythematosus (SLE) with thrombotic thrombocytopenic purpura. The most common presenting clinical sign was fever (231).

Exserohilum

Exserohilum may also cause infections in both immunocompromised and immunocompetent hosts (91,232–235). Reported cases are mainly from warm tropical and subtropical regions of the world. Patients typically present with infections of the skin and soft tissue, cornea, paranasal sinuses including allergic fungal sinusitis, lungs, bone, and brain. Species implicated include *E. rostratum*, *E. longirostratum*, and *E. mcginnsii*. Corneal infection is usually secondary to trauma, and none of the reported cases occurred in immunosuppressed individuals. In patients with lesions limited to the skin and/or subcutaneous tissue, trauma is the usual inciting event. Lesions can present as papules, plaques, vesicles, nodules, or ecthyma gangrenosum. Though some skin infections became systemically invasive, most cases of invasive infection were acquired via inhalation, with subsequent dissemination via the bloodstream to other organs mainly in immunosuppressed patients.

Fonsecaea

Fonsecaea pedrosoi is one of the leading causes of chromoblastomycosis (151,153). *F. pedrosoi* and *F. compacta*, which are endemic to various tropical parts of the world, are the most common infecting species (151,153,236). Males in rural areas are most often affected, with painless nodular or verrucous lesions predominating on the extremities. Lesions tend to appear weeks to months after the initial trauma, which tends to be minor and often passes unnoticed. Examination of skin scrapings or tissue histology reveals the typical muriform or sclerotic bodies, and culture is required for correct identification of the etiologic agent.

Phialophora

Phialophora verrucosa has been reported as a cause of chromoblastomycosis (151,153). Occurring predominantly in the tropics, chromoblastomycosis occurs after traumatic inoculation, usually to the extremities (lower greater than upper), of mostly male farmers and other rural workers. Lesions are slowly growing, vegetating, nodular, verrucous, or mixed nodular-verrucous. *P. verrucosa* has also been reported in a case of fatal hemorrhage due to invasive tracheal infection in a BMT patient with prolonged neutropenia (237).

Ramichloridium

Ramichloridium mackenzii (previously *Ramichloridium obovoideum*) is a dematiaceous mold that has been reported from the Middle East, where it appears to be endemic and possibly geographically restricted. *Ramichloridium* is also considered neurotropic, and reports of cerebral abscesses in both immunocompetent and immunodeficient patients have described uniformly fatal outcome despite aggressive surgical and antifungal interventions (238–242). Only one case of nonfatal *R. mackenzii* cerebral abscess, in a kidney transplant recipient, has been reported (243).

ZYGOMYCOSSES

The agents of zygomycosis are members either of the order Entomophthorales or of the order Mucorales. These organisms are characterized by sparsely septate hyphae in tissue. The hyphae are broad, variable in diameter, and polymorphic, with irregular branching, and in the case of the Mucorales, may invade blood vessels with thrombosis, tissue infarction, and necrosis (4,6,90,244). The molds of the order Entomophthorales are usually found in tropical areas, in soil, decaying vegetation, on insects, and as saprobes in the gastrointestinal tract of reptiles, amphibians, and mammals. Of the Entomophthorales, *Basidiobolus* and *Conidiobolus* species are pathogenic to humans, causing subcutaneous infections of the extremities and trunk, and of the nasal submucosa, respectively (245). Members of the order Mucorales are found in soil, decaying vegetation, fruits, foodstuffs, and animal excreta in a wide geographic distribution. The portal of entry for infection is likely pulmonary with eventual dissemination to other sites,

though primary cutaneous infection has been reported (246). The Mucorales cause the majority of cases of human zygomycosis, with *Rhizopus*, *Mucor*, *Rhizomucor*, *Absidia*, *Apophysomyces*, and *Cunninghamella*, among others, found in the literature (36,245). The most commonly reported *Zygomycete* of human infection is *Rhizopus*.

Risk factors for zygomycosis include diabetes mellitus, malnutrition, malignancy, and use of voriconazole (36,247–252). Iron overload and deferoxamine therapy have been associated with a higher risk of zygomycosis (248,253–257). Iron is an important virulence factor for *Zygomycetes*, and when deferoxamine binds to iron in the host, it serves as a siderophore for this mold.

In a large series study, 65% of infections occurred in males. Underlying conditions included diabetes in 36%, no underlying condition in 19%, and malignancy in 17% (of which 95% were hematologic). The site of infection varied based on the population, but overall, the most frequent locations were rhinocerebral 48% (particularly in diabetics and intravenous drug users), lung 24% (neutropenic patients, SOT patients), skin 19% (penetrating wounds), gastrointestinal 7%, and disseminated infection 3% (burns, prematurity, diabetes) (248). Deep extension to bone, tendon, or muscle occurred in 24%, and hematogenous dissemination from skin to other organs in 20%. Overall mortality approached 54%, but varied with the site of infection: in disseminated disease mortality was 96%; in rhinocerebral and localized cerebral disease it was 62%; and in gastrointestinal infection it was 85% due to bowel perforation. Survival was 3% with no therapy, approximately 60% for antifungal or surgical treatment alone, and 70% for combination surgical and antifungal therapy.

In SOT recipients, zygomycosis is associated with corticosteroid treatment, with 78.9% of infected patients having received a cumulative dose of ≥ 600 mg of prednisone (250). Clinical presentations were as mentioned above and were similar among the various organ transplant groups. Interestingly, all kidney transplant recipients had infection of the allograft in one series (250). The genus most frequently isolated was *Rhizopus* (73%) followed by *Mucor* (13%). In a series of 263 consecutive BMT patients, 1.9% developed invasive zygomycosis over 10 years, 80% thereof >100 days after transplant (253). Interestingly, no cases of disseminated infection occurred. Iron overload, neutropenia, and GVHD were reported as risk factors for death in BMT recipients. Breakthrough zygomycosis after voriconazole administration (as prophylaxis, empirical, preemptive, and targeted therapy for IA), in patients following allogeneic BMT, and following intensive chemotherapy in patients with hematologic malignancies, has been increasingly reported (36,247,249,251,252,258). Most of these infections occurred late in the posttransplant/chemotherapy period, and lungs and sinuses were the most frequently affected sites. *Rhizopus* was again the most common genus isolated in culture (36,249). Overall mortality for zygomycosis breaking through voriconazole therapy was very high, with a 69% to 73% attributable mortality reported (249,251). Infections with *Cunninghamella bertholletiae*, though rare, have been reported in patients following both BMT and SOT (246,259–265).

ENDEMIC MYCOSES

The endemic mycoses are a group of thermally dimorphic fungi characterized by growing as a mycelial form at 25°C but as a yeast or yeast-like form at 37°C. The major etiologic agents of endemic mycoses are *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, and *Penicillium marneffeii*, each one with a distinct geographic distribution. These fungi are usually present in the soil, and inhalation of conidia may lead to systemic infection, with clinical manifestation of disease varying in relation to the intensity of exposure and the immune status of the host (6). Disease manifestation may occur on primary exposure or through reactivation of a latent focus when there is a decrease in cell-mediated immunity. In the SOT population, though the overall incidence is low, the most frequent endemic mycosis reported in a prospective study was *Histoplasma capsulatum*, with almost two-thirds presenting as disseminated disease (266). Most occurred at a median of 13.7 months posttransplant and appeared unrelated to rejection episodes.

Blastomyces

Blastomyces dermatitidis is the dimorphic fungus causing blastomycosis (also called North American blastomycosis). *B. dermatitidis* grows on decaying organic material. In North America, the

fungus is found in the south central and southeastern states, states bordering the Mississippi and Ohio River basins, the Canadian provinces and Midwest states that border the Great Lakes, and areas of Canada and New York along the St. Lawrence River, although cases west of the Mississippi River Valley have also been reported (267,268). Africa is also considered an endemic region for blastomycosis (6). The portal of entry is via inhalation of conidia; in the alveoli, transformation to the yeast form takes place with an inflammatory response generating granulomata. Specific cell-mediated immunity is the major host defense system to prevent dissemination. Most infected individuals are asymptomatic. The clinical presentation of pulmonary blastomycosis is varied and includes flu-like illness, acute pneumonia, subacute or chronic respiratory illness or fulminant ARDS; verrucous or ulcerative cutaneous lesions have also been reported (6,269).

In a large case series, the incidence of blastomycosis in an endemic area was 23.88/100,000 admissions or 0.62 cases/100,000 population (270,271). The average patient age was approximately 40 years, with 75% of the patients in the 25–64 years age group. Approximately 65% of the patients were male. The overall distribution of organ involvement was pulmonary 90%, cutaneous/subcutaneous 20%, osseous 15%, CNS 1–3%, lymph nodes 3%, and genitourinary 1%. Multiple organs were involved in almost one-third of the cases, and the mortality rate in one Canadian study reached 6.3% (271).

Though the numbers are small, cases of blastomycosis have been reported in SOT recipients (272–274). One review of cases from 1966 to 1991 found only four definite and one probable report of blastomycosis in transplant recipients (273). Infection most often originated in the lung then disseminated, with skin lesions present in two patients.

Coccidioides

Coccidioides immitis is the etiologic agent of coccidioidomycosis. This organism is found in the soil in the southwestern United States, Mexico, Central America (Guatemala, Honduras, Nicaragua), and South America (Argentina, Paraguay, Venezuela and Colombia) in areas of arid to semi-arid climate (6,275,276). Environmental conditions that favor its growth are heat, low altitudes, sparse flora, and alkaline soils. The portal of entry is via inhalation of the arthroconidia, and a single arthroconidium is able to produce respiratory infection. Arthroconidia germinate to produce spherules and endospores, which are released when the spherules rupture. The spherules are surrounded by neutrophils and macrophages, leading to granuloma formation. The organism is resistant to killing by neutrophils and macrophages, and a cell-mediated response is essential. Defects in cell-mediated immunity are associated with dissemination. More than half of the *Coccidioides* infections are asymptomatic, with about 40% having a self-limited flu-like illness in the ensuing weeks after exposure (6,276,277). Other manifestations include pneumonia and disseminated disease to other sites including the blood, meninges, joint, bone, skin, and urogenital tract (275,278–282).

Immunocompetent patients may present with infection after activities or natural events that lead to soil disruption. Between 1998 and 2001, the incidence of coccidioidomycosis in Arizona, an endemic area, was 43/100,000 population, representing an increase of 186% since 1995 (283). The epidemic was associated with a winter season that followed a prolonged drought, and the presence of hot and dusty conditions likely facilitated aerosolization of spores. Though most patients (85%) presented with mild influenza-like illness, approximately 8% developed severe disease (284). Risk factors for severe pulmonary disease include diabetes, recent cigarette smoking, and older age, while risk for dissemination include Asian or black race, pregnancy, and immunosuppression.

Miliary coccidioidomycosis is the initial presentation in about 1% of immunocompetent patients (285). Two distinct patterns of miliary coccidioidomycosis have been noted with equal distribution, one acute with primarily respiratory symptoms lasting ≤ 1 week, and another chronic with symptoms lasting 5–12 weeks, in patients ethnically predisposed and with multiple sites of involvement. Initial symptoms include cough, dyspnea, fever, chills, and chest pain. Sixty percent of patients developed dissemination to various organs and 40% died. Early recognition and prompt treatment are therefore crucial to avoid mortality in miliary coccidioidomycosis, and a high index of suspicion is important because early in the course of illness, skin test results are negative, and serology is unrevealing.

Coccidioides fungemia has been rarely reported, and of 33 adult reported cases, 31 were male and 29 were seropositive for HIV (281). Seventy-four percent of patients died during the admission or shortly thereafter, with a mean survival of only eight days. Antifungal therapy did not seem to have any effect on survival, probably because in most instances, treatment was instituted late due to technique-related delays in diagnosis. Survival also did not depend on CD4 count, lymphocyte count, age, or other risk factors.

Infections in SOT recipients have an incidence varying from 3.8% to 8.7% in highly endemic areas (275,278–280,286–290). The clinical presentation in SOT recipients is variable; those with pulmonary involvement may have an acute illness with fever, cough, and dyspnea, whereas others progress to respiratory distress, altered sensorium, dissemination to other organs, and disseminated intravascular coagulation with multiorgan failure (286,287,289–291). In a review of coccidioidomycosis in transplant recipients, antirejection therapy was associated with an increased risk of disease (275). The risk after transplant is also increased if there is a prior history of coccidioidomycosis, or there is any positive serologic finding in the period just before transplant. As with histoplasmosis, coccidioidomycosis in SOT recipients may occur as a primary infection following transplant after exposure in endemic areas, or through reactivation of latent infection. Patients who have resided or traveled to endemic areas are at risk for reactivation, which occurs about four to six months posttransplant (275,288). In highly endemic areas, some centers therefore test for *C. immitis* and prophylactically treat those with a positive serology or prior history of coccidioidomycosis before transplantation (292,293). This obviously does not preclude transmission via a donated organ. Such reports describe fulminant infections occurring very early in the posttransplant period, usually within two to three weeks (286,287,289). In a nonendemic area, the absence of clinical suspicion along with lack of detailed information about the donor, such as travel history, may lead to a delay in diagnosis. In contrast to SOT, coccidioidomycosis in BMT recipients has not been widely reported, even in endemic areas. In a retrospective review of autologous recipients, the low incidence was attributed to possible underreporting of disease and possibly reduced sensitivity of coccidioidal serology in patients with malignancy (294).

Histoplasma

Two varieties of *Histoplasma* may cause disease in humans: *Histoplasma capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii*. The mycelial form of *Histoplasma* in the environment is found in soils with high nitrogen content, e.g., soil contaminated with droppings from fowl, in roosts, caves, and old buildings. In the United States, the areas endemic for *H. capsulatum* var. *capsulatum* include the Ohio, Mississippi, and St. Lawrence River valleys. The organism is found throughout most of Latin America as well. *H. capsulatum* var. *duboisii* is found in tropical Africa (6). The portal of entry of infection is via inhalation of microconidia. The transformation into the yeast form takes place intracellularly in neutrophils and macrophages. Circulating yeasts are cleared by the reticuloendothelial system. When a specific cell-mediated response develops, macrophages are then able to kill the organism, and the host develops a granulomatous necrotizing inflammatory response. Most people remain asymptomatic following primary exposure, with more than 50% of the population in endemic areas having a positive skin test indicating exposure (295). Immunocompromised patients, children, and immunocompetent individuals after exposure to a large inoculum may develop symptoms after primary infection including acute self-limited pulmonary histoplasmosis, progressive pulmonary histoplasmosis, or progressive disseminated histoplasmosis.

Disruption of the soil (e.g., roto-tilling, construction, or landfill work) leads to aerosolization of topsoil and dust. Areas with bird or bat guano, where soil conditions favor growth of *Histoplasma*, can lead to large outbreaks of histoplasmosis in immunocompetent individuals (296–298). Symptoms consist of headache, fatigue, fever, cough, myalgias, chills, and chest pain, with the majority of cases having five or more symptoms.

Series of histoplasmosis in SOT and BMT recipients in both nonendemic and endemic areas have been published. It is worth noting that transmission of *Histoplasma capsulatum* through donated organs has been reported (299,300). In one case, reactivation from donor-transmitted histoplasmosis occurred four years after transplantation (300). However, histoplasmosis is generally not found in transplant patients even in endemic regions, suggesting that, in the absence

of an outbreak, histoplasmosis is a rare infection even in the face of immunosuppression (301). In one study, the estimated incidence of histoplasmosis in an SOT population in a nonendemic area was approximately 0.4% (302). Most patients presented with a nonspecific febrile illness, and the infections were judged to be due to endogenous reactivation. All patients had concurrent CMV infection or had received augmented immunosuppression prior to histoplasmosis dissemination, suggesting that reactivation in these cases was due to a severely immunosuppressed state. Primary infection due to inhalation of conidia was thought to account for outbreaks of histoplasmosis among kidney transplant patients in endemic areas (303,304). Fever, cough, and dyspnea or hoarseness were common presenting symptoms, and dissemination occurred in most cases.

Paracoccidioides

Paracoccidioides brasiliensis is the etiologic agent of paracoccidioidomycosis, also referred to as South American blastomycosis. This dimorphic fungus is found in several countries in Latin America from Mexico to Argentina, with Brazil, Colombia, Venezuela, Ecuador, and Argentina reporting the greatest number of cases (6,305). The precise ecologic niche of *P. brasiliensis* remains undefined though the conditions of endemic regions include mild temperatures, many forests, high humidity with plenty of water, rainy summers, and short winters. The areas are also notable for tobacco and coffee farming (306). Naturally acquired infection occurs in armadillos; in humans, it is presumed that the portal of entry may be either via inhalation or traumatic inoculation, with most patients involved in farming activities. The organism undergoes conversion to the yeast form in the lung parenchyma from where it can disseminate. The characteristic appearance of the organism is as multiple budding yeasts in a pilot wheel configuration. Polymorphonuclear leukocytes and cell-mediated immunity play a role in host defense against the organism. Most primary infections are self-limited. The organism has the ability to remain dormant for long periods of time and cause clinical disease at a time when host defenses are impaired. In the subacute form (present in young or in immunocompromised individuals), the disease may manifest with minimal pulmonary symptoms, with hypertrophy of the reticuloendothelial system, or with bone marrow dysfunction. In the chronic or adult form, the sole manifestation might be respiratory symptoms, such as cough, sputum production, dyspnea; fever, weight loss, malaise, and asthenia are also reported. Radiographic images are variable, with infiltrates, nodules, cavity, or fibrosis seen; occasionally, a large mass termed paracoccidioma is seen. Extrapulmonary sites include the skin and mucosa (around the mouth and nose as well as lower extremities), reticuloendothelial organs, adrenals, bone, and CNS (305,307–311).

Infections in SOT patients have been reported in endemic areas (312–315). In one study of 71 renal transplant recipients from an endemic area who died from infectious causes, fungi represented 27.5% of infections, with *P. brasiliensis* representing 4.5% of these (313). Some case studies report presentation many years after transplant. *Paracoccidioides* infection has also been reported in patients with HIV (310,311,316,317).

Penicillium marneffeii

Penicillium marneffeii is the only dimorphic fungus in the genus *Penicillium*. Other *Penicillium* species are hyaline, saprophytic molds which rarely cause infection and are known to contaminate plates in microbiology labs, whereas *Penicillium marneffeii* infections are frequently reported in HIV-infected individuals. This fungus is geographically restricted to Southeast Asia and China though reports of infection have come from Europe, Australia, and the United States in HIV-infected and other immunosuppressed travelers (4–6,56,244,318). *P. marneffeii* is isolated from bamboo rats and their burrows; the rodents themselves not only harbor but sometimes succumb to *P. marneffeii* (319,320). However, the question still remains as to whether human disease is zoonotically or environmentally transmitted (318). The portal of entry is the respiratory tract. Pulmonary macrophages and blood monocytes then take up the organism, where intracellularly, it divides by binary fission, showing a characteristic morphology of septate elliptical yeast with prominent cross-walls. Host response is dependent on polymorphonuclear leukocytes and cell-mediated immunity, and when either or both are lacking, dissemination ensues (321). Though clinical presentation may resemble other infections, such as histoplasmosis, cryptococcosis, tuberculosis, leishmaniasis, and melioidosis, the diagnosis is not difficult if

P. marneffei is suspected. The organism may be cultured from specimens of skin lesions, blood, bone marrow, or lymph node biopsy and will grow on conventional media, where it produces a characteristic soluble red pigment that diffuses into the agar.

Penicillium marneffei infections rarely occur in immunocompetent individuals and, prior to the HIV epidemic, penicilliosis was only sporadically reported in the literature. However, between 1991 and 1997, 1173 cases of penicilliosis were reported in HIV-infected patients in Thailand (322). In HIV patients, the clinical picture is one of fever, weight loss, diarrhea, cough, molluscum-like skin lesions, plus generalized lymphadenopathy and hepatosplenomegaly. Skin lesions typically are papules with central necrosis involving the extremities, trunk, face, and mucocutaneous surfaces. Pulmonary presentation includes pleural effusion, interstitial pneumonia, and diffuse alveolar infiltrates. Lytic bone lesions or arthritis of the large joints and small joints of fingers can occur. Anemia, leukopenia, and thrombocytopenia are often present (318,322–326). Most patients respond to treatment with itraconazole within one week, with complete resolution of cutaneous lesions after three weeks of treatment (326). Cases of *P. marneffei* have been rarely reported in SOT and BMT recipients, with presentation similar to that in HIV patients (323,327–331).

Sporothrix

Sporothrix schenckii is the agent of sporotrichosis. Direct skin inoculation with contaminated soil or thorn plants such as roses leads to a subacute or chronic cutaneous and subcutaneous infection, with nodular lesions that follow the lymphatics and occasionally ulcerate (332,333). The lesions have been mistaken for pyoderma gangrenosum (332). The yeast form of the organism, which may be visualized under microscopic examination, is often cigar-shaped, though some varieties may produce large budding cells (6).

Large series of patients with sporotrichosis have been reported from Brazil and India (334,335). In these series, both fixed cutaneous lesions and lymphangitic/lymphocutaneous forms are described. In a series of 304 patients with sporotrichosis, 96% of the patients had *S. schenckii* recovered in culture, whereas only 32% of cases from India had growth of the organism (334,335). Occupational exposure is frequent, particularly in those jobs involving agricultural activities (farming, horticulture, and forestry). Exposure through hobbies such as carpentry, beekeeping, hunting, and fishing were also reported. Interestingly, while in the study from Brazil, males constituted 68.4% of the patients (210/306), in India females predominated. This was probably due to increased agricultural or horticultural exposure in these women. Upper extremities were predominantly affected, followed by the lower limbs. Though cutaneous and lymphangitic/lymphocutaneous are the most common presentations, unusual manifestations have been reported, involving buttock, abdomen, face, neck, presternal, periumbilical, and pubic region, while extracutaneous involvement was noted as osteomyelitis, oral lesions, and primary conjunctival *S. schenckii* infection (336). In a zoonotic outbreak described between 1998 and 2001, 178 cases of culture-proven sporotrichosis were diagnosed (337). Females predominated, and professional or domiciliary contact with cats was reported in 90.7% of patients, with many reporting traumatic injury preceding the symptoms. Sporotrichosis has been reported only in isolated cases of SOT patients (338–340).

YEASTS

Candida

Candida is now one of the leading causes of nosocomial infection in the United States. In one prospective nationwide surveillance study that included 24,179 cases of bloodstream infections reported between 1995 and 2002, 9.5% were due to *Candida*, thus positioning this organism as the fourth most commonly isolated blood pathogen (341). In intensive care units (ICUs), *Candida* was the third most commonly isolated blood pathogen. Although *C. albicans* remains the most common species causing disease, *C. glabrata* has been increasingly reported as a cause of candidemia in adults (342–344). In fact, non-*albicans* *Candida* species as a group are now responsible for approximately half of all infections. For example, of 1890 *Candida* isolates recovered in the surveillance program mentioned above, *C. albicans* was the most common species recovered, accounting for 54% of cases, followed by *C. glabrata* (19%), *C. parapsilosis*

(11%), *C. tropicalis* (11%), and *C. krusei* (2%) (341). A similar distribution was reported from a separate National Nosocomial Infections Surveillance (NNIS) investigation that only included ICUs (342). In neonatal ICUs, the distribution seems to be slightly different in that *C. parapsilosis* is typically the second most commonly isolated species rather than *C. glabrata* (345).

The explanation for the increase in non-*albicans* *Candida* infections has yet to be fully elucidated, but one of the most likely explanations is the increasing use of antifungal prophylaxis (346,347). For example, the Transplant Associated Infection Surveillance Network (TRANSNET) prospectively monitored over 32,000 SOT and BMT recipients for invasive fungal infections between 2001 and 2006. *Candida* was the most common invasive fungal infection in the SOT population, and the *Candida* species distribution mirrored that of prior epidemiologic reports. However, in the BMT population where fluconazole prophylaxis is routinely employed, *Aspergillus* has now replaced *Candida* as the most common invasive fungal infection. Further, in BMT recipients, *C. glabrata* was the most common infecting *Candida* species (39%) followed by *C. krusei* (18%) and then *C. albicans* (16%) (348). The association between *C. parapsilosis* and neonatal candidiasis is less easily explained. It is known that *C. parapsilosis* has the propensity to adhere to foreign material, including intravascular catheters so often used in pediatrics and neonates. In one neonatal study, patients infected with *C. parapsilosis* were more likely to have received >3 days of third-generation cephalosporins compared with those infected with *C. albicans*. (345,349–356). Exposure of the neonate to *Candida* is vertical and horizontal, and studies have examined the potential link between *C. parapsilosis* infections in neonates and vaginal colonization in the birth mother, as well as skin colonization on the neonate and on the hands of healthcare workers. Though highly suggestive, so far, no definite conclusive link has been established for *C. parapsilosis* (354,355,357–360).

Much effort has been put into defining risk factors associated with the development of invasive candidiasis (IC) in various populations (Table 3). For example, in patients admitted to a surgical ICU for more than 48 hours, acute renal failure, total parenteral nutrition, and central venous catheters were significantly associated with the development of candidemia (361). Other factors that have repeatedly been associated with a risk for invasive candidiasis include receipt of immunosuppressive therapy, cancer and chemotherapy, transplantation, high acuity of illness, increased length of hospital stay, *Candida* colonization at multiple sites, diabetes, and broad spectrum antibiotics. Unfortunately, using the presence of a single factor to predict infection is not effective since the factors occur frequently in hospitalized patients. A predictive rule for invasive candidiasis in adult ICU patients was developed that consistently identified a population with 9.9% incidence of infection (362). The definition included patients admitted to a medical or surgical ICU for greater than or equal to 48 hours, and receiving an antibiotic or having a central venous catheter in place during the first four days of admission, and found to have any two additional risk factors including: total parenteral nutrition or dialysis on ICU days 1–4, or major surgery, or pancreatitis, or receiving steroids or other immunosuppressive agent within seven days of ICU admission. Although the definition was highly selective, recruiting only 11% of patients admitted to the ICU, the definition lacked sensitivity (34.1%) for the individual patient (362). Other populations at increased risk for candidemia include infants born at 26 weeks, independent of birth weight, and patients who have undergone abdominal surgery (363). Historically, 80% of HIV-infected patients developed mucosal candidiasis; however, the introduction of highly active antiretroviral therapy (HAART) has resulted in a marked reduction in AIDS-related IC (2).

The clinical presentation of systemic *Candida* infection is variable and nonspecific, and patients with *Candida* infections may or may not appear seriously ill (364). In a retrospective review of 476 episodes of candidemia, only 7% (37/478) of patients had sepsis syndrome on the date of the first positive blood culture. This was the same for both neutropenic and nonneutropenic patients (365).

Although deep-seated infections such as endocarditis, endophthalmitis, disseminated infection with skin lesions, peritonitis, and chronic disseminated disease have all been well described, fever is the most frequent clinical manifestation (62%, 419/678) (366). In fact, fever is often the only clinical clue of invasive candidiasis, and in neutropenic patients, fever that persists for five days or more despite broad-spectrum antibiotics at adequate doses, and for which a noninfectious cause is not discernible, suggests the presence of invasive candidiasis

Table 3 Risk Factors for Invasive Candidiasis in Adults and Neonates

Risk factors for adults and neonates

Acute renal failure/hemodialysis/peritoneal dialysis
 Broad spectrum antibiotics
 Cancer and chemotherapy
 Central venous catheter (CVC)
 Colonization with *Candida*
 Corticosteroids
 Diabetes mellitus
 Endotracheal intubation/mechanical ventilation
 Immunosuppressive drugs
 Neutropenia
 Pancreatitis
 Prior surgery
 Prolonged hospital stay
 Total parenteral nutrition (TPN)
 Transplant (SOT, HSCT)
 Trauma

Additional risk factors for neonates

Age at first enteral feed
 DIC / shock
 Low APGAR scores
 Prematurity
 Use of H2 blockers
 Very low birth weight

Source: From Refs. 344,345,350,354,355,361,362,366,478–481.

(367). Not all patients with candidemia have the same risk of visceral dissemination. Patients with neutropenia have a much higher rate of this complication, and in a review of almost 70 cases of hepatosplenic candidiasis reported in the literature, characteristics include persistent fever in a neutropenic patient whose leukocyte count is returning to normal. The fever is often coupled with abdominal pain, an elevated alkaline phosphatase level, and less commonly, rebound leukocytosis (368). Characteristic “bull’s eye” lesions (target-like abscesses) may be seen with ultrasound, MRI, or CT examination of the liver and spleen, but these lesions are not generally detectable radiographically until neutrophil recovery has occurred.

Although not common, when present, a characteristic macronodular rash may be isolated (extremities, abdomen) or may cover the entire body and is frequently confused with a drug reaction (369). In a review of 53 documented systemic candidiasis cases, 36% (19/55) had skin lesions. Interestingly, 80% (15/19) of patients that developed skin manifestations were neutropenic (370).

Historically, endophthalmitis was documented to be present in up to 30% of patients with candidemia. Typical lesions of candidal endophthalmitis are whitish chorioretinal spots with filamentous borders protruding into the vitreous and causing vitreal haze. The percent of patients with actual vitreal involvement appears to be decreasing with the earlier initiation of antifungal therapy (371,372). In a study of 118 patients with candidemia examined within 72 hours of a reported positive blood culture, 9% (11) were documented to have chorioretinal lesions consistent with fungal chorioretinitis, but no patient had vitreal involvement (endophthalmitis) (373). Data from a randomized trial comparing fluconazole with amphotericin B for the treatment of candidemia in nonneutropenic patients revealed an incidence of candidal endophthalmitis of only 1% (374). If a fundoscopic examination is performed at the time that candidemia is either suspected or proven, the majority of patients with positive findings will be asymptomatic (375). Progressive disease is associated with decreased vision, eye discomfort, foreign body sensation, floaters, and eye redness and pain in cases with advanced iritis (376). Based on a rabbit model, an appropriate immune response seems to be necessary for *Candida* endophthalmitis to become manifest (377). Accordingly, neutropenic patients rarely develop clinically apparent candidal endogenous endophthalmitis.

Cryptococcus

Cryptococcus neoformans is an encapsulated yeast that reproduces by multilateral budding (6,378–381). *C. neoformans* has been isolated from fruit, trees, and soil enriched by bird droppings, whereas *C. gattii* has been found in eucalyptus trees, and possibly firs and oaks as seen in a recent outbreak from British Columbia (382,383). Aerosolization of infectious particles from disruption of a contaminated environment (soil or trees) leads to inhalation into the respiratory tract as the portal of entry; from here, extrapulmonary spread may ensue. Though the lung and CNS are considered the two primary sites of infection, three other sites frequently involved are skin, prostate, and eye.

Cell-mediated immunity plays a major role in host defenses with granulomatous inflammation essential for containment of the organisms, and conditions that impair cell-mediated immunity render patients more vulnerable to this pathogen. However, it is important to note that in the immunocompetent population, *C. neoformans* and *C. gattii* infections have been reported (383–391).

In the pre-HAART era, cryptococcosis was often seen in HIV-infected individuals, frequently as the presenting AIDS-defining illness. In a population survey conducted in the United States between 1992 and 1994, 86% of *Cryptococcus* cases occurred in HIV-positive individuals (392). Smoking and outdoor activities such as building and landscaping were associated with a higher risk of cryptococcosis, whereas fluconazole in the preceding three months conferred protection. Since the advent of HAART, the incidence of cryptococcosis has significantly decreased in developed countries. In the United States, the incidence of cryptococcosis in patients who have HIV/AIDS has decreased from 24–66 per 1000 in 1992 to 2–7 per 1000 in 2000 (393). In France, there was a 46% decrease of the incidence of cryptococcosis during the post-HAART era (1997–2001) (394). Unfortunately, in developing countries where access to HAART is limited, cryptococcosis continues to be a frequent opportunistic infection (387,395,396).

In immunocompetent hosts, pulmonary and extrapulmonary sites (predominantly the CNS) are affected, and presenting symptoms include cough, fever, chills, dyspnea, anorexia, and possibly night sweats. Pulmonary imaging shows patchy airspace consolidation or nodules, predominantly in the periphery, with cavitation occurring in 40% (388). Pulmonary and CNS infections are the most common presentations in HIV-positive individuals as well, but other forms such as cutaneous, genitourinary, ocular, and fungemia are also reported (381,397,398). HIV patients infected with *C. gattii* most often present with symptoms of meningitis although vomiting, cough, dyspnea, and night sweats were also reported (387). Immune reconstitution inflammatory syndrome (IRIS) occurring after initiating HAART may lead to worsening clinical or radiologic features (381). Lymphadenitis, CNS findings such as meningitis and mass lesions, and pulmonary cavitation have been reported as part of immune reconstitution in patients with cryptococcosis (399,400).

In SOT recipients, most cryptococcal infections develop late in the posttransplant period compared with other fungal pathogens (401). In large databases, cryptococcosis accounted for 1% to 4% of invasive fungal infections (402–404). Time to diagnosis after transplant varied from 7 to 21 months, depending on the organ transplanted, and the overall incidence was significantly higher in heart transplant recipients compared to lung, liver, kidney, and small bowel recipients. In one series, 38% of transplant patients with cryptococcosis had isolated pneumonia, 35% had isolated meningitis, and 24% had disseminated disease (403). In another review, meningitis was the most common presentation (55%) followed by skin and/or osteoarticular involvement (13%) and pneumonia (6%) (404). The risk for disseminated disease appears to be highest in liver transplant and lowest for lung transplant recipients. In liver transplant recipients, the risk of dissemination was 80% in patients with hepatitis C and 71% in those with alcoholism (405).

Among SOT recipients with cryptococcal pneumonia, dyspnea (95%), cough (76%), and fever (62%) were the predominating symptoms. However, asymptomatic pulmonary cryptococcosis has also been reported in SOT recipients (406). In such cases, incidental findings on radiographic imaging (ground-glass infiltrates, multiple nodules, or mass with cavitation) led to the histopathological or microbiologic diagnosis. Also of note, up to 25% of SOT recipients with disease limited to the lung had negative serum cryptococcal antigen tests. Positive serum antigen tests were more frequent in disseminated disease (100%) and meningitis (86%) than in

isolated pneumonia, suggesting that the latex agglutination test is unreliable for diagnosis of pulmonary cryptococcosis and more invasive methods may be needed (403).

Cryptococcus laurentii is a rare human pathogen in immunocompromised patients. As with *C. neoformans*, this infection is most commonly seen in those with impaired cell-mediated immunity (407). Based on one review, it appears the presence of an indwelling device confers a significant risk for infection with *C. laurentii*. Infections of the blood and CNS are most frequently reported though infection of lung and other sites have also been described.

Malassezia

The lipophilic yeasts *Malassezia furfur*, *M. pachydermatis*, *M. sympodialis*, and *M. globosa* cause infections such as tinea versicolor, infectious folliculitis, and catheter-related fungemia (408–411). More invasive infection has been described in immunocompromised hosts, neonates, burn patients, and those receiving intravenous lipids (4,56,90,408,412,413).

Tinea versicolor (pityriasis versicolor) is caused by *Malassezia*, but as taxonomy has evolved, new species have been implicated as the etiologic agent (414). *M. furfur* was originally claimed to be the etiologic agent, but now *M. globosa* and *M. sympodialis* are also involved, with *M. globosa* occurring in temperate climates (411,415–417). A distinct presentation was reported in 12 patients with atrophic dermatitis found to have tinea versicolor (409). The lesions, atrophic plaques and papules, resembled other dermatologic conditions such as mycosis fungoides, SLE, and steroid atrophy. Histology revealed hyphae and spores in an atrophied epidermis and dermis, along with other characteristics that prompted the authors to propose the name atrophying tinea versicolor. *Malassezia* folliculitis, due to *M. furfur* or *M. pachydermatis*, has been reported in heart transplant, kidney transplant, and BMT recipients (418–421). The rash can present as an acneiform eruption or as folliculitis with a papular or papular-pustular appearance. Fever may precede the rash in BMT recipients (278,420,421).

Malassezia, both *M. furfur* and *M. pachydermatis*, has been implicated in fungemia in adults, children, and infants receiving intravenous lipids or with prolonged catheterization (410,412,413,422,423). The central venous catheter is most often considered the portal of entry though other sites, such as upper respiratory tract, lung, and urine, may be colonized or infected. Fungemia manifests as fever refractory to antibiotics. Patients may also present with respiratory findings, pulmonary infiltrates, and thrombocytopenia, the latter especially in infants (413,423). In one series of 3044 BMT recipients, infection with *M. furfur* was reported in 6 patients over a 25-year period. These infections occurred at a median of 59 days posttransplant and the majority of the infections (5/6) were in allogeneic recipients. The spectrum of disease ranged from infection of the mucosal surface and skin (folliculitis) to catheter-related fungemia. No patient in this group had pulmonary involvement (421).

Trichosporon

Trichosporon species are opportunist yeasts that cause infections in immunocompromised patients (4,56,90). Distinct subgroups with different morphologic, biochemical, and genetic profiles are members of the genus, which may lead to confusion. Further, it is still under discussion whether *Trichosporon beigelii*, formerly called *T. cutaneum*, and *T. asahii* are the same organism (424,425).

Trichosporon beigelii is a saprophytic yeast usually found in the soil that can be cultured from human skin, stool, and urine. This organism has been implicated as the etiologic agent of white piedra, an infection of the distal end of the hair shaft, as well as causing cutaneous infections in immunocompetent patients (425–427). In immunosuppressed patients, particularly in those with underlying malignancy, it has been implicated as the cause of fungemia, renal insufficiency, pulmonary infiltrates, cutaneous lesions, chorioretinitis, and chronic hepatic trichosporonosis (428–437). The majority of patients had hematologic diseases though other immunosuppressive conditions, such as SOT, BMT, solid tumor, HIV infection, burns, and peritoneal dialysis, have also been shown to predispose to infection with the organism (438). Approximately 50% of *Trichosporon* infections was determined to be disseminated, involving two or more organs with or without fungemia.

Pneumocystis

Pneumocystis pneumonia (PCP), due to *P. jiroveci* (previously *P. carinii* f. sp. *hominis*), remains one of the most feared complications of immunosuppression due to its significant morbidity and mortality (439,440). Though the genus *Pneumocystis* has been known for years, taxonomic assignment of *Pneumocystis* has placed it with either fungi or protozoa, with phylogenetic data and characteristics that would lend credence to having it belong to either (441). Furthermore, DNA sequence analyses have revealed DNA diversity of the organism in different host species. This has led to a renaming of the species that infects humans to *P. jiroveci* (439,442). The term PCP, however, is still used to refer to *Pneumocystis pneumonia* in humans. The organism has different morphologic forms in its life cycle including trophozoites, cysts, and intracystic bodies (sporozoites), and the portal of entry appears to be inhalational though the infective form is unknown (443). The incidence of disease relates to the degree of immunosuppression, with impairment in T-cell-mediated responses lending increased susceptibility. Patients with HIV/AIDS, with CD4 less than 200 cells/mm³, who are not receiving HAART or who do not receive PCP prophylaxis, and patients iatrogenically immunosuppressed after transplant are especially vulnerable (444,445).

Pneumocystis jiroveci is widespread though its source in nature has not been identified (445). As the organism is host specific, transmission likely occurs from human to human (446–448). Exposure seems to occur in early childhood, with antibodies to *Pneumocystis* detected in 85% of children up to 20 months of age (449,450). Under usual circumstances, little or no respiratory symptoms develop during this primary exposure, though some infants may present with a self-limiting upper respiratory tract infection, and some children have presented with bronchiolitis (451–453). Adults have also been found to be colonized with *P. jiroveci*, and patients with chronic lung disease may be at increased risk of colonization (454–457). Thus, both children and adults may play a role in the epidemiology of *Pneumocystis*, serving as potential reservoirs. Immunosuppressed individuals may be newly exposed to *P. jiroveci* or experience reactivation of latent *Pneumocystis* infection, and disease may result from either recent acquisition or reactivation of long-standing, dormant organisms (458). Nosocomial transmission of PCP has been reported (448).

PCP was the first AIDS-defining illness, and as the HIV epidemic has evolved, the epidemiology of PCP has also changed, especially after the advent of HAART (440,459). In patients with HIV/AIDS, PCP usually presents as a subacute onset of dyspnea, nonproductive cough, and occasionally low-grade fever. The patient may present with tachypnea and tachycardia but unremarkable lung exam. Hypoxemia is present in the more severe cases, reflecting a high burden of organism and host inflammatory response. Chest imaging often reveals bilateral perihilar interstitial infiltrates, which can become diffuse, alveolar, or confluent if the disease progresses (445,460). Imaging with CT scan reveals ground glass opacities in a perihilar, patchy or geographic distribution, with thickening of the interlobular septa. Affected areas may be interspersed with normal parenchyma. Less often, CT of *Pneumocystis pneumonia* will reveal large cystic lesions, often multiple, which carry a high risk of pneumothorax (461). Other less common imaging findings include mass lesions, nodules, consolidation, pleural effusion, and lymph node involvement (460).

Although PCP has historically presented in patients with HIV/AIDS, it has also been reported in patients with other underlying immunosuppressive conditions such as hematologic malignancies, inflammatory and autoimmune diseases, and in patients receiving cytotoxic drugs or prolonged corticosteroid therapy, particularly after BMT and SOT (440,462–471). A 2% incidence of PCP has been reported in lung transplant recipients in the absence of prophylaxis (471). Concomitant conditions which may play a role in increasing the risk of PCP in both SOT and BMT recipients, include the presence of CMV, rejection (including GVHD), and an increase in immunosuppressive therapy (441,469–473). The mean time to diagnosis is 20 weeks posttransplantation, with 54% of patients developing PCP within six months of transplant. The incidence in the first year posttransplant is 8 times higher than subsequent years. The incidence also varies by transplanted organ, with lung recipients having the highest (22 cases per 1000 patient years) and kidney recipients having the lowest (0.8 cases per 1000 patient years) rates (471). PCP in transplant patients mirrors the presentation in HIV/AIDS patients, although atypical pneumonias with no infiltrates, unilateral infiltrates, alveolar

infiltrates, granulomata, nodules, and cavities, and even asymptomatic infection, have been reported (467,474–476).

In general, PCP rarely develops in patients receiving prophylaxis, and observed cases are mostly attributed to noncompliance (477). In the rare instances when breakthrough PCP infection in patients on prophylaxis with adequate systemic absorption occurs, the infection may be atypical in presentation and require lung biopsy for diagnosis (441).

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3 Experimental Animal Models of Invasive Fungal Infections

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INTRODUCTION

Animal models play an important role in antimicrobial drug discovery. The initial standard approach to discovery and development of antimicrobial agents, including antifungals, is to screen activity of a large number of various compounds (e.g., chemical libraries) against reference organisms *in vitro*. This step is known as primary screening. When a potential candidate is identified, antifungal activity against a large panel of clinical isolates is undertaken. Candidate compounds, which demonstrate an appreciable antimicrobial activity in these tests, are selected and their properties characterized further using many different test systems *in vitro* and *in vivo* (e.g., pharmacokinetic properties). The *in vivo* efficacy of a candidate compound can be dramatically affected by pharmacokinetics and pharmacodynamics of the drugs (1). Hence, no matter how sophisticated drug screening and development may be, an essential step in the discovery and development of new antimicrobial and antifungal therapies, before testing in human, is evaluation of the drug for its antimicrobial efficacy and toxicity in animal models. In this regard, showing that a candidate compound is active *in vitro* does not necessarily guarantee that it is active *in vivo*. Importantly, some compounds that possess outstanding activity *in vitro* turn up to be very toxic when introduced into animals. Therefore, evaluation of candidate drugs in animal models is a critical step in predicting the efficacy and toxicity of antifungal agents in humans.

This chapter describes animal models of medically important fungal infections including candidiasis, aspergillosis, and cryptococcosis as well as recent catheter-associated biofilm *in vivo* models developed by the author's group. This chapter also describes a guinea pig model that has utility in evaluating antifungals targeting superficial fungal infections (*dermatophytosis*).

CANDIDIASIS

Hematogenously Disseminated Models

A suitable animal model is essential to delineate the efficacy of any therapeutic agent, optimize its mode of delivery, and assess drug–drug interactions when combined, in the treatment of candidiasis. Systemic infection models for candidiasis have been established in mice, rats, guinea pigs, and rabbits. Among these animals, the mouse is the most popular species for evaluating the efficacy of antifungal agents followed by rabbit, rat, and guinea pig. The reason why the murine model is often used is: (i) mice can be easily infected, (ii) disseminated candidiasis in the mouse produces disseminated infection in a manner similar to that developed in immunocompromised patients (e.g., kidney and brain invasion), (iii) a large body of literature exists regarding antifungal therapy in this model, (iv) the mouse model is the best system for large-scale evaluation of anti-*Candida* agents, (v) the mouse is the lowest member of the phylogenetic tree in which an infection can be produced that resembles human candidiasis, (vi) only small quantities of candidate compounds are needed for initial screening, and (vii) the mouse model is more economical with respect to purchase, per diem, and husbandry costs. Finally, well-known background and variety strains exist, such as outbred mice, inbred mice,

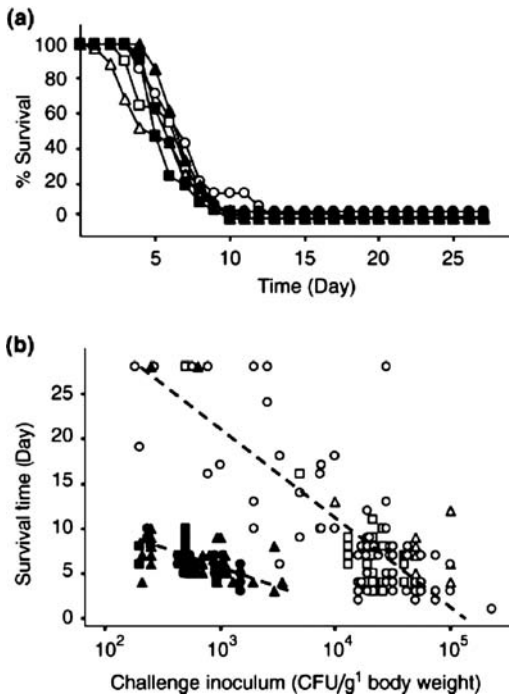


Figure 1 (A) Kaplan–Meier survival curves and (B) survival times (times to termination) of individual mice as a function of challenge inoculum for female BALB/c (open symbols) and DBA/2 (closed symbols) mice inoculated i.v. with *Candida albicans* SC5314 (circles), CAF2–1 (triangles), or CAI-4 + Clp10 (squares). Dashed lines shown are regression lines calculated for each mouse strain without regard to *C. albicans* challenge strain (10).

and specific gene mutant mice. Outbred mice such as CD-1 mice (or ICR mice) (2) and CF1 (3) mice, or inbred mice including BALB/c (4), C57BL/6 (5), and C3H/He (6), are generally used for evaluating the efficacy of candidate antifungals in the treatment of hematogenously disseminated candidiasis. Moreover, immunocompetent and immunocompromised models have been employed to evaluate the efficacy of antifungal agents. Immunosuppression can be induced with cyclophosphamide (7), 5-fluorouracil (8), or gold sodium thiomalate (9).

Challenge of mice in this model is generally achieved by infecting the animals by intravenous injection (i.v.) through the tail vein. Challenge dose used to infect animals is determined based on the virulence of the *Candida* strain used to challenge them and the susceptibility of the mouse strain to the infection. In general, an i.v. inoculum higher than 10^6 *Candida albicans* is rapidly lethal while an inoculum less than 10^4 gives a low-grade, chronic infection with spontaneous resolution (1). MacCallum and Odds (10) demonstrated the effect of challenge dose on infection outcomes. They examined the relationship between challenge dose and survival time for two mouse species (BALB/c and DBA/2) and *C. albicans* strain (SC5314) and found the range 2×10^4 to 1×10^5 organisms/g body weight for BALB/c and 2×10^2 to 1×10^3 organisms/g body weight for DBA/2 lead to reproducible survival times in the range of 2–10 days (Fig. 1). While in neutropenic mice, the minimal lethal dose may decrease by two logs or more, low virulent strains require much higher inoculum to cause a lethal infection up to 5×10^7 colony-forming units per mouse (11).

Drugs being evaluated for their efficacy in the treatment of systemic candidiasis are administered via various routes, such as intraperitoneally (i.p.), orally (p.o.), or intravenously. Intraperitoneal route is more convenient to use than the i.v. route for repeated administration, since tail vein may collapse especially when more than once daily dosing is used. Some antifungal agents, such as voriconazole and terbinafine, are rapidly cleared from mice (voriconazole causes autoclearance in rodents). In such cases, alternative guinea pig models have been used (12).

Survival rates and tissue fungal burden, particularly the kidneys and also increasingly the brain, are the main endpoints used to assess efficacy of an agent in the treatment of systemic candidiasis. Other read-outs, although used infrequently include body weight (13) and physiological characters of mice (such as blood pressure, heart rate, and body temperature as well as blood chemistry parameters) (14).

Vaginal Candidiasis Models

Mouse and rat models of vaginal candidiasis have been used for testing the efficacy of topical or systematic antifungal agents. Different kinds of animal strains, such as mice [BALB/c (15), C57BL/6, CBA/J (16), DBA/2, and C3H/HEN] and rats [Sprague-Dawley (17), CD, Wistar, and Alderley Park], have been used in these models. Calderon et al. reported that differences in host factors influence the susceptibility of the different strains to vaginally administered *C. albicans*. These authors compared BALB/c, CD-1, DBA/2, AKR/j, C3H/HeN, A/J, C57BL/6, and CBA/J mice strains for their susceptibility to *Candida* infections. Of these mice strains, only CD-1 mouse showed resistance to vaginal candidiasis (18). Therefore, all of the different mouse strains tested, with the exception of CD-1 strain, can be used in in vivo screening of potential antifungal agents for treating vaginal candidiasis.

Vaginitis in rodents is inducible only under conditions of a pseudo-estrus (19). Estradiol valerate is injected subcutaneously to induce and maintain the pseudo-estrus condition (15,17). Animals are anesthetized and inoculated intravaginally with 1×10^5 to 5×10^6 cells/mouse (15) or 10^7 cells/rat (17). Topical drugs are administered with a small volume of oil-based vehicle such as polyethyleneglycol using a pipetman or a gavage needle, while p.o. treatment is administered using a gavage needle.

To monitor the course of infection and the efficacy of antifungal agents in the treatment of fungal candidiasis, fungal cells are recovered from lavage fluid or homogenized tissue. Vaginal lavage is obtained by gentle to moderate agitation with phosphate buffered saline. Alternatively, vaginal infection is monitored by obtaining a swab from the vagina and used for semi-quantitative evaluation. The vaginal lumen is sampled with a sterile cotton swab or a calibrated (1 μ L) plastic loop and either plated onto Sabouraud dextrose agar (SDA) plates or phosphate buffered saline (PBS) for serial dilution and plating.

Oral Candidiasis Model

Oropharyngeal *Candida* infection is a serious problem for patients with AIDS, diabetes mellitus, those receiving broad-spectrum antibiotics, steroids or immunosuppressive drugs, as well as radiation for head and neck cancer. There are several variants of oral candidiasis such as pseudomembranous, erythematous, angular cheilitis, and hyperplastic type (20,22). Among these variants, pseudomembranous candidiasis (thrush) is the best-known form of mucosal candidiasis and is mainly encountered in HIV-infected patients. *C. albicans* is the predominant species to cause oral candidiasis. However, recent data shows that *C. glabrata* is increasing in incidence particularly in head and neck cancer patients treated with radiation (23).

Rats and mice have been most used to develop oral candidiasis models because of their variety and convenience to manipulate. Since establishing an oral candidal infection is challenging in immunocompetent animals, a variety of approaches have been employed to establish the infection. These approaches include use of (i) broad-spectrum antibiotic therapy (e.g., tetracycline) (21), (ii) carbohydrate-rich diets (24), (iii) topical use of corticosteroids (25), (iv) corticosteroid inhalation (26), (v) estrogen injection (27), (vi) trauma (28), (vii) iron deficiency (29), (viii) diabetes (30), (ix) xerostomia (30), and (x) immunosuppressive therapy (31).

Oral infection is accomplished by means of a cotton swab rolled over all parts of the mouth (32), while drug efficacy is assessed by measuring the number of *C. albicans* organisms (CFUs) in oral swabs or homogenized tissue. Histopathological examination is also employed to assess the efficacy of drugs (32).

Rabbit *Candida* Biofilm Model

Central venous catheters infected with *Candida* biofilms are problematic since biofilms are nearly totally resistant to common antifungal agents (33–35). Therefore, developing and evaluating new drugs against *Candida* biofilm by using animal models is quite valuable. While rabbits and rats have been used to develop catheter-associated *Candida* biofilm models (36), rabbits are more commonly used in evaluating the efficacy of antifungal agents in the treatment of catheter infections caused by candidal biofilms (37). For example, the author's group showed that using a rabbit model of catheter-associated *C. albicans* biofilm, lipid-based amphotericin B, and echinocandins (anidulafungin, caspofungin, and micafungin) are effective in the treatment of biofilms formed on catheter intraluminally when used as antifungal lock therapy (38). In brief, the rabbit model involves surgically placing a silicone catheter in the external jugular vein of New Zealand white rabbits under anesthesia (Fig. 2). To form a biofilm, an inoculum of

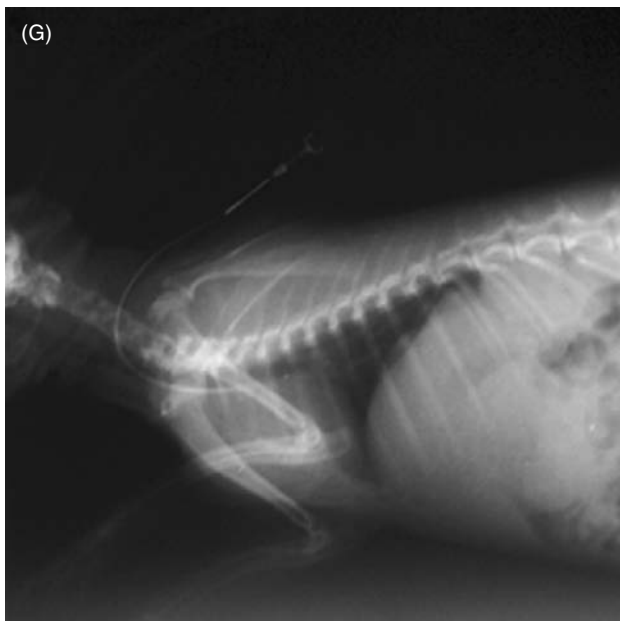
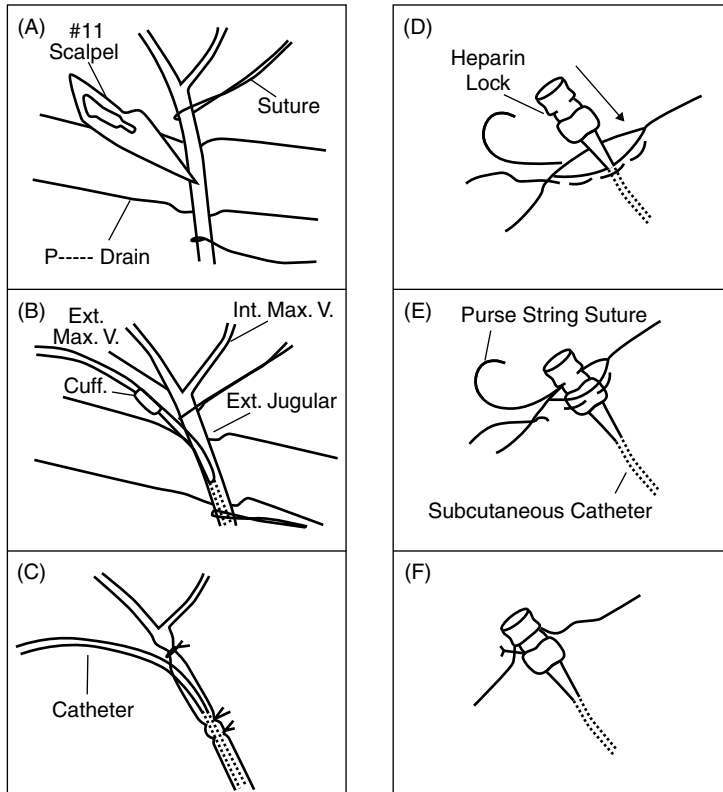


Figure 2 Surgical placement of the intravenous catheter. (A–C) Catheter insertion into the external jugular vein; (D–F) attachment of the heparin lock device to skin; (G) postoperative venogram of catheter placement (38).

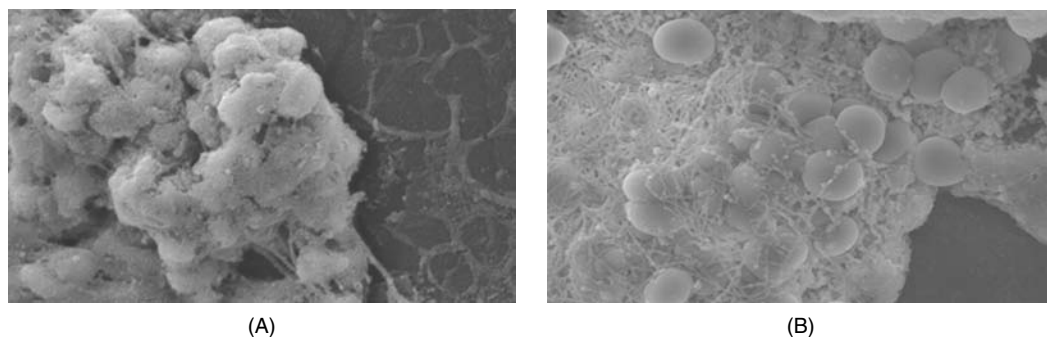


Figure 3 Mature in vivo biofilm formation during model development. Scanning electron micrographs of *C. albicans* biofilms adherent to the intraluminal surface of catheters showing no difference in biofilm architecture at seven days postinfection (magnification, x6500) (A) and three days postinfection (magnification, x2500) (B) are shown (38).

Candida cells are locked in the internal lumen of the catheter, allowed to dwell for 24 hours and then removed. To evaluate the efficacy of a candidate antifungal in the treatment of catheter-associated biofilm, a solution of the agent is locked in the lumen for between 2 and 8hr/day for 7 days. Upon completion of therapy, blood cultures are obtained and the catheters are removed for quantitative culture (CFU determination). Additionally, the ability of the agent to eradicate the biofilm is also analyzed using scanning electron microscopic analyses (Fig. 3).

Mouse Subcutaneous *Candida* Biofilm Model

While using mice to evaluate the ability of lock solutions to treat intraluminal biofilms is challenging, owing to difficulties encountered in placing a catheter in their tiny vein, this animal has utility as a subcutaneous model in evaluating the effectiveness of catheter coating in preventing biofilm formation (39). This model employs BALB/c mice. The mice are anesthetized, their backs shaved, a midline incision is made in the skin above the midthoracic spine, and a pocket is made subcutaneously (s.c.) by blunt dissection. The author's group used this s.c. model and evaluated the ability of amphogel, a dextran-based hydrogel into which amphotericin B is adsorbed, in killing *C. albicans* biofilm. Amphogels or hydrogels without amphotericin B were inoculated with *C. albicans*, then implanted s.c. in mice and allowed to form a biofilm. Animals were sacrificed, disks removed for enumeration of cells and microscopic examination. The data showed that no fungal survival was observed with amphogels, whereas dextran hydrogels were heavily colonized. Additionally, SEM analysis showed that amphogel surfaces did not have any *Candida* cells or biofilm attached [Fig. 4(C)]. In contrast, dextran hydrogels without amphotericin B were covered with *Candida* biofilm [Fig. 4(E)], *Candida* blastospores, and white blood cells [Fig. 4(F)] (39).

ASPERGILLOSIS

To test the efficacy of antifungal agents in invasive aspergillosis, some animal models including rabbits (40), guinea pigs (41), rats, and mice were employed. In order to mimic immunocompromised hosts and to facilitate the establishment of infections (as *Aspergillus* tends to be less pathogenic than *C. albicans*), immunosuppressive agents such as cyclophosphamide and/or a corticosteroid are given to the animals. To render the animals neutropenic, cyclophosphamide is usually administered before and after challenging them with the fungi (42). Cortisone acetate is also used to produce an immunocompromised host (43). To prevent bacterial superinfections, animals receive several broad spectrum antimicrobial agents before and after animal challenge (44). This chapter describes rats and mice models of invasive pulmonary aspergillosis. For description of aspergillosis in rabbits, refer to the work of Walsh et al. (45).

Rat Pulmonary Aspergillosis Model

Aspergillus fumigatus is delivered to the lung by several methods: (i) intratracheal (surgical): the trachea of the animal is surgically opened and then the conidial suspension is injected into the

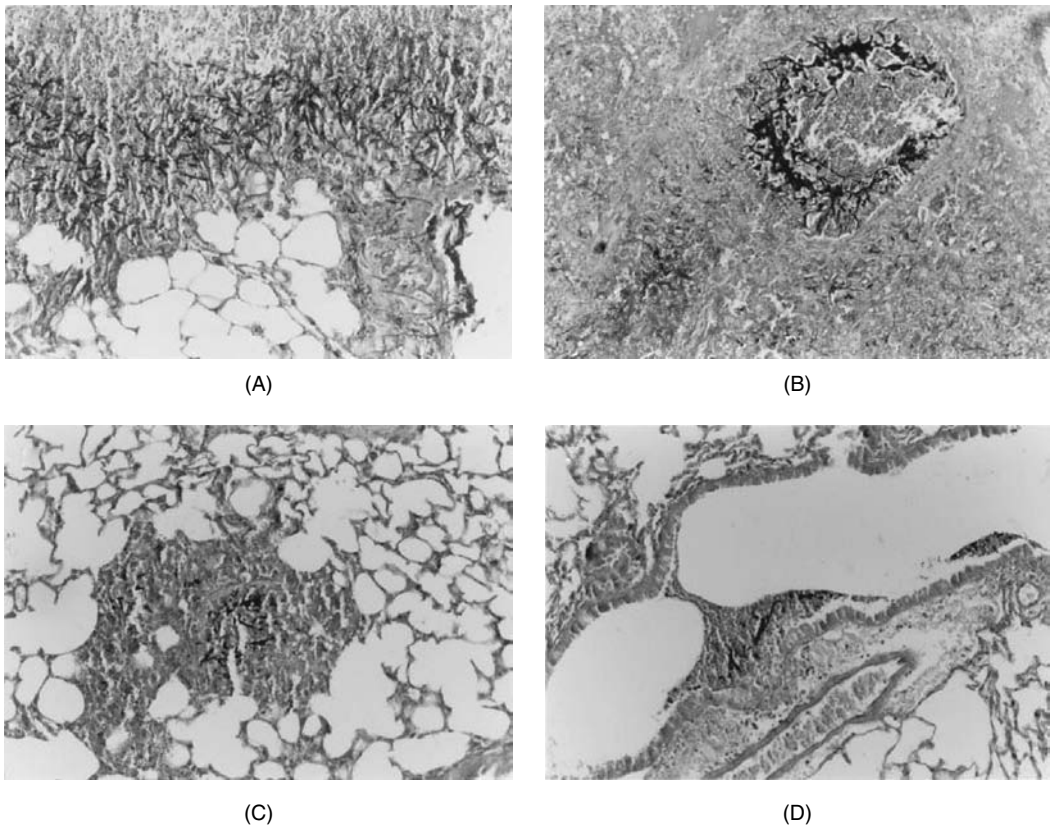


Figure 4 Histopathological findings for lung tissues of mice treated with 5% glucose (A), AmBisome at 2.0mg/kg (B), PEG-L-AmB at 2.0mg/kg (C), and 34A-PEG-L-AmB at 2.0mg/kg (D) on day 3 after injection. The tissues were stained with periodic acid-Schiff stain (53).

trachea with a tuberculin syringe (46), (ii) intratracheal (nonsurgical): a tube is nonsurgically intubated, then a cannula is passed through the tube and conidial suspension is introduced (47) to the lung of the animals, and (iii) intranasal: a conidial suspension is delivered with a micropipette to the nares of the rat (42). Antifungal agents are given to the animals via i.v. (48), p.o. (49), i.p. (48), or inhalation. The effect of antifungal treatment is evaluated by survival rate, tissue fungal cells burden, or histopathological examination (42).

Mouse Pulmonary Aspergillosis Model

There are several routes for conidial inoculation in murine models of aspergillosis, including (i) intratracheal: a conidial suspension is delivered to surgically exposed trachea under anesthesia by using a syringe with a small size (25–26 gauge) needle (50), (ii) intranasal: a single droplet of a conidial suspension is slowly instilled on both nares (51), and (iii) inhalational: mice are exposed to aerosolized conidial suspension (12mL) for one hour in an inhalational chamber (52). Antifungal agents are administered by i.v., i.p., or p.o. after infection. To evaluate the effects and toxicities of the drugs, several parameters such as survival rate, pulmonary fungal burden, histopathology (Fig. 5) (53), and drug distribution are examined.

Systemic Aspergillosis Model

For the systemic aspergillosis model, the conidial suspension is inoculated via the lateral tail vein of immunosuppressed (induced by cyclophosphamide with/without corticosteroid) rats (54) and mice (55). Guinea pigs have also been used in systemic aspergillosis models (56).

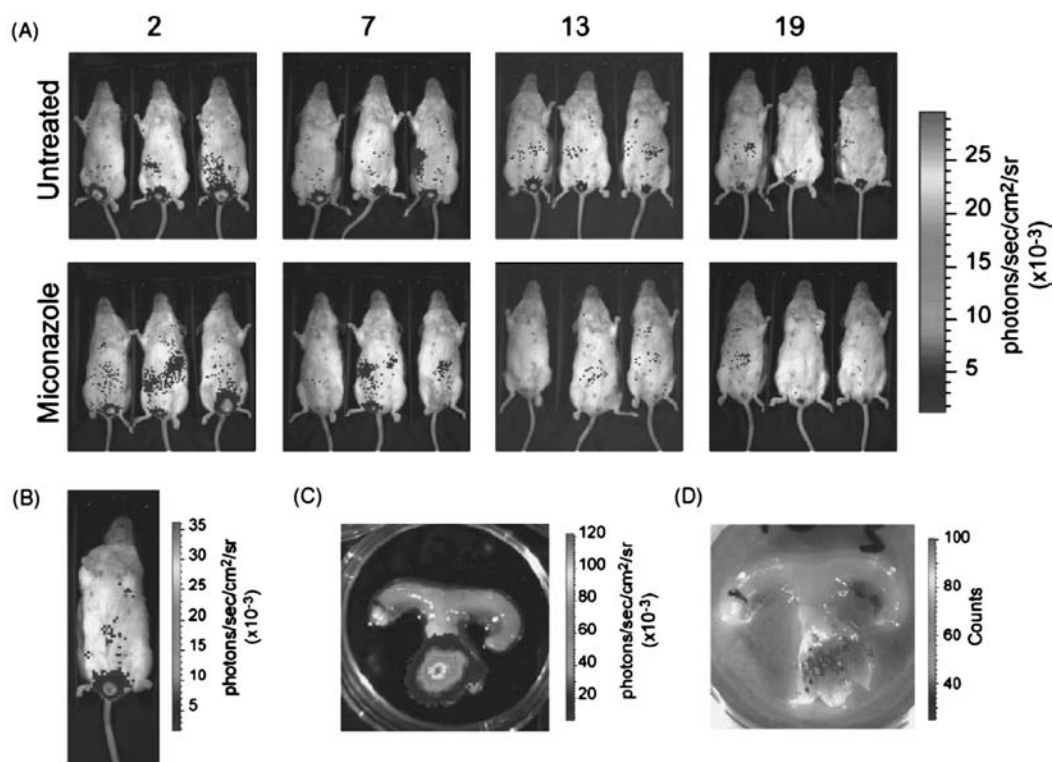


Figure 5 (See color insert) Bioluminescent *C. albicans* ATCC 90234 strain in a mouse vulvo-vaginal candidiasis model, with and without antifungal treatment. **(A)** Groups of three pseudo-estrous mice were infected in the vaginal lumen with approximately 5×10^5 CFU of *Candida*, and were imaged on subsequent days following anesthesia with 2–3% v/v isoflurane and vaginal lavage with 50mL 16mg/mL luciferin in PBS in the IVIS 100™ Imaging System, and representative images are shown at four different days postinfection. Groups of mice were treated with topical miconazole (lower group) or were left untreated as controls (*Top*). **(B)** Single untreated mouse imaged 30 days postinfection prior to end of experiment. **(C)** Excised vaginal/uterine tissue from mouse in **(B)** removed postmortem, the lumen opened to display the inner surface, and imaged directly after application of a luciferin solution. **(D)** Same vaginal/uterine imaged at high resolution with the close-up attachment lens.

CRYPTOCOCCOSIS

Animal models of cryptococcal meningitis and pneumonia are often examined to assess the efficacy of antifungal agents to these life-threatening diseases. Among the experimental animals, mice have been widely used to assess the efficacy of antifungal agents because their susceptibility to *Cryptococcus* and ability of using a large number of animals allow comparison of a variety of treatment regimens.

Mouse Cryptococcal Meningitis Model

Murine models of cryptococcal meningitis have been widely used to evaluate the efficacy of antifungal agents in the treatment of this disease. Murine models are used because (i) mice can be easily infected, (ii) disseminated cryptococcosis in the mouse produces meningoencephalitis in a manner similar to that developed in AIDS patients (Fig. 6), (iii) a large body of literature exists regarding antifungal therapy in this model, (iv) the mouse model is the best system for large-scale evaluation of anticryptococcal agents, (v) the mouse is the lowest member of the phylogenetic tree in which an infection can be produced that resembles human cryptococcosis, (vi) only small quantities of candidate compounds are needed for initial screening, and (vii) the mouse model is more economical with respect to purchase, per diem, and husbandry costs.

To deliver the cells to intracranial space, pericranial approach is employed. Under brief anesthesia, the animals are challenged intracranially with *C. neoformans* using a tuberculin syringe through a 27-gauge needle. The needle is pushed through the skull with a rotating

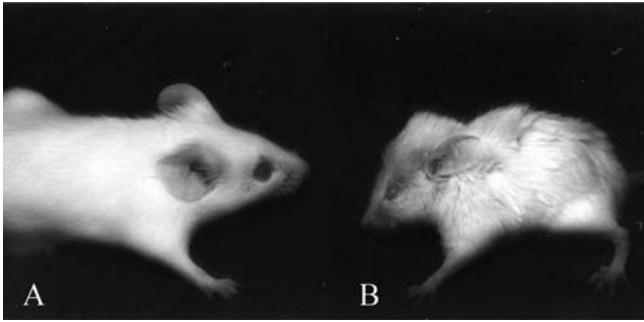


Figure 6 Intracranial model of Cryptococcal infection; (A) Healthy Mouse, (B) Infected mouse.

movement into the posterior half of the skull, about 6 mm lateral to mid-line in order to avoid the superior sagittal sinus. A sleeve attached to the needle guides the penetration of the needle and prevents too deep penetration of the needle, which could cause death of the animal (57). Several parameters, such as survival rate, fungal burden in the tissues (brain, CSF, lung, etc.), histopathology, and pharmacokinetics, are evaluated to assess the efficacy of antifungal agents.

Other Animal Models for Cryptococcal Meningitis

Rabbits (58), rats (59), and guinea pigs (60) have also been used to evaluate the efficacy of antifungals in the treatment of cryptococcosis. The advantage of using large animals (e.g., rabbits) include (i) reproducible infection which mimics the human infection in histopathology and response to treatment; (ii) ease of drug administration and withdrawal of body fluids, including CSF, which facilitate pharmacokinetics/pharmacodynamic studies; (iii) drug pharmacokinetics/pharmacodynamics can be studied concurrently with the efficacy studies; (iv) immunosuppression is an important feature of the model which is similar to many human cases of cryptococcal infection; (v) a fewer number of animals are required because this model has been relatively consistent and statistical comparison can be made with small numbers, (vi) it provides an alternative species for pharmacokinetics analysis, and (vii) comparison with most old and new antifungal agents is already available. However, since rabbits and guinea pigs are less susceptible to *C. neoformans*, they are treated by steroids to maintain the infection. *C. neoformans* cell suspension is inoculated intracisternally to these animals. In addition, guinea pigs can be infected via pericranial puncture (60).

Mouse Pulmonary Cryptococcosis

To establish a cryptococcal infection of the lungs, *C. neoformans* cell suspension is inoculated to the mouse either intratracheally or nasally. For the intratracheal infection, a 30–50 μL of cell suspension is placed in the trachea distal to the vocal cords, using a blunted 25-gauge needle followed by injection of 200 μL of air at the same site to disperse the instilled organisms (61). For the intranasal infection, a single droplet (50 μL) of yeast cell suspension is instilled on both nares (62). Gilbert et al. showed the time course of experimental murine model of cryptococcal pneumonia induced by intranasal fungal inoculation (62). Although the total number of organisms in the lungs is dependent on the size of the initial inoculum, the greatest increase in growth took place with smaller inocula during the first 7 to 10 days (62). Lungs appeared to become saturated with organisms by about two to three weeks, with most alveoli containing one or more yeast cells when examined histologically (62). At this time, cryptococcal cells could be found in other organs as well, especially the brain, liver, and kidneys (62). Over the four-week period, the level of cryptococcal cells in the lungs, brain, and liver with the two largest inocula used to challenge the animals increased 10- to 100-fold or more (62).

DERMATOPHYTOSIS

Guinea pigs have been used in in vivo models to assess the efficacy of antifungals in the treatment of dermatophytosis caused principally by the three fungal genera that belong to the dermatophytes, namely *Trichophyton*, *Microsporium*, and *Epidermophyton* (63).

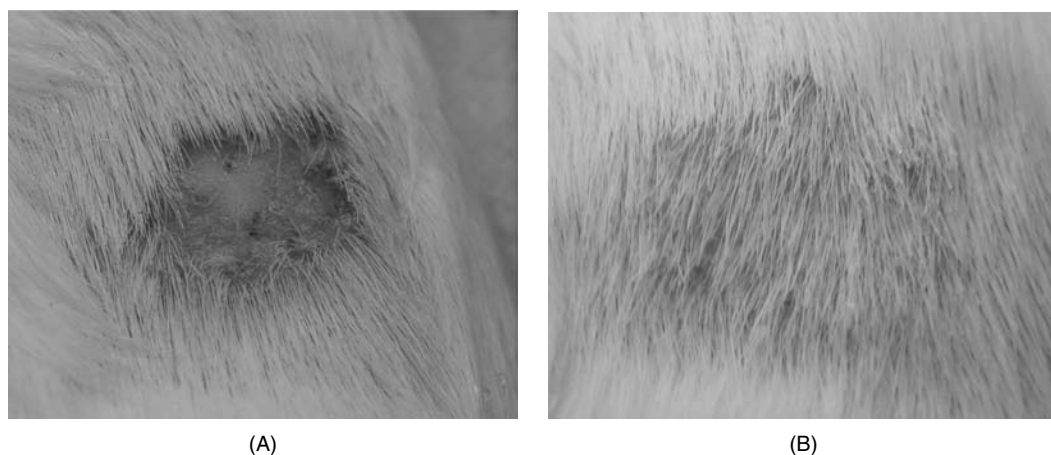


Figure 7 (A) Untreated showing: hair loss, redness, scaling and (B) Treatment showing: normal hair growth, no redness, no scaling.

Animals are anesthetized intramuscularly and an area of skin on the left side of the guinea pig's back is clipped, shaved, and a square drawn on the guinea pig. The marked area is abraded with sandpaper, and infected with a standardized suspension (1×10^7) of *Trichophyton mentagrophytes* conidia using a sterile pipette-tip and rubbed thoroughly. Animals can be treated topically or systemically. Both clinical and mycological criteria are used to evaluate the efficacy of potential antifungals. Clinical evaluation involves daily monitoring of changes in redness (mild, moderate, or severe), ulceration, scaling, or hair-loss at the site of inoculation. These signs are used in the clinical assessment of efficacy of different treatments and control regimens. Clinical efficacy is scored on a scale from 0 to 5 as follows: 0 = no signs of infection; 1 = few slightly erythematous areas on the skin; 2 = well-defined redness, swelling with bristling hairs, bald patches, scaly areas; 3 = large areas of marked redness, incrustation, scaling, bald patches, ulcerated in places; 4 = partial damage to the integument, loss of hair; and 5 = extensive damage to the integument and complete loss of hair at the site of infection (Fig. 7).

Mycological evaluation of efficacy (also known as the hair root invasion test) is used to assess mycological cure resulting from antifungal treatment. In brief, following clinical assessment, hairs are removed from each animal in the treatment and control groups, and then planted on the surface of potato dextrose agar Petri dishes. Following incubation, hairs showing fungal growth at the hair root are counted (Fig. 8). Mycological evaluation is based on the number of culture positive hair obtained from each animal.

In addition, the efficacy of an agent is assessed using histopathology analysis, where skin biopsy samples are obtained from a representative animal. Next, the tissue is fixed with 10% neutral buffered formalin, embedded in paraffin, and processed for histopathological examination. Fungal elements are visualized using Grocott Methenamine Silver (GMS) stain. Tissue is examined for the presence of fungal elements, inflammation, and tissue destruction using a light microscope.

NONINVASIVE MONITORING OF INFECTION IN LIVING ANIMAL MODELS

Animal models commonly used to evaluate efficacy of antimicrobial agents including antifungals utilize post mortal recovery of the infected tissue, homogenization, plating on agar plates, and counting CFUs. Such an approach requires a large number of animals to be sacrificed (which is frowned upon by animal review committees), and comparison of data sets from different groups of animals introduces unavoidable large variations. Moreover, these conventional models are expensive, time consuming, and labor intensive. Therefore, alternative models that use noninvasive approaches are encouraged.

One such approach is the use of noninvasive monitoring of infection in living animals using bioluminescent gene-tagged organisms (64,65). The usefulness of employing such a model

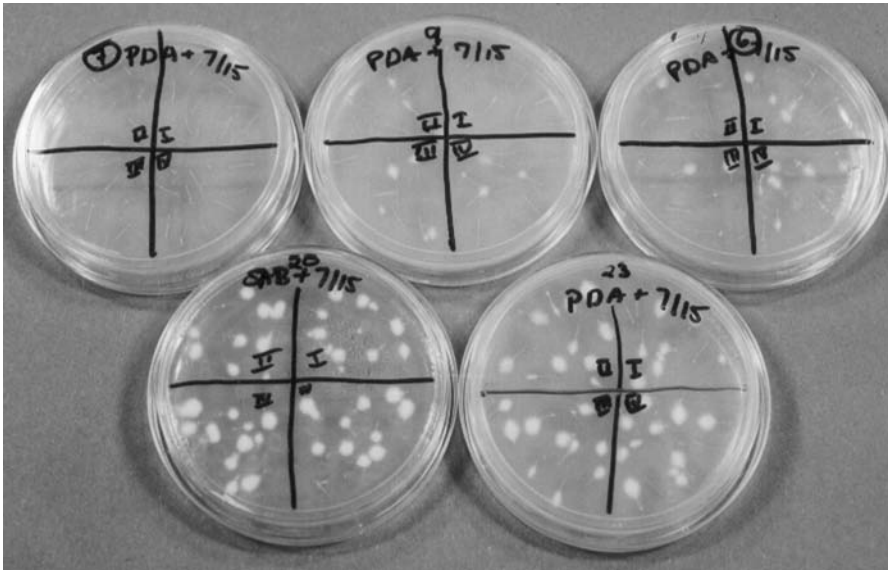


Figure 8 Hair root invasion test: Note the reduction of fungal growth in hairs obtained from the treated guinea pigs (top). Untreated controls showed abundant fungal growth (bottom) in the hair root when cultured in PDA plates.

with candidiasis has been demonstrated by Doyle et al., who used a *C. albicans* strain that functionally expresses the firefly luciferase in infected animals (15). *C. albicans* clinical isolates, which are stably transformed with a codon-optimized luciferase gene to constitutively express luciferase, are infected systemically or vaginally to mice. Mice infected with this luciferase-expressing strain are imaged following luciferin injection at a number of time points post infection using an IVIS 100™ CCD camera system. The efficacy of the antifungal drug miconazole was tested in this model, and clearance in animals was apparent by both direct imaging and fungal load determination (15).

INVERTEBRATE HOST

Invertebrate organisms have been increasingly used as “in vivo” assays for antifungal drug efficacy studies because of their low cost and simplicity (66) (Table 1). For example, *Drosophila melanogaster* have been used as *Aspergillus* (67) and *Candida* (68) infection models. *Drosophila* is infected with fungi either by injection, rolling, or ingestion methods. For injection assay, the dorsal side of the thorax of CO₂-anesthetized *Drosophila* flies is punctured with a thin sterile needle that is dipped in concentrated solutions of fungal cells. For rolling assay, CO₂-anesthetized *Drosophila* flies are rolled for two minutes on yeast extract glucose plates that contain a carpet of fungal cells. For ingestion assay, flies are placed in special fly-food vials containing yeast extract–peptone–dextrose agar medium, on which a lawn of fungal cells grew. Antifungal drugs-containing foods are given to the animals for the treatment (68).

Galleria mellonella (the greater wax moth) caterpillar is used for cryptococcal infection. A 10- μ L Hamilton syringe is used to inject 10 μ L of inoculum into the hemocoel of each caterpillar via the last left proleg. Antifungal drugs are injected using the same technique that was used for fungal challenge (69).

Silkworms have been used to evaluate the efficacy of antifungals in the treatment of *Candida* infections. Suspensions of *C. albicans* or *C. tropicalis* in Sabouraud dextrose medium are injected into the hemolymph through the dorsal surface of the silkworm. Antifungal drugs are injected into the hemolymph or by the intramidgut route (70).

Although these studies provide an alternative to using live animals, these invertebrate models have a number of limitations, which include neither drug levels being measured nor pharmacokinetic analysis undertaken. Although high-performance liquid chromatography (HPLC) analysis and bioassay methods are feasible in invertebrates, such studies are more

Table 1 Invertebrate Animal Models for the Study of Antifungal Agents

Invertebrate	Fungus	Infection	Treatment	Evaluation	Reference
<i>Drosophila melanogaster</i> (Oregon R fly, WT and Toll mutant)	<i>Aspergillus fumigatus</i>	Injection Rolling Ingestion	VRC via drug-containing food	Survival rate Tissue fungal burden (PCR) Tissue drug concentration (Bioassay) Histopathological and SEM analysis	67
<i>Drosophila melanogaster</i> (Oregon R fly, WT and Toll mutant)	<i>C. albicans</i> <i>C. parapsilosis</i> <i>C. krusei</i>	Injection Ingestion	FLC via drug-containing food	Survival rate Tissue fungal burden (CFU) Histopathology	68
<i>Galleria mellonella</i> (in the final instar larval stage)	<i>C. neoformans</i> <i>C. laurentii</i>	Injection	AMB, FLU and 5-FC via injection	Survival rate Tissue fungal burden (CFU)	69
Silkworms	<i>C. albicans</i> <i>C. tropicalis</i>	Injection	AMB and FLU via drug-containing food or injection	Survival rate	70

cumbersome, imprecise, and technically challenging in these models compared with mammal models (66). Furthermore, little is known regarding the metabolism and elimination pathway of drugs and potential for drug–drug interactions in mini-host models (66). However, despite these limitations, invertebrates are attractive models for the mass screening of candidate antifungal compounds that require subsequent validation in mammalian systems (66).

CONCLUSION

Animal models for evaluating the efficacy of antifungal agents in the treatment of various mycoses with demonstrated utility are available. Because of ethical concerns (advocating limiting the number of animals in each treatment), new alternative models, such as using bioluminescent pathogens, have been developed. Showing that a compound is effective *in vivo* is an important step in drug discovery since it shows the potential of activity in humans and identifies toxicity issues early. A compound that fails to demonstrate efficacy in animals is unlikely to show potency in humans. However, showing activity *in vivo* does not guarantee success in patients.

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4 Antifungal Drug Resistance: Significance and Mechanisms

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INTRODUCTION

Resistance to commonly used antifungals (e.g., azoles, polyenes, echinocandins, allylamines) is a significant problem in nosocomial infections (including invasive and superficial mycoses), as well as those associated with indwelling devices like central venous catheters, urinary catheters, and contact lenses (fungal keratitis). Fungal resistance has been reported even for newer antifungals, such as the echinocandins, underscoring the importance of gaining insight into the mechanisms of antifungal resistance. This chapter briefly describes the methods used to evaluate antifungal susceptibility of fungi, reviews the significance of antifungal resistance, and summarizes recent advances in identification of the underlying mechanisms.

METHODS USED TO EVALUATE ANTIFUNGAL SUSCEPTIBILITY IN VITRO

Antifungal agents are broadly categorized as fungistatic, which inhibit but do not kill fungi, and fungicidal, which kill fungal organisms. Common methods of evaluating in vitro antifungal susceptibility of fungi involve determination of the minimum inhibitory concentration (MIC), minimum effective concentration (MEC), or time-kill assay (1,2). Fungicidal agents are evaluated based on their minimum fungicidal concentration (MFC), which is the drug concentration that results in at least a 3-log (or 99.9%) reduction in colony-forming units (CFUs) compared to the starting fungal inoculum.

Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) of an antifungal agent is defined as the minimum concentration of the drug resulting in 80% (or 50% in some cases) inhibition of fungal growth relative to growth in the absence of the drug. MIC values for antifungal agents can be determined using microdilution, disk diffusion, or Epsilometer test (E-test) methods (see Table 1 for representative list of recent studies). Agents with low MICs are considered active, while those with higher MICs indicate reduced susceptibility of the organism and/or antifungal resistance (based on existing breakpoint guidelines for a given drug).

Microdilution Method

The most commonly used method to determine the MIC of antifungal agents is the microdilution-based method, in which a standardized number of organisms (e.g., 10^4 cells/mL) are exposed to serially diluted concentrations of the test agent in a 96-well format. The drug concentration resulting in 50% or 80% growth inhibition (compared to drug-free control well) represents the MIC of the agent against the tested organism. In studies testing large number of isolates, antifungal activity is commonly represented by MIC₉₀, or the concentration of drugs that inhibit growth of 90% of the isolates tested. The microdilution-based method is widely used, and standardized methods to determine MIC of antifungals against yeasts and molds (M 27A-3 and M 38-A2) have been developed and validated through the Clinical and Laboratory Standards Institute (CLSI) (3–5).

Table 1 Representative Studies Describing Use of Different Method to Evaluate Antifungal Susceptibilities

Testing method	Drugs tested	Organism	Study conclusion	Reference
Broth microdilution	Amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole and caspofungin	<i>Candida</i> spp.	Caspofungin was active against the majority of isolates	189
	Flucytosine, fluconazole, itraconazole, posaconazole, voriconazole and caspofungin	<i>Candida</i> spp.	In vitro susceptibility of 375 <i>C. albicans</i> isolates and biofilm production	190
	Voriconazole, posaconazole, and fluconazole	<i>Candida</i> and <i>Cryptococcus</i> spp.	Both voriconazole and posaconazole were more active than fluconazole against all <i>Candida</i> spp. and <i>C. neoformans</i>	191
Disk diffusion	Lemongrass oils and citral (main component of lemongrass oil)	<i>Candida</i> spp.	Lemongrass oil and citral have a potent in vitro activity against <i>Candida</i> spp.	192
	Fluconazole and voriconazole	<i>Candida</i> spp.	Voriconazole was very active in vitro against <i>C. glabrata</i> and <i>C. krusei</i>	193
	Fluconazole and voriconazole	<i>Cryptococcus</i> spp., <i>Saccharomyces</i> spp., <i>Trichosporon</i> spp., and <i>Rhodotorula</i> spp.	Voriconazole exhibits broad-spectrum against opportunistic yeast pathogens but has reduced activity against less common species	7
	Voriconazole	<i>C. krusei</i>	No evidence of increasing resistance of <i>C. krusei</i> to voriconazole from 2001 to 2005	71
	Ciclopirox, terbinafine, griseofulvin, fluconazole, itraconazole, posaconazole, and ravuconazole	<i>Trichophyton</i> spp., <i>Microsporium canis</i> , <i>Epidermophyton floccosum</i>	Correlation between microdilution and disk diffusion methods was variable	12
	Voriconazole, posaconazole, itraconazole, amphotericin B, and caspofungin	<i>Absidia corymbifera</i> , <i>Aspergillus</i> spp., <i>Alternaria</i> spp., <i>Bipolaris spicifera</i> , <i>Fusarium</i> spp., <i>Mucor</i> spp., <i>Paecilomyces lilacinus</i> , <i>Rhizopus</i> spp., and <i>Scedosporium</i> spp.,	Identified optimal testing conditions for mold disk diffusion testing	9

Table 1 Representative Studies Describing Use of Different Method to Evaluate Antifungal Susceptibilities (Continued)

Testing method	Drugs tested	Organism	Study conclusion	Reference
E-test	Ketoconazole and itraconazole	<i>Candida</i> spp.	Increase in <i>Candida</i> bloodstream infections were due to non- <i>albicans Candida</i> spp.	44
	Posaconazole	<i>Candida</i> spp.	Posaconazole exhibited excellent in vitro activity against <i>Candida</i> strains	15
	Voriconazole	<i>Trichophyton rubrum</i>	Voriconazole was the most and fluconazole was the less-active drug	194
	Posaconazole	<i>Candida</i> spp.	Disk diffusion zone diameters are highly reproducible and correlate well with both the E-test and the microdilution method	16

Disk Diffusion Assay

Disk diffusion assay, available as a standardized CLSI method (M-44A) for fluconazole susceptibility testing (6), involves placing disks that contain different concentrations of drugs on agar media plates (supplemented with 2% glucose and 0.5 μg of methylene blue per mL) seeded with the fungus. The plates are incubated for specific time periods (usually 24–48 hours); drug activity is indicated by clearance zone around the disks, and the zone diameter is measured to calculate the MIC. Disk diffusion method has been used in several studies (7–14) including the ARTEMIS DISK Global Antifungal Surveillance Study (7,13), which evaluated in vitro susceptibility of fluconazole by the CLSI M44-A disk diffusion method for thousands of *Candida* and non-*Candida* yeasts collected in 40 countries over a 10-year period (1997–2007).

Epsilometer Strip Test

The Epsilometer test (E-test; AB Biodisk, Solna, Sweden) is also used to determine the in vitro efficacy of antifungal agents. MICs are determined from the point of intersection of a growth inhibition zone with a calibrated strip impregnated with a gradient of antimicrobial concentration and placed on an agar plate lawned with the microbial isolate under test. Several studies have demonstrated good correlation between MIC values obtained using the E-test and broth macro/microdilution testing methods (15–21).

Minimum Effective Concentration

Antifungal susceptibility of *Aspergillus* and other filamentous fungi is often evaluated by determining the MEC, which is defined as the lowest drug concentration causing a morphological effect (e.g., abnormally swollen and highly branched hyphal tips, cells with distended balloon shapes, and stubby growth with thick cell walls) (22). The MEC assay is often related to inhibition of glucan synthase activity in vitro, especially for filamentous fungi, and is increasingly being used to determine echinocandins susceptibilities of filamentous fungi (23–25).

Time-Kill Assay

Time-kill assays are used to evaluate the effect of an agent on the rate and extent of antifungal activity over time, thus providing a measure of its in vitro pharmacodynamics (26–28). In this method, a standardized cell suspension (usually 5×10^5 cells/mL) is exposed to different concentrations of drug combinations for different time intervals. After a specified treatment time, cells are retrieved, plated onto agar medium, and incubated to allow growth. The number

of CFUs for each incubation time point per milliliter (CFUs/mL) are determined, and plotted as a function of time—resulting in “time-kill” curves for each drug combination tested.

EPIDEMIOLOGY OF ANTIFUNGAL RESISTANCE

Resistance Against Azoles

Historically, a large body of knowledge is available for resistance against azole antifungals, mainly because these are also the most commonly used antifungal agents. In particular, majority of resistance has been reported for fluconazole and itraconazole, while the newer third-generation triazoles (voriconazole, posaconazole, etc.) exhibit excellent activities against all *Candida* spp. Most cases of azole resistance are associated with prior exposure to the agent or infection with non-*albicans* *Candida* spp. Risk factor analyses have reported that prior surgery and systemic antifungal exposure were significantly associated with candidemia due to non-*albicans* *Candida* spp. and a potentially fluconazole-resistant *Candida* spp. (29–31).

Azole Resistance Among Candida Species

Azole resistance among *C. albicans* isolates is uncommon, but is known to occur. In a recent study, Li et al. (32) collected 21 *C. albicans* isolates from three HIV-infected patients over a period of three years and reported fluconazole resistance in five (24%) isolates, while four (19%) isolates exhibited dose-dependent susceptibility. Pulsed-field gel electrophoresis analysis of the isolates revealed that identical isolates were obtained for individual swab samples. Furthermore, for each patient, identical isolates were recovered at different time points. Based on these studies, these investigators associated long-term fluconazole therapy with development of fluconazole resistance.

Azole resistance has been reported to occur frequently among non-*albicans* *Candida* spp. International surveillance programs conducted in the late 1990s reported development of azole resistance among *C. glabrata* and *C. krusei* isolates obtained from patients in the United States, Canada, South America, and Europe (33–36). Similar trends in resistance have continued, as shown by more recent studies. For example, Richter et al. (37) performed susceptibility testing on vaginal yeast isolates collected from 429 patients with suspected vulvovaginal candidiasis (1998–2001), and reported resistance to itraconazole (MIC $\geq 1\mu\text{g/mL}$) among *C. glabrata* (74.1%), *C. krusei* (58.3%), *Saccharomyces cerevisiae* (55.6%), and *C. parapsilosis* (3.4%) isolates. In another study, Ruan et al. (38) evaluated the impact of *C. glabrata* fungemia on ICU patients over a five-year period (2000–2005), and reported that among 147 episodes of candidemia occurring in adult ICUs, *C. glabrata* was the second most common species (accounting for 30% episodes) and 11% of these isolates exhibited fluconazole resistance. Other recent studies have also pointed to the continued occurrence of fluconazole resistance among non-*albicans* *Candida* spp. such as *C. glabrata* (39–44), *C. tropicalis* (45–47), *C. dubliniensis* (48), *C. nivariensis* (49), *C. haemulonii* (50), *C. guilliermondii* (51). Borman et al. (49) reported that clinical isolates of the newly identified yeast species (*C. nivariensis*, which is genetically related to *C. glabrata*) are less susceptible than *C. glabrata* isolates to itraconazole, fluconazole, and voriconazole, and also have significantly higher flucytosine MICs than *C. glabrata* strains.

Some studies have suggested geographical distribution of antifungal resistance, although a conclusive association remains to be demonstrated. In this regard, Pfaller et al. (52) studied variation in species and strain distribution and antifungal susceptibility of 408 isolates of *Candida* spp. obtained from the National Epidemiology of Mycoses Survey (NEMIS), and reported variation in susceptibility to itraconazole and fluconazole: MIC_{90s} of itraconazole ranged from 0.25 $\mu\text{g/mL}$ in Texas to 2.0 $\mu\text{g/mL}$ in New York. Similarly, the MIC₉₀ for fluconazole was higher for isolates from New York (64 $\mu\text{g/mL}$) compared to the other sites (8–16 $\mu\text{g/mL}$). In a separate study, Pfaller et al. (53) evaluated azole susceptibility of *Candida* isolates obtained from bloodstream infections in the United States, Canada, and Latin America, and reported that isolates from Canada and Latin America were generally more susceptible to both triazoles than U.S. isolates. Arendrup et al. (54) reported surveillance results of fungemia in Denmark during the period 2004–2006, and showed that the number of isolates with reduced fluconazole susceptibility was >30% in 2006, with 12 (14%) *C. glabrata* isolates resistant to voriconazole in 2006. Tortorano et al. (55) performed an analysis of multi-institutional surveys of *Candida* BSIs

performed in Europe, including the large prospective survey by the European Confederation of Medical Mycology (2089 episodes from seven countries), and suggested limited role of species with decreased susceptibility to azoles in causing bloodstream infections and a low proportion of antifungal resistance in Europe. Although these studies indicate that antifungal resistance may be linked to specific regions or countries, more detailed investigations into this possibility need to be performed.

Azole Resistance Among Cryptococcus Isolates

Several studies have shown that *Cryptococcus neoformans* can develop resistance against azoles. Brandt et al. (56) used CLSI microdilution and E-test methodologies to determine antifungal susceptibilities of *C. neoformans* over a six-year period from 1992 to 1998 (56), and showed that although MIC ranges of fluconazole, itraconazole, flucytosine, and amphotericin B did not change during this time period for majority of the isolates, isolated incidences of resistance development were noted. For example, in serial isolates from seven patients (12% of all serial cases identified in the study) the increase in the MIC was at least fourfold, while for isolates from another patient there was a 32-fold decrease in the fluconazole MIC over a one-month period. Datta et al. (57) evaluated susceptibility of clinical isolates of *C. neoformans* against fluconazole and itraconazole at a tertiary care center in India using CLSI methodology, and showed that MIC₉₀ values for fluconazole and itraconazole was 16 and 0.125 µg/mL, respectively, with MIC/MFC ratios for fluconazole and itraconazole ≥1:32, indicating possible azole tolerance.

In an early study, Mondon et al. (58) reported isolation of *C. neoformans* isolates that exhibited unusual patterns of resistance to fluconazole and voriconazole from seven isolates from two different geographical regions: one isolate from an Israeli non-AIDS patient and six serial isolates from an Italian AIDS patient, who had suffered six recurrent episodes of cryptococcal meningitis. Each isolate produced cultures with heterogeneous compositions in which most of the cells were susceptible, but cells highly resistant to fluconazole (MICs ≥ 64 µg/mL) were recovered at a variable frequency. These investigators reported that “heteroresistance” phenotype was innate and unrelated to drug exposure since the Israeli patient had never been treated with azoles or any other antimycotic agents. However, in a subsequent study, Yamazumi et al. (59) evaluated fluconazole susceptibility among 107 clinical isolates of *C. neoformans* (MIC between 0.25 and 32 µg/mL), and showed that exposure to fluconazole can induce heteroresistance. Hsueh et al. (60) demonstrated that three *C. neoformans* isolates (4%) were resistant to fluconazole (MICs ≥ 16 µg/mL), while two (3%) isolates were resistant to 1 µg/mL of amphotericin B. Bii et al. (61) reported the existence of azoles resistance in *C. neoformans* isolates obtained from Kenya, and associated it with the increasing use of fluconazole in HIV-infected patients in the sub-Saharan Africa (11.2% resistant to fluconazole, 38.7% isolates resistant to itraconazole). Sar et al. (62) used E-test method to evaluate fluconazole susceptibility of *C. neoformans* isolates obtained from HIV/AIDS patients, who were given amphotericin B as initial therapy followed by fluconazole as maintenance therapy (2000–2002). These investigators showed that MIC₉₀ of fluconazole changed significantly during the test period, with the number of resistant isolates increasing from 2.5% to 14%, indicating development of resistance against this agent. Taken together, these studies clearly demonstrate that *C. neoformans* isolates can acquire resistance against azoles.

Azole Resistance Among Filamentous and Other Fungi

Although azole resistance is uncommon among *Aspergillus* isolates, recent reports suggest this trend may be changing. Panagopoulou et al. (63) showed that *Aspergillus* isolates obtained from a tertiary care center over a 12-month period exhibited relatively reduced susceptibility to itraconazole, voriconazole, and posaconazole. Hsueh et al. (60) reported that in a surveillance study in Taiwan, four (4.2%) of the *Aspergillus* isolates exhibited itraconazole MICs of 8 µg/mL. Rodriguez-Tudela et al. (64) suggested that *Aspergillus* spp. may demonstrate cross-resistance against azoles. Anecdotal evidence suggests a rise in zygomycosis in association with voriconazole use in immunosuppressed patients. Kontoyiannis et al. (65) performed prospective surveillance of patients with zygomycosis, invasive aspergillosis, and patients without fungal infection, combined with molecular typing and in vitro susceptibility testing of *Zygomycetes* isolates. These investigators found that all *Zygomycetes* isolates (74% of which belonged to the

genus *Rhizopus*) were resistant to voriconazole. However, the new azole, posaconazole exhibits excellent activity against *Zygomycetes*. A recent study reported isolation of *F. solani* isolates that showed high azole MICs (66).

Azole resistance has also been reported among other fungi (e.g., *Rhodotorula* spp., *Trichosporon* spp.) (67,68). For example, Diekema et al. (67) showed that *Rhodotorula* isolates collected in surveillance programs between 1987 and 2003 exhibited resistance to fluconazole (MIC₅₀ >128 µg/mL). However, all the new and investigational triazoles tested were active in vitro, with ravuconazole being the most active (MIC₅₀ = 0.25 µg/mL).

Resistance Against Flucytosine

Primary resistance to flucytosine has been reported for *C. albicans*, but more commonly for *C. krusei* or *C. tropicalis* isolates. Subsequent to the identification of genetically clonal clades of *Candida* isolates, resistance associated with clades was first demonstrated for flucytosine, and almost exclusively restricted to *C. albicans* isolates belonging to clade I (69). Pfaller et al. (70) used CLSI guidelines to evaluate primary resistance against flucytosine in >8000 clinical isolates of 18 *Candida* spp. obtained from more than 200 medical centers worldwide between 1992 and 2001. These investigators reported that while flucytosine was very active against most of the *Candida* isolates tested (92–100% of all species were susceptible), *C. krusei* isolates exhibited intrinsic resistance against this agent (only 5% of the *krusei* isolates were susceptible, MIC₉₀ = 32 µg/mL). In a more recent study describing the results of the ARTEMIS trial, Pfaller et al. (71) used the disk diffusion and broth microdilution methods to determine antifungal susceptibilities of clinical *C. krusei* isolates, and showed that while most isolates were susceptible to voriconazole or echinocandins, they exhibited decreased susceptibilities to amphotericin B (MIC₉₀ = 4 µg/mL) and flucytosine (MIC₉₀ = 16 µg/mL). In a recent survey of candidemia in Germany, a total of 25 isolates (4.5%), all of which were identified as *C. tropicalis*, showed resistance against flucytosine. Flucytosine resistance has also been reported for *C. neoformans*, as shown by Bii et al. (61) who demonstrated that 21% of *C. neoformans* isolates obtained from Kenya were resistant to flucytosine (MIC₉₀ = 64 µg/mL).

Resistance Against Polyenes

Amphotericin B Resistance Among Candida Isolates

Amphotericin B has a wide spectrum of activity against fungi, and resistance is relatively uncommon. However, incidences of resistance against this agent have been reported. Blignaut et al. (69) demonstrated amphotericin B resistance among *C. albicans* isolates belonging to the "SA" clade in South Africa. These investigators reported that 8.4% of South African oral yeast isolates were naturally resistant to amphotericin B. In another report, Pfaller et al. (71) used disk diffusion and broth microdilution to show that most *C. krusei* isolates tested in their study exhibited reduced susceptibilities to amphotericin B (MIC₉₀ = 4 µg/mL). Another case of amphotericin B resistance among non-*albicans* *Candida* isolates was recently demonstrated by Yang et al. (72), who showed that 16 of the 17 amphotericin B-resistant isolates obtained in a four-year period in Taiwan were non-*albicans* *Candida* spp. Similarly, Colombo et al. (73) reported resistance to amphotericin B (MIC ≥ 2 µg/mL) in 2.5% of isolates (two strains of *C. albicans*, two of *C. parapsilosis*, and one of *C. krusei*). In a separate study, Colombo et al. (74) reported that amphotericin B resistance in *C. rugosa* isolates obtained from an outbreak in six hospitalized patients in a tertiary care teaching hospital in Sao Paulo, Brazil.

Amphotericin B Resistance Among Aspergillus and Other Fungi

In recent years, several cases have been reported documenting amphotericin B among *Aspergillus* spp. *Aspergillus terreus* is intrinsically resistant to amphotericin B, and isolates of *A. ustus* and *A. lentulus* have been noted increasingly as causes of invasive aspergillosis in tertiary care centers in the United States (75,76). Panagopoulou et al. (63) showed that *Aspergillus* isolates obtained from a tertiary care center in Greece over a 12-month period exhibited reduced susceptibility to amphotericin B. Lass-Flörl et al. (77) evaluated the epidemiology and outcome of infections

due to *A. terreus* in Austria over a 10-year period, and showed that infections due to this fungus were associated with a lower response rate to amphotericin B therapy (20%), compared with 47% for patients with non-*A. terreus* infections ($P < 0.05$). Hsueh et al. (60) reported that *A. flavus* was less susceptible to amphotericin B, with MIC₅₀ of 1 µg/mL and MIC₉₀ of 2 µg/mL, which were twofold greater than those for *A. fumigatus* and *A. niger*. However, all *Aspergillus* isolates were susceptible to voriconazole, including isolates with reduced susceptibility to amphotericin B and itraconazole.

Although less common, amphotericin B resistance has been reported for other fungi also. Hsueh (60) showed in an earlier study that two (3%) isolates of *C. neoformans* were not inhibited by amphotericin B at 1 µg/mL. *Scedosporium prolificans* is intrinsically resistant to amphotericin B, and resistance to this drug has also been demonstrated among *S. apiospermum*, *Fusarium* spp., and *Sporothrix schenckii* (78,79).

Resistance Against Echinocandins

Although several surveillance studies have shown that echinocandin has broad spectrum activity against most fungi (80–82), smaller-scale studies and case reports have reported the occurrence of echinocandins resistance, especially among *Candida* isolates. One of the earliest reports demonstrating fungal resistance against echinocandins was by Laverdiere et al. (83), who reported progressive loss of cross-echinocandin activity (increased MICs of caspofungin, micafungin, anidulafungin) against four *C. albicans* isolates obtained at the initiation and during micafungin therapy from a patient with advanced HIV infection and chronic oesophagitis. Echinocandin resistance among *C. albicans* isolates is generally associated with esophageal candidiasis/HIV as the underlying disease, while among non-*albicans* isolates, the underlying conditions commonly noted are leukemia, transplants, and endocarditis. In a separate study, Forrest et al. (84) performed a five-year retrospective review of cases at a tertiary care center and reported significant correlations between increased caspofungin usage and an increased incidence of *C. parapsilosis* candidemia. Similarly, Pasquale et al. (85) recently reported echinocandin resistance in *C. tropicalis* isolates.

Although caspofungin, micafungin, and anidulafungin belong to the same class of compounds (echinocandins), their activity and efficacy potential can vary greatly. For example, Villareal et al. (86) showed that a *C. glabrata* isolate obtained from a patient who failed caspofungin was nonsusceptible to caspofungin (MIC = 8 µg/mL) while remaining susceptible to anidulafungin (MIC = 0.125 µg/mL). Other studies have also showed that the three echinocandins are not alike, when tested against *C. krusei* or *C. parapsilosis* (87–89). Moudgal et al. (89) evaluated the antifungal susceptibility of a series of *C. parapsilosis* isolates from a patient who failed caspofungin treatment, and showed that although the MICs increased for both caspofungin and micafungin, the isolates remained susceptible to anidulafungin. In an expanded study, our group (90) recently assessed in vitro activity of the three echinocandins and two triazoles (fluconazole and voriconazole) against 77 *C. parapsilosis* and 13 *C. albicans* isolates (obtained from patients, healthcare workers, and the hospital environment) using the CLSI M27-A2 method. It is found that *C. parapsilosis* isolates obtained from burn unit patients were more susceptible to anidulafungin than to caspofungin or micafungin, while isolates obtained from healthcare workers or environmental sources were susceptible to all antifungals examined. *C. albicans* isolates were susceptible to the antifungals tested. These studies clearly demonstrate that echinocandin cross-resistance occurs among *C. parapsilosis* isolates.

Wierman et al. (91) reported isolation of a set of clinical *C. glabrata* isolates from a patient with disseminated candidiasis who failed therapy with caspofungin and micafungin, but responded to amphotericin B and anidulafungin. Antifungal susceptibility testing showed that the *C. glabrata* isolates were cross-resistant to caspofungin and micafungin but susceptible to anidulafungin. In another study, Cota et al. (92) evaluated the activities of anidulafungin and caspofungin against 18 *C. glabrata* isolates with reduced caspofungin activity, and showed that isolates not susceptible to caspofungin were killed by anidulafungin, with MFC values ranging between 1 and 128 µg/mL for caspofungin compared to 0.25 and 8 µg/mL for anidulafungin.

Echinocandin resistance has also been reported for other fungi. *Aspergillus* isolates resistant to echinocandins has been associated with biofilm formation (discussed in later section) (93). In one recent study, Suzuki et al. (94) reported breakthrough cryptococcosis in a patient with systemic lupus erythematosus (SLE) receiving micafungin. Moreover, echinocandins have no activity against *Zygomycetes* or against *Trichosporon*, *Scedosporium*, and *Fusarium* spp. Therefore, resistance against echinocandins, while uncommon, has been reported in several cases, and more careful monitoring of echinocandin susceptibility among fungi is warranted.

Resistance Against Allylamines

Resistance against terbinafine, the commonly used allylamine, is rare. However, Mukherjee et al. (95) reported the first instance of terbinafine resistance in dermatophytes. The *in vitro* antifungal susceptibilities of six clinical *T. rubrum* isolates obtained sequentially from a single onychomycosis patient who failed oral terbinafine therapy (250 mg/day for 24 weeks) were determined by broth microdilution and macrodilution methodologies. Strain relatedness was examined by random amplified polymorphic DNA (RAPD) analyses. Data obtained from both broth microdilution and macrodilution assays were in agreement and revealed that the six clinical isolates had greatly reduced susceptibilities to terbinafine. The MICs of terbinafine for these strains were $>4 \mu\text{g/mL}$, whereas they were $<0.0002 \mu\text{g/mL}$ for the susceptible reference strains. Consistent with these findings, the minimum fungicidal concentrations (MFCs) of terbinafine for all six strains were $>128 \mu\text{g/mL}$, whereas they were $0.0002 \mu\text{g/mL}$ for the reference strain. The MIC of terbinafine for the baseline strain (cultured at the initial screening visit and before therapy was started) was already 4000-fold higher than normal, suggesting that this is a case of primary resistance to terbinafine. The results obtained by the broth macrodilution procedure revealed that the terbinafine MICs and MFCs for sequential isolates apparently increased during the course of therapy. RAPD analyses did not reveal any differences between the isolates. The terbinafine-resistant isolates exhibited normal susceptibilities to clinically available antimycotics including itraconazole, fluconazole, and griseofulvin. However, these isolates were fully cross-resistant to several other known squalene epoxidase inhibitors, including naftifine, butenafine, tolnaftate, and tolciclate, suggesting a target-specific mechanism of resistance.

MECHANISMS OF RESISTANCE

The antifungal activity of different classes of drugs is mediated by different mechanisms of action. Thus, the primary mode of action of azoles is inhibition of fungal ergosterol biosynthesis (by inhibiting lanosterol 14α -demethylase enzyme), while polyenes bind to ergosterol present in fungal membranes, leading to leakage of cellular components and cell death. Flucytosine is transported into the fungal cell, where it is deaminated to 5-fluorouracil, which is then phosphorylated and incorporated into RNA, resulting in inhibition of protein/DNA synthesis. Echinocandins inhibit synthesis of β -glucan, a key component of the fungal cell wall, resulting in the collapse of the fungal cell. Allylamines (e.g., terbinafine) bind squalene epoxidase thus inhibiting fungal ergosterol biosynthesis (see Refs. 96 and 97 for a detailed description of mode of action of different antifungals). Fungi can develop resistance against these antifungal agents using one or more mechanisms including reduced availability of the drug, alteration/complementation of drug target, etc. (Fig. 1). These mechanisms are described briefly below.

Mechanism of Azole Resistance

Major mechanisms mediating resistance against azoles involve alteration in membrane sterol composition, increased demethylase and sterol levels, reduction of azole permeability, efflux of the drug, modification in the target enzyme, and/or reduced access to the target.

Reduced Drug Permeability

Changes in membrane lipid composition and/or fatty acids have been suggested as mechanisms mediating azole resistance in *C. albicans* (98–102). Such changes can be induced by different mechanisms, including altered biosynthesis of membrane lipids (such as ergosterol and sphingolipid), binding to sterol biosynthesis enzymes (e.g., demethylase, sterol desaturase), and modulation of membrane transporters. The net effect of changes in membrane lipid

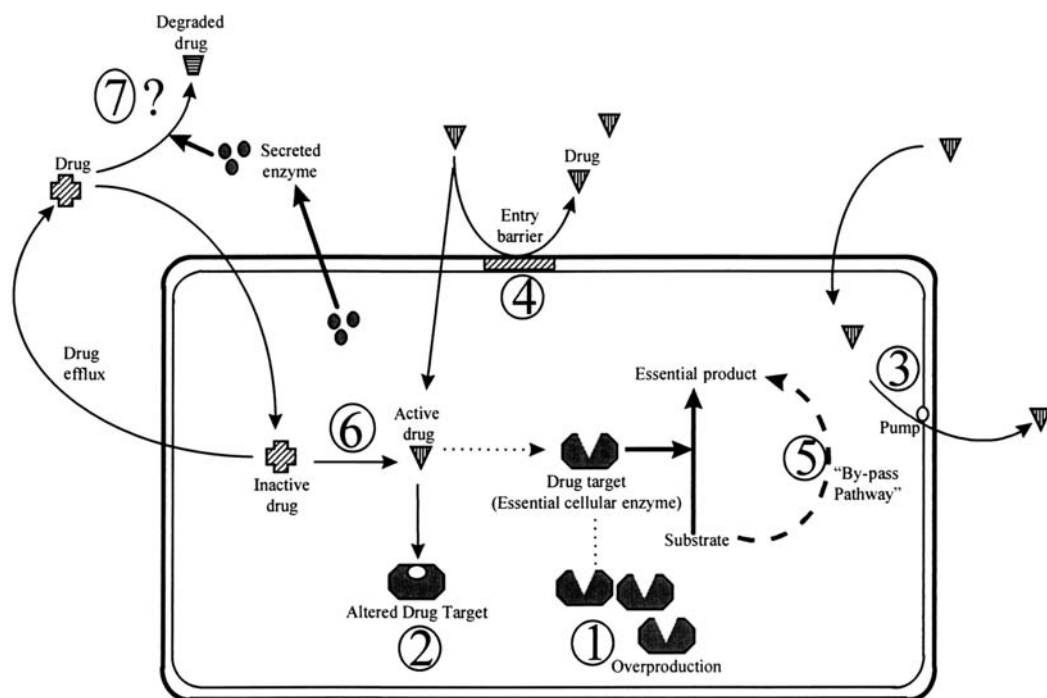


Figure 1 Mechanisms by which microbial cells might develop resistance. 1. The target enzyme is overproduced, so that the drug does not inhibit the biochemical reaction completely. 2. The drug target is altered so that the drug cannot bind to the target. 3. The drug is pumped out by an efflux pump. 4. The entry of the drug is prevented at the cell membrane/cell wall level. 5. The cell has a bypass pathway that compensates for the loss-of-function inhibition due to the drug activity. 6. Some fungal “enzymes” that convert an inactive drug to its active form are inhibited. 7. The cell secretes some enzymes to the extracellular medium, which degrade the drug. *Source:* Adapted from Ref. 96, reproduced with permission from American Society for Microbiology.

composition is to alter the membrane permeability, thus restricting the amount of azole that can enter the cell.

Mago and Khuller (98) showed that exposure to cerulenin (a specific inhibitor of fatty acid and sterol biosyntheses) inhibited growth and lipid synthesis in *C. albicans*, an effect that was reversed by exogenous addition of fatty acids. These fatty acid-supplemented cells contained altered levels of phospholipids and sterols, and were more resistant to miconazole, showing that alteration in fatty acid composition is a mechanism by which *C. albicans* is rendered resistant to azoles. Changes in sterol composition [e.g., replacing membrane ergosterol with fecosterol (99)] have been observed in azole-resistant *C. albicans* strains, and been proposed to be a mechanism of azole resistance in fungal cells. Furthermore, Hitchcock et al. (103) showed that in a *C. albicans* isolate resistant to both polyene and azole groups of antifungals, ergosterol was replaced by methylated sterol (lanosterol, 24-methylene-24,25-dihydrolanosterol, and 4-methylergostadiene-3-ol), which results in double resistance by preventing polyene binding and reducing azole permeability. Other studies have also provided evidence that cross-resistance to fluconazole and amphotericin B among *C. albicans* can be explained by similar defects in sterol $\Delta 5,6$ -desaturation (104). In a separate study, Kohli et al. (105) showed that *C. albicans* strains serially passed through increasing concentrations of fluconazole led to acquisition of resistance, overexpression of *CDR1* and *CDR2* genes, and alterations in membrane fluidity and asymmetry.

However, changes in membrane sterol composition cannot always explain azole resistance in fungal cells, as shown in different studies (106). In one such study, Hitchcock et al. (106) demonstrated that although the 14α -sterol demethylase enzyme in an azole-resistant *C. albicans* strain was less sensitive to a triazole than two azole-sensitive strains, there was no direct correlation between the IC_{50} values for triazole inhibition of the demethylase and IC_{50} values

for growth, and suggested that the basis of azole resistance in this strain may be linked to altered or absent azole targets. Lamb et al. (107) separately showed that azole resistance in *C. albicans* strain NCPF 3363 was associated with reduced intracellular accumulation of drug and not reduced affinity for the target site.

Alteration in Target Enzymes

Azole resistance has also been associated with alteration in activity of cytochrome P450-dependent 14α -demethylase and that of other ergosterol biosynthesis enzymes, such as $\Delta 5-6$ desaturase (108–111). In this regard, Vanden Bossche et al. (112) demonstrated increased microsomal cytochrome P450 content and subcellular ergosterol synthesis from mevalonate or lanosterol in azole-resistant *C. glabrata*, indicating that the level of P450-dependent 14α -demethylation of lanosterol was higher in these cells, and contributed to resistance. Azole resistance in *C. krusei* has been linked to reduced susceptibility of 14α -demethylase because of reduced binding affinity (113). Overexpression of CYP51A1 in *C. albicans* and *C. glabrata* may also account for a decreased susceptibility to azole antifungal agents (114).

Accumulation of Toxic Intermediates

Inhibition of sterol biosynthesis pathway can also result in accumulation of intermediates that are toxic to the fungal cells. In this regard, Marichal et al. (101) showed that two azole-resistant *C. albicans* isolates (C48 and C56) overexpressed efflux pumps and contained increased intracellular levels (20–30%) of 14α -methyl-ergosta-8,24(28)-diene- $3\beta,6\alpha$ -dio 1 (3,6-diol). Itraconazole treatment of C43 resulted in a dose-dependent inhibition of ergosterol biosynthesis and accumulation of 3,6-diol (up to 60% of the total sterols), eburicol, lanosterol, obtusifolol, 14α -methyl-ergosta-5,7,22,24(28)-tetraene-3betaol, and 14α -methyl-fecosterol. These investigators also showed that itraconazole exposure led to increased levels of obtusifolione, a toxic 3-ketosteroid earlier shown to accumulate after itraconazole treatment in *C. neoformans* and *Histoplasma capsulatum*, and that obtusifolione accumulation correlated with inhibition of growth of these azole-resistant strains.

Both reduced permeability and activity of demethylase have been suggested to explain azole resistance among *A. fumigatus* isolates (115,116). Denning et al. (116) demonstrated at least two mechanisms of resistance to be responsible for itraconazole resistance in three clinical isolates of *A. fumigatus* (AF72, AF91, and AF92) obtained from two patients. These investigators showed that isolate AF72 had reduced ergosterol content, greater quantities of sterol intermediates, a similar susceptibility to itraconazole in cell-free ergosterol biosynthesis, and a reduced intracellular itraconazole concentration. In contrast, isolates AF91 and AF92 had slightly higher ergosterol and lower intermediate sterol concentrations, fivefold increased resistance in cell-free systems to the effect of itraconazole on sterol 14α -demethylation, and intracellular itraconazole concentrations found in susceptible isolates. These studies showed that at least two mechanisms—one involving reduced permeability of itraconazole and the other due to a more direct effect on enzyme activity—were mediating resistance in these isolates. In a subsequent study, Manavathu et al. (115) evaluated itraconazole susceptibility of two resistant *A. fumigatus* isolates, and showed that intracellular accumulation of itraconazole in azole-resistant isolates was reduced by up to 80% compared to the susceptible parent, suggesting that the reduced accumulation of itraconazole is more likely associated with diminished drug permeability and not with drug efflux. Moreover, the respiratory inhibitor carbonyl cyanide *m*-chlorophenyl hydrazone reduced the intracellular accumulation of itraconazole by around 36% in the parent and in the mutant strains, demonstrating that uptake of itraconazole in *A. fumigatus* is an energy-dependent process.

Recently, Willger et al. (117) showed that azole resistance in *A. fumigatus* is modulated by a sterol-regulatory element-binding protein, SrbA. A mutant strain lacking this protein SrbA was hyper-susceptible to fluconazole and voriconazole, suggesting that SrbA plays a role in modulating fungal susceptibility to azoles, most likely by regulating ergosterol biosynthesis.

Modification of Drug Target: Mutation and Overexpression

Mutations in drug target have been associated with azole resistance in several studies. Marichal et al. (118) identified mutations in cytochrome P450 14α -demethylase (Erg11p, Cyp51p) that play critical roles in azole resistance among fungi. Xu et al. (119) evaluated the relationship

between mutations in the *ERG11* gene of 15 fluconazole-resistant and 8 fluconazole-susceptible *C. albicans* isolates obtained from non-AIDS patients, and demonstrated 18 silent mutations and 19 missense mutations. These investigators showed that six missense mutations occurred in resistant isolates: G487T (A114S), T916C (Y257H), T541C (Y132H), T1559C (I471T), C1567A (Q474K), and T1493A (F449Y), of which the first four are known to contribute to fluconazole resistance, while the role of the last two have not been investigated.

Modification of the target enzyme is a common mechanism identified for azole resistance in *Aspergillus* spp., primarily through amino acid substitutions in the drug target Cyp51 (encoding 14 α -lanosterol demethylase) (120–122). In azole resistant *A. fumigatus* isolates, the most frequent amino acid substitutions occur at the positions Gly 54, Gly 138, Met 220, and Leu 98, coupled with a tandem repetition in the gene promoter (64,120,121). Other amino acid substitutions identified in Cyp51 genes of voriconazole-resistant isolates include N22D and M220I in *A. fumigatus* (122). Among resistant *A. flavus* isolates commonly identified substitutions include K197N, Y132N, T469S, K197N, D282E, and M288L (123). Another mechanism by which fungi become resistant is by expressing multiple copies of the drug target. An example of this approach is evident in the study by Oshero et al. (124), who showed that *A. nidulans* and *A. fumigatus* isolates that are resistant to itraconazole induce overexpression of the P450 14 α -demethylase gene.

Transporter-Mediated Drug Efflux

A major mechanism of azole resistance is induction or overexpression of drug efflux pumps (*Candida* drug resistance, CDR) and transporters (major facilitator superfamily, MFS), which mediate clearance of the drug from fungal cells. Several studies have demonstrated that drug efflux mediates resistance to azoles (125–129). Prasad et al. (125) cloned and sequenced the *C. albicans* *CDR1* gene, and showed that transformation of a hypersensitive *S. cerevisiae* strain with *CDR1* resulted in resistance to miconazole, cycloheximide, and chloramphenicol. Role of multiple efflux mechanisms in azole resistance was also shown by Albertson et al. (130), who demonstrated that a set of fluconazole-resistant *C. albicans* strains contained elevated amounts of *CDR1* mRNA, and some of these isolates also contained increased amounts of mRNA encoding Ben^r, an MFS transporter. These studies suggested that fluconazole resistance may involve energy-dependent drug efflux associated with increased expression of Ben^r and/or Cdr1.

Different studies have demonstrated that multiple mechanisms can be operative in fungal cells and contribute to azole resistance in *C. albicans* (131–133). White (131) evaluated mRNA levels in a series of 17 clinical isolates taken from a single HIV-infected patient over two years, during which time the levels of fluconazole resistance of the strain increased over 200-fold. These investigators reported increased mRNA levels of *ERG16* (which encodes the 14 α -demethylase enzyme), *CDR1*, and *MDR1* in this series, which correlated with increases in fluconazole resistance of the isolates. In a second study, Franz et al. (132) reported the isolation of five *C. albicans* isolates from two AIDS patients with oropharyngeal candidiasis, from recurrent episodes of infection that became gradually resistant against fluconazole during treatment. Isolates from patient 1 exhibited enhanced expression of *MDR1* and constitutively high expression of *ERG11*, which correlated with a stepwise development of fluconazole resistance. In the isolates from patient 2, increased *MDR1* mRNA levels and the change from heterozygosity to homozygosity for a mutant form of the *ERG11* gene correlated with continuously decreased drug susceptibility, reduced drug accumulation, and increased resistance in activity of sterol 14 α -demethylase. Exposure of cells to fluconazole can also induce expression of *CDR1*, which can contribute to development of azole resistance (134).

Sanglard et al. (135) showed that *C. glabrata* *CDR1* (Cg*CDR1*) is involved in the resistance of clinical isolates to azole antifungal agents (135), while Torelli et al. (136) recently implicated upregulation of another ATP-binding cassette transporter, CgSNQ2, in azole resistance among *C. glabrata* isolates. Furthermore, Thakur et al. (137) showed that a nuclear receptor-like pathway regulates multidrug resistance in *C. glabrata*.

In a separate study, Katiyar and Edlind (138) showed that in azole-resistant *C. krusei* cells, expression of two ATP cassette-binding (ABC) transporters (*ABC1* and *ABC2*) increased at stationary phase. This increase correlated with decreased susceptibility to miconazole. Furthermore, *ABC1* was upregulated following a brief treatment of *C. krusei* with miconazole and

clotrimazole (but not other azoles), and the unrelated compounds albendazole and cycloheximide. The latter two compounds antagonized fluconazole activity versus *C. krusei*, supporting a role for the *ABC1* transporter in azole efflux. Finally, miconazole-resistant mutants selected in vitro demonstrated increased constitutive expression of *ABC1*. Based on these expression data, genetic and functional characterization of the *ABC1* transporter to directly test its role in *C. krusei* azole resistance would appear to be warranted. Molecular mechanisms of azole resistance in *C. dubliniensis* include increased drug efflux, modifications of the target enzyme, and alterations in the ergosterol biosynthetic pathway (48,139).

Efflux pumps have been shown to contribute to drug resistance in *Aspergillus* and *Cryptococcus* spp. Overexpression of efflux pumps play a critical role in itraconazole resistance among *A. fumigatus* isolates, with *Afumdr3*, *Afumdr4*, and *AtrF* playing critical roles, especially in the early stages of resistance acquisition (122,140,141). Posteraro et al. (142) cloned and sequenced an ABC transporter-encoding gene, *C. neoformans* AntiFungal Resistance 1 (*CnAFR1*), from a fluconazole-resistant *C. neoformans* isolate, and demonstrated that the isogenic knock-out mutant *cnaf1* (in which the *CnAFR1* gene was disrupted) was highly susceptible to fluconazole, while reintroduction of the functional gene in *cnaf1* resulted in restoration of the resistance phenotype. These studies clearly showed that efflux pumps play important roles in drug resistance among different fungal species.

Mechanism of Polyene Resistance

Altered Membrane Lipid Composition

Much of amphotericin B resistance is related to changes in sterols in the cell membranes since ergosterol is the molecule interacting with this drug. Such changes include decreased ergosterol production and altered ergosterol products caused by mutation in the sterol biosynthesis pathway.

Walsh et al. (143) showed that amphotericin B resistance in *A. terreus* is linked to decreased levels of membrane ergosterol. These investigators used a persistently neutropenic rabbit model of invasive pulmonary aspergillosis due to *A. terreus* and *A. fumigatus*, and investigated possible mechanisms of resistance in *A. terreus* using microbicidal time-kill assays, colorimetric MTT assays of hyphal damage, and sterol composition analysis of the fungal cell membrane by gas-liquid chromatography (GLC). Both time-kill and MTT assays showed that *A. terreus* was resistant to the fungicidal effects of amphotericin B. Membrane sterol composition analysis revealed that the amphotericin B-resistant *A. terreus* contained reduced ergosterol levels (20.3%), and increased levels of zymosterol (17.1%) and squalene (17.5%). These investigators suggested that the depletion of ergosterol in the amphotericin B-resistant *A. terreus* contributes substantially to diminished binding of amphotericin B to the cytoplasmic cell membrane, resulting in polyene resistance. The substituted nonergosterol cytoplasmic membrane sterols and lipids (e.g., zymosterol and squalene) may have further reduced affinity for AmB, resulting in diminished binding. These studies indicated that amphotericin B resistance in *A. terreus* isolates is likely due to reduction in membrane ergosterol levels. In a separate study, defective Δ -8,7 isomerase was found to be associated with decreased intercalation of the drug with the membrane, resulting in amphotericin B resistance in *C. neoformans* (78,144–146).

Modifications in Drug Target and Binding

Amphotericin B resistance can also result from alteration in the drug's target. In a recent study, Vandeputte et al. (147) recently reported that a missense mutation in *ERG6* gene (Cys–Phe substitution) correlated with reduced polyene susceptibility (determined using disk diffusion method) of a clinical *C. glabrata* isolate that grew as pseudohyphae. This isolate lacked ergosterol and accumulated late sterol intermediates, indicative of a defect in the final steps of the ergosterol pathway. Functional complementation of the mutation restored susceptibility to polyenes and a classical morphology, demonstrating the role of *ERG6* in amphotericin B in *C. glabrata*.

Ikeda et al. (148) suggested that melanin, a virulence factor in *C. neoformans*, also mediates antifungal resistance. These investigators induced melanin formation by growing laccase-active strains of *C. neoformans* and *C. albidus* in L-DOPA, and observed no change in MIC of

amphotericin B and fluconazole for these cells. However, live cells were detected in wells containing amphotericin B-inhibited cells, and contained melanin. In contrast, melanization did not protect *C. albicans* from being killed by amphotericin B. Time-kill analysis of the effect of amphotericin B on *C. neoformans* revealed that higher number of melanized cells survived in the first few hours than nonmelanized cells. Binding studies suggested that melanin in the cell walls binds amphotericin B, thus reducing its effective concentrations and consequently minimizing exposure of *C. neoformans* cells to this agent.

Zaragoza et al. (149) showed recently that enlargement of the polysaccharide capsule of *C. neoformans* resulted in protection against resistance to reactive oxygen species (ROS) induced by catalase-independent hydrogen peroxide, suggesting that the capsule can act as a scavenger of ROS, thus protecting the cells from phagocytosis. Interestingly, these investigators reported that capsule enlargement also conferred resistance to amphotericin B.

Mechanism of Flucytosine Resistance

Mechanism of flucytosine resistance in fungal cells is well documented, and is commonly mediated by modification in cytosine permease and ribosyl transferase activities (150–153). Additional mechanisms include failure to metabolize flucytosine to 5FUTP and 5FdUMP, or from the loss of feedback control of pyrimidine biosynthesis (114,154). The homozygous resistant strain *fcy1/fcy1* (lacking functional UMP pyrophosphorylase) was associated with decreased UMP pyrophosphorylase activity that resulted in poor conversion from 5-flucytosine to FUMP, whereas resistance in *fcy2/fcy2* strains was associated with decreased cytosine deaminase activity (155,156). Hope et al. (153) evaluated flucytosine resistance mechanisms in 25 *C. albicans* strains by identifying and sequencing the genes *FCA1* (encoding cytosine deaminase), *FUR1* (encoding uracil phosphoribosyltransferase; UPRT), *FCY21* and *FCY22* (encoding two purine–cytosine permeases). These investigators showed an association between a polymorphic nucleotide and resistance to flucytosine within *FUR1* (with a C301T nucleotide substitution), which resulted in R101C substitution in UPRT. A single resistant isolate, lacking this *FUR1* polymorphism, contained instead a homozygous polymorphism in *FCA1* that resulted in a G28N substitution in cytosine deaminase. Single nucleotide polymorphism has also been linked to clade-specific resistance in *C. albicans* clades. In this regard, Dodgson et al. (157) evaluated flucytosine resistance patterns in *C. albicans* clades and showed that a single nucleotide change (C301T) in *FUR1* can lead to flucytosine resistance in clade I isolates. The flucytosine MICs for strains with no copies, one copy, and two copies of the mutant allele were ≤ 0.25 , > 0.5 , and > 16 $\mu\text{g}/\text{mL}$, respectively. Vlant and Diallinas (158) recently cloned and characterized the *A. nidulans fcyB*, encoding the closest homologue to the yeast *Fcy2p/Fcy21p* permeases. A *fcyB* null mutant lacked all known purine transporters, and was resistant to flucytosine. These investigators showed *FcyBp* to be a low-capacity, high-affinity, cytosine–purine transporter, with scavenging of cytosine–purine as its main function. In a recent study, Papon et al. (159) showed that inactivation of the *FCY2*, *FCY1*, and *FUR1* genes in *C. lusitaniae* produced two patterns of resistance to flucytosine. Mutant *fur1* demonstrated resistance to 5-fluorouracil, whereas mutants *fcy1* and *fcy2* demonstrated fluconazole resistance in the presence of subinhibitory flucytosine concentrations.

Flucytosine–fluconazole cross-resistance has also been reported (159,160). In one such study, Noel et al. (160) demonstrated that the cross-resistance involved a fluconazole uptake transporter in purine–cytosine permease-deficient *C. lusitaniae* clinical isolates. Genetic analyses showed that resistance to flucytosine was derived from a recessive mutation in a single gene, whereas cross-resistance to fluconazole seemed to vary like a quantitative trait. Kinetic transport studies with flucytosine showed that flucytosine resistance was due to a defect in the purine–cytosine permease.

Mechanism of Allylamine Resistance

Resistance against allylamines (e.g., terbinafine) is mostly due to changes in the squalene epoxidase enzyme, which catalyzes the conversion of squalene to epoxysqualenes in the ergosterol biosynthesis pathway (161).

Modification of Drug Target: Overexpression and Mutation

Expression of increased number of copies of squalene epoxidase had been shown to lead to terbinafine resistance in *Aspergillus* isolates (162). Liu et al. (162) identified the gene responsible for terbinafine resistance as the *A. fumigatus* squalene epoxidase gene (*ERG1*). In a separate study, Rocha et al. (163) showed that a F389L substitution in *ergA* confers terbinafine resistance in *Aspergillus*. Osborne et al. (164) characterized a new clinical strain of *T. rubrum* highly resistant to terbinafine and showed that resistance to terbinafine in this strain is caused by a missense mutation in the squalene epoxidase gene leading to the amino acid substitution F397L.

Degradation of Drug

Graminha et al. (165) demonstrated that terbinafine resistance in UV-induced *A. nidulans* mutants was mediated by salicylate 1-monooxygenase (*salA*), a naphthalene-degrading enzyme, since transformation of sensitive strain with this gene rendered it resistant. Moreover, *salA* transcript accumulation analysis showed terbinafine-dependent induction in the wild-type strain. These investigators suggested that terbinafine resistance in the resistant isolate could be due to degradation of the naphthalene ring contained in terbinafine.

Mechanism of Echinocandin Resistance

Echinocandin resistance can be mediated by mutations in the *FKS1* gene, adaptive or lower level drug tolerance, and stimulation of chitin synthase gene (166).

Modification in Drug Target: Mutations and Altered Substrate

Mutations in two distinct *FKS1* regions, Hotspot 1 (HS1) and Hotspot 2 (HS2), have been linked to echinocandin resistance. The region around Ser645 (within HS1) is considered to be the major contributor to echinocandin resistance, with the highest frequency of substitution. Amino acid substitutions in HS1 and HS2 were evaluated following DNA sequence analysis of *FKS1* genes from susceptible and resistant *Candida* spp. (167). In a recent study, Garcia-Effron et al. (168) reported that a naturally occurring Fks1p P600A substitution (immediately distal to the hot spot 1 region) was responsible for reduced echinocandin susceptibility of *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis*.

Although mutations in the *FKS1* gene are common mechanisms, all cases of echinocandin resistance cannot be explained by this phenomenon. For example, our analysis of echinocandin cross-resistant *C. parapsilosis* isolates (90) showed that although anidulafungin and caspofungin possess equivalent activity against the caspofungin-susceptible *C. parapsilosis* strain, they differed in their ability to damage the caspofungin nonsusceptible strain [cellular damage and distortion of morphology was induced by lower concentrations of anidulafungin (1 $\mu\text{g}/\text{mL}$) than that of caspofungin (16 $\mu\text{g}/\text{mL}$)]. To determine whether the nonsusceptibility of *C. parapsilosis* isolates to caspofungin could be due to mutations in the *FKS1* gene, the sequence of the 493bp portion of this gene associated with echinocandin resistance was compared, and no differences were found in the amino acid pattern within the targeted region. Therefore, differences in the activity between anidulafungin and the other echinocandins cannot be attributed to mutations within the *FKS1* gene (90), and the observed nonsusceptibility to caspofungin is due to some other mechanism.

Some studies have suggested that modulation of echinocandin susceptibility is associated with differential expression of overlapping set of genes involved in *FKS* regulation, compensatory chitin synthesis, protein mannosylation, and the protein kinase C1 (Pkc1)-dependent cell integrity pathway (169,170). Oshero et al. (171) proposed overexpression of *SBE2* (which encodes Sbe2p, a Golgi protein involved in the transport of cell wall components) as an adaptive mechanism of resistance against echinocandins. These investigators showed that overexpression of Sbe2p resulted in caspofungin resistance in *S. cerevisiae*, and that deletion of *SBE2* rendered the yeast hypersensitive to caspofungin, thus showing that overexpression of Sbe2p imparts caspofungin resistance.

Recently, Walker et al. (172) reported that treatment of *C. albicans* with low levels of echinocandins stimulated expression of the gene encoding chitin synthase (CHS), increased

activity of this enzyme, elevated chitin content, and rendered the cells less susceptible. The role of substrate availability in echinocandin resistance is also underscored by the study performed by Feldmesser et al. (173), who showed that inactivity of caspofungin against *C. neoformans* is largely due to difference in glucan structure in this fungus, which contains both 1,3 β -D-glucan and 1,6 β -D-glucan in the cell wall.

Drug Efflux

Echinocandins are poor substrates for most multidrug efflux transporters, and several studies have argued against the role of drug efflux pumps in echinocandin resistance (174–176). However, one study showed that efflux pumps were upregulated in azole–echinocandin cross-resistant isolates (177). Therefore, it is likely that more than one mechanism of resistance could be operative in such cross-resistant isolates. For example, it is possible that mutation in *FKS1* and overexpression of efflux pumps may be operational in the azole–echinocandin cross-resistant isolates, thereby accounting for the broad cross resistance.

Biofilm Formation as a Mechanism of Resistance

Fungal cells including *Candida*, *Cryptococcus*, *Aspergillus*, and *Fusarium* spp. have been shown to form biofilms on surfaces such as catheters, dentures, contact lenses, and wells of microtiter plates (93,178–182). Biofilms are communities of cells encased in self-produced extracellular matrix (ECM), and are characterized by resistance against commonly used antifungal agents as well as common biocides (178,179,183), prompting the notion that growth as a biofilm also represents a mechanism by which fungi become drug resistant. This section briefly summarizes the mechanisms by which fungal biofilms are rendered drug resistant.

Phase-Dependent Mechanisms Mediate Drug Resistance in Fungal Biofilms

Ramage et al. (184) reported that efflux pumps including Cdr1p, Cdr2p, and Mdr1p were not involved in drug resistance associated with mature *C. albicans* biofilms formed on 96-well microtiter plates. In a subsequent study, Mukherjee et al. (185) compared the mechanism of antifungal resistance in biofilms at early and mature phases. These investigators showed that in early phase biofilms, efflux pumps contributed to antifungal resistance, while in mature phase biofilms, resistance was associated with changes in levels of ergosterol biosynthesis intermediates. The role of efflux pumps in biofilm-associated resistance was confirmed in a separate study by Mateus et al. (186), who showed that expression of *MDR1* and *CDR1* genes was significantly lower in daughter cells from 48-hour biofilms than in firmly adherent cells (two hours after attachment), demonstrating that efflux pump expression in adherent cultures is transient. These studies clearly demonstrated that antifungal resistance in *Candida* biofilms is due to multiple mechanisms in a phase-dependent manner.

Role of Capsule in Biofilm Resistance

Recent studies have characterized the role of biofilm formation in drug resistance profile of *C. neoformans* (187,188). Martin and Casadevall (188) showed that while exposure of *C. neoformans* to amphotericin B or echinocandin prevented biofilm formation, fluconazole, or voriconazole did not have any effect on biofilm-forming ability of this organism. Interestingly, *C. neoformans* biofilms exhibit reduced susceptibility to host antimicrobial peptides, amphotericin B, and caspofungin than planktonic cells, and the presence of melanin in fungal cells resulted in further reduction of susceptibilities to these drugs (187,188). *C. neoformans* biofilms were found to be susceptible to amphotericin B and caspofungin at concentrations >2 and $16 \mu\text{g/mL}$, respectively, but resistant to fluconazole and voriconazole. It is notable that although amphotericin B and caspofungin reduced biofilm formation by *C. neoformans* cells, the concentrations used were high and were above the levels achievable in vivo after systemic administration.

CONCLUSIONS

Antifungal resistance is mediated by a variety of mechanisms, which vary by both species and genera. Given the fact that antifungal resistance mechanisms as well as susceptibility patterns

among fungi are greatly influenced by the species, it is critical to identify the organisms under investigation to the species level. With recent advances in technology, such identification is becoming more easily accessible and reliable, and may lead to the better identification of the mechanism of resistance, which in turn will be an important tool to overcome such resistance.

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5 Antifungal Prophylaxis: An Ounce of Prevention is Worth a Pound of Cure

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INTRODUCTION

Given the difficulties inherent in diagnosing and treating invasive fungal infections (IFIs), much attention has been given to the role of prophylaxis against fungal infections. Key components of a successful prophylactic strategy include the following: identification of appropriate high-risk patients, identification of which fungi are most likely to cause infection, effort to decrease risk of infection through nonpharmacologic mechanisms (e.g., laminar air flow rooms to decrease risk of invasive mold infections in hematopoietic stem cell transplant recipients), and selection of the appropriate drug at the appropriate dose to provide effective prophylaxis while minimizing side effects and adverse drug reactions. This chapter will provide evidence for prophylaxis against both yeast and mold infections in high-risk settings.

CANDIDA PROPHYLAXIS

Prophylaxis against *Candida* infections is a key component of patient care in certain high-risk situations, particularly in patients hospitalized in intensive care unit (ICU) settings, preterm infants, those receiving hematopoietic stem cell transplantation, and those with certain hematologic malignancies (i.e., acute myelogenous leukemia and myelodysplastic syndromes) and solid organ transplant recipients. In addition to the major risk groups mentioned above, risk factors for the development of invasive candidiasis (IC) also include receipt of broad-spectrum antibacterial agents, disruption of gastrointestinal integrity (mucositis, gastrointestinal surgery or perforation, administration of total parenteral nutrition, presence of central venous catheters, burns, and mechanical ventilation (1,2). Colonization with *Candida* species at multiple sites remains a somewhat controversial risk factor; however, many studies have noted that while the positive predictive value of *Candida* colonization for development of IC may be low, the negative predictive value is quite high (1). Thus, one should note *Candida* colonization but not necessarily institute routine surveillance cultures of stool or the oropharynx to document colonization.

In addition to prophylaxis against IC, notable additional issues include the inclusion of mold-active agents in prophylaxis (as opposed to fluconazole prophylaxis) and, for allogeneic stem cell transplant recipients, the extension of prophylaxis to the post-engraftment period during the time of acute and chronic graft-versus-host disease (GVHD) prophylaxis or treatment. As the clinical situation differs markedly for each group mentioned above, prophylactic strategies are typically evaluated in a risk-group-specific fashion.

Risk Groups

Intensive Care Unit

Due to evidence of increased risk of developing IC amongst persons hospitalized in ICUs, the concept of prophylaxis in this setting has received considerable attention. The greatest difficulty in addressing the role of prophylaxis in the ICU is identification of the appropriate patients to receive prophylaxis. Additional key issues relating to ICU antifungal prophylaxis include the choice of agent, route of administration (if applicable, i.e., azoles). Goals of prophylaxis in this setting also vary, with the most obvious goal being reduction of episodes of IC, with secondary goals being reduction of overall mortality, avoidance of toxicity, and avoidance of induction of drug resistance (3). Although the concept that *Candida* colonization precedes and thus predicts disease is somewhat controversial (1); some studies of antifungal prophylaxis in the ICU also express the goal of reducing *Candida* colonization. In some settings, investigators

have attempted to calculate a “colonization index (CI)” (ratio of the number of culture-positive surveillance sites for *Candida* spp. to the number of sites cultured) for each patient. Typically, patients undergo culture of the nose, throat, stool, urine, and/or protected tracheal aspirate on admission to the ICU and then weekly. Once data is obtained, then a threshold CI of >0.4 or 0.5 is used as an indication to institute prophylaxis (4,5). While this approach identifies patients who are at potentially high risk for developing IC, it is also labor-intensive and costly. At this time, practice guidelines for prophylaxis in the ICU are still being defined.

The bulk of the studies evaluating the role of anti-*Candida* prophylaxis in the ICU is single-center studies. Key early trials were conducted in surgical ICUs with considerably high rates of IC. An early study enrolled 43 extremely high-risk patients with refractory gastrointestinal perforation or leakage in a randomized, prospective, double-blind placebo-controlled trial of fluconazole 400 mg IV per day versus placebo (6). The observed rate of *Candida* peritonitis was reduced from 35% in the placebo group to 4% in the fluconazole group ($p = 0.02$), highlighting the potential for fluconazole prophylaxis in a highly select group of patients. Subsequently, a larger randomized, double-blind, placebo-controlled trial was performed in a large academic surgical ICU (7). Inclusion criteria for this study were broad and included any patient predicted to have an ICU stay of at least three days. Patients were randomized to receive fluconazole 400 mg/day or placebo. Receipt of fluconazole prophylaxis decreased the rate of IC from 15.3% in the placebo group to 8.5% in the fluconazole group ($p = 0.07$). Other endpoints included time to onset of fungal infection which showed marked benefit with fluconazole versus placebo ($p = 0.01$). Adjusted overall risk of IFI reduced by 55% with fluconazole compared with placebo (RR 0.45; 95% CI = 0.21–0.98). A follow up to this study analyzed the rate of infection and species type in the pre- and postfluconazole prophylaxis era. This study found that the infection rate in the time period prior to initiation of universal prophylaxis was 1.94/1000 patient days versus in the postprophylaxis era, infection rate was 0.76/1000 patient days (OR 0.44; 95% CI = 0.25–0.78; $p = 0.004$). Importantly, this retrospective look at *Candida* epidemiology revealed a change in predominant flora to azole-resistant isolates in this ICU (8). This evaluation occurred in the two years following institution of fluconazole prophylaxis as standard of care in this particular ICU, and would bear repeating after a longer duration as well. Finally, a large randomized trial involving 220 patients evaluated fluconazole 100 mg/day versus placebo in medical or surgical ICU patients, who were on or greater than day 3 of ICU hospitalization (9). Patients in this study were all mechanically ventilated and had undergone selective digestive decontamination. Although administration of low dose fluconazole as prophylaxis in this study reduced the absolute number of episodes of IC (8.9% of placebo group versus 3.9% of fluconazole group, $p = 0.2$), reductions in frequency and intensity of candidal colonization was also found in the fluconazole group as compared to the placebo group (9).

The major detractor from the findings in the above-mentioned trials is that each was conducted at a single center. As the incidence and epidemiology of IC varies greatly between institutions, prophylactic measures deemed successful in one setting may not be applicable to other institutions. Meta-analyses of well-constructed, randomized, controlled trials provide a broader insight into the role of *Candida* prophylaxis in the ICU setting. A meta-analysis of 12 trials evaluating either fluconazole (8) or ketoconazole (4) versus placebo as prophylaxis in high-risk ICU patients showed that when combined, fluconazole/ketoconazole reduced total mortality by one-quarter (relative risk 0.76, 95% CI = 0.59–0.97) and invasive fungal infections by about one-half (relative risk 0.46, 95% CI = 0.31–0.68) (10,11). An additional meta-analysis of six randomized, controlled trials of either fluconazole (four), itraconazole (one), or ketoconazole (one) versus placebo concluded that the use of an azole reduced the risk of IC by 75% amongst nonneutropenic adults hospitalized in an ICU (12). For the purposes of the meta-analysis, the risk factors for IC were defined as having three or more of the following: fungal colonization, diabetes mellitus, solid tumor, abdominal surgery, presence of a central venous catheter for more than three days, antibacterial exposure, intubation, or receipt of a solid organ transplant with anticipated more than five days ICU postoperative stay. Additionally, fewer fungal infections (816 patients, 0.20, 0.13–0.32), fewer episodes of candidemia (604 patients OR 0.28, 95% CI = 0.09–0.86), nonbloodstream IFIs (OR 0.26, 0.12–0.53), and superficial fungal infections (0.22, 0.11–0.43) were noted in the group receiving prophylaxis versus the placebo group. Overall mortality reduction was similar between groups receiving prophylaxis versus

those receiving placebo (0.74, 0.52–1.05), as were adverse events (1.28, 0.82–1.98). As stated in the editorial for the above-discussed meta-analysis, “focused studies in selected high-risk groups have shown that meaningful prophylaxis is possible (6,7), but generalizing the idea has not yet been possible” (13). At this time, the Infectious Diseases Society of America (IDSA) recommends that physicians consider prophylaxis against IC in ICUs where “high” rates of IC persist despite adequate infection control measures” (14). Despite the availability of multiple meta-analyses of prophylaxis trials, identification of the appropriate patients for prophylaxis and which agent is optimal are the key issues for development of effective IC prophylactic strategies for the ICU.

As not every ICU is a particularly “high risk unit” where universal prophylaxis may be the best option, other authors advocate the use of a “*Candida* score” incorporating known risk factors for IC to predict which patients may benefit from fluconazole prophylaxis (15,16). The *Candida* prediction score developed by Ostrosky-Zeichner and colleagues was based on data generated from reviewing 2890 patients, who were hospitalized for four or more days in an ICU (medical or surgical). Persons were excluded if they were on antifungal agents at the time of admission to the ICU or if the status of antifungal therapy was unknown. This dataset recorded an incidence of IC of 3% (88/2890). Evaluation of multiple predictors derived a prediction rule that predicted 34% of cases (rate of 9.9%, RR 4.36, sensitivity 0.34, specificity 0.9, PPV 0.01, NPV 0.97). A patient defined as “high risk” using this rule would meet the following criteria:

- Major criteria: Abx days 1–3 OR CVC days 1–3
- Need 2 minor criteria: TPN days 1–3, surgery days –7 to 0, pancreatitis days –7 to 0, steroids days –7 to 3, immunosuppression days –7 to 0

This rule requires prospective validation, but provides framework for clinical trial design.

As clinical prediction rules are often faulted for being difficult to implement clinically, simpler risk predictors that can be applied to a variety of ICU settings have been developed as well. Modeling of individual risk factors versus background rates of candidemia in any given ICU provides a conceptual framework for prophylaxis protocols (17), as well as defines a number-needed-to-treat (NNT) of approximately four persons to prevent one *Candida*-related death, provided a drug efficacy of 65%. Importantly, this paradigm provides outstanding framework for clinical trials of prophylaxis in the ICU setting. Shortcomings of this framework include the inclusion of only three risk factors, as well as requiring physicians to have up-to-date information regarding background rates of candidemia in the ICU.

Solid Organ Transplantation

Additional situations where prophylaxis against candidal infections is utilized are in abdominal organ transplant recipients (liver, pancreas, small bowel). For a full discussion of the role of prophylaxis against IC and other fungal infections in solid organ transplant recipients, see chapter 25 “Prophylaxis and Treatment of Invasive Fungal Infection in Neutropenic Cancer and Hematopoietic Stem Cell Transplant”.

Preterm Infants

Low birth weight and very low birth weight infants represent a high risk group for the development of IC. For a full discussion of the epidemiology, prophylaxis, and treatment of IC in preterm infants, see chapter 26 “Infants: Yeasts and Beasts in Early life”.

CANDIDA AND MOLD PROPHYLAXIS

Certain clinical situations, specifically patients with acute leukemia/hematologic malignancies or those who undergo hematopoietic stem cell transplantation, expose patients to risk of both IC and invasive mold infections. Clinicians caring for these patients must consider risk for both types of infection, as portals of entry differ (gastrointestinal tract or IV catheter for yeast vs. inhalation for molds), as do the susceptibility to various potential prophylactic agents. Guidelines for who should receive prophylaxis are more well established for these clinical situations as compared to ICU prophylaxis; however, much still remains to be learned regarding the optimal agent, dose, and duration.

Risk Groups

Hematologic Malignancies

Risk factors for development of invasive fungal infections differ amongst persons with hematologic malignancies, with the lowest risk being those persons undergoing autologous transplantation (Table 1). Patients at highest risk for IFI include those with profound and persistent neutropenia ($<0.1 \times 10^9/L$ for over three weeks), those undergoing allogeneic unrelated or mismatched unrelated transplant, those colonized by *C. tropicalis* and those receiving high dose corticosteroids or certain types of chemotherapy (high-dose Ara-C) (18). Of all hematologic malignancies, acute myelogenous leukemia and myelodysplastic syndrome are amongst the highest risk for development of IFIs, including both candidiasis and mold infections (18). This is likely due to intrinsic defects in myeloid cells as well as decreased numbers of functional myeloid cells and mucositis. Fluconazole prophylaxis during the period of neutropenia for patients with hematologic malignancies has been given a C1 recommendation (19) (poor evidence to show support for a recommendation) (20). Similarly, itraconazole prophylaxis has been given a B1 recommendation (moderate evidence from ≥ 1 randomized controlled trial) based on a trial comparing itraconazole oral solution to placebo in 405 neutropenic patients with hematologic malignancies. Proven and suspected deep fungal infection occurred in 24% of itraconazole recipients and in 33% of placebo recipients, a difference of nine percentage points (95% CI = 0.6–22.5%; $p = 0.035$). Fungemia due to *Candida* species was documented in 0.5% of itraconazole recipients and in 4% of placebo recipients, a difference of 3.5 percentage points (95% CI = 0.5–6%; $p = 0.01$) (21). An additional open label trial comparing fluconazole to itraconazole oral suspension in 494 patients with high-risk hematologic malignancies demonstrated equivalence between the two agents, with overall low rates of IFI (1.6% for the itraconazole group and 2.0% for the fluconazole group) (22). In both trials, itraconazole oral solution was well tolerated and effectively prevented proven and suspected deep fungal infection as well as systemic infection and death due to *Candida* species. Owing to issues of bioavailability and the need for adequate serum levels for prophylactic activity, itraconazole usage should be limited to the oral solution, as opposed

Table 1 Risk Stratification Scheme for Invasive Fungal Infections in Patients with Hematologic Malignancies or Hematopoietic Stem Cell Transplantation

Low risk

- Autologous transplant
- Childhood ALL (except for *Pneumocystis carinii pneumonia*)
- Lymphoma

Intermediate risk

Low-intermediate

- Moderate neutropenia ($0.1-0.5 \times 10^8/L < 3$ wk, lymphocytes $<0.5 \times 10^8/L +$ antibiotics)
- Older age
- Central venous catheter

High-intermediate

- Colonized > 1 site or heavy at one site
- Neutropenia < 0.5 to $> 0.1 \times 10^8/L > 3-5$ wk
- AML
- TBI

- Allogeneic matched sibling donor BMT

High risk

- Neutrophils $< 0.1 \times 10^8/L > 3$ wk
- Colonized by *Candida tropicalis*
- Allogeneic unrelated or mismatched donor BMT
- GVHD
- Neutropenia $< 0.5 \times 10^8/L > 5$ wk
- Corticosteroids > 1 mg/kg and neutrophils $< 1 \times 10^8/L > 1$ wk
- Corticosteroids > 2 mg/kg > 2 wk
- High-dose Ara-C
- Fludarabine?

to capsules (20). Additionally, a meta-analysis of antifungal prophylaxis trials demonstrated the efficacy of prophylaxis in key subgroups of patients along several parameters. This comprehensive analysis included 38 trials (total 7014 patients; study agents, 3515 patients; control patients, 3499 patients). Overall, when comparing patients receiving prophylaxis to patients receiving placebo, there were reductions in the use of parenteral antifungal therapy [prophylaxis success: odds ratio (OR), 0.57; 95% CI = 0.48–0.68; relative risk reduction (RRR), 19%; number requiring treatment for this outcome (NNT), 10 patients], superficial fungal infection (OR, 0.29; 95% CI = 0.20–0.43; RRR, 61%; NNT, 12 patients), invasive fungal infection (OR, 0.44; 95% CI = 0.35–0.55; RRR, 56%; NNT, 22 patients), and fungal infection–related mortality (OR, 0.58; 95% CI = 0.41–0.82; RRR, 47%; NNT, 52 patients). As expected in these highly complex patients, overall mortality was not reduced based on use of antifungal prophylaxis (OR, 0.87; 95% CI = 0.74–1.03). However, subgroup analyses showed reduced mortality in studies of patients who had prolonged neutropenia (OR, 0.72; 95% CI = 0.55–0.95) or were HSCT recipients (OR, 0.77; 95% CI = 0.59–0.99). From the multivariate meta-regression analyses performed, key predictors of treatment effect identified were HSCT, prolonged neutropenia, acute leukemia with prolonged neutropenia, and higher azole dose (23). A key point learned from the prophylaxis data in these patients is that need is not universal in all patients with hematologic malignancies who undergo cytotoxic chemotherapy, but that certain subsets of patients benefit greatly.

Posaconazole, a broad-spectrum triazole, has recently been demonstrated to reduce IFI and mortality in patients with newly diagnosed or relapsed acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS), who were treated with intensive chemotherapy (24). Notably, this study enrolled only the highest risk patients (MDS or AML). Patients enrolled in this study received posaconazole, 200 mg thrice daily ($n = 304$) or a standard azole regimen [either fluconazole, 400 mg once daily ($n = 240$) or itraconazole, 200 mg twice daily ($n = 58$), choice determined by the investigative site] with each cycle of chemotherapy until complete remission or for up to 12 weeks. Use of posaconazole as compared to use of fluconazole or itraconazole was associated with fewer total IFI during the treatment phase (2% vs. 8%; $p = 0.0009$) and fewer episodes of invasive aspergillosis (1% vs. 7%; $p = 0.0001$) (25). In addition to the efficacy of posaconazole for prevention of IFIs in this group of patients, a mortality benefit was found as well. At 100 days postrandomization, there was a survival benefit in favor of posaconazole in terms of all cause (15% vs. 22%; $p = 0.0354$) and IFI-related death (2% vs. 5%; $p = 0.0209$). The rates of adverse events were similar between the two treatment groups.

Hematopoietic Stem Cell Transplantation

Owing to overt immune suppression, as well as disruption of gastrointestinal integrity during intensive chemotherapy, persons undergoing hematopoietic stem cell transplantation (HSCT) are at risk for developing IFIs during the pre-engraftment phase of transplantation. Typically, the risk for IC is highest during the pre-engraftment period. An additional risk period for invasive mold infections occurs during the time of acute GVHD, as the disease itself as well as the agents used for prophylaxis and/or treatment induce immune suppression.

Several large trials have demonstrated the efficacy of fluconazole versus placebo for the prophylaxis against IC during the period of neutropenia following conditioning for allogeneic and autologous stem cell transplantation. Given the overwhelming evidence from multiple well-constructed clinical trials, the Infectious Diseases Society of America, The Centers for Disease Control and Prevention, and the American Society for Blood and Marrow Transplant currently recommend prophylaxis against IC with fluconazole 400 mg IV or PO daily for hematopoietic stem cell transplant recipients from the time of neutropenia through the time of engraftment (26). Concern about fluconazole-resistance amongst *Candida* isolates has prompted the evaluation of echinocandin antifungals for prophylaxis in this setting as well. Based on a randomized, controlled trial of prophylaxis with micafungin (50 mg/day) versus fluconazole (400 mg IV/day), micafungin was found to be superior to fluconazole for prophylaxis during the neutropenic period in patients undergoing allogeneic HSCT (proportion free of IFI at four weeks 80% in micafungin group vs. 73.5% in fluconazole group, 95% CI = 0.9–12%, $p = 0.03$). Data from this randomized trial led to the approval of micafungin by the US FDA for prophylaxis against IFI in stem cell transplant recipients during the period of neutropenia to engraftment (27).

As the risk period for infection does not end with engraftment, the effects of extension of fluconazole prophylaxis through day +75 following autologous (12%) and allogeneic (88%) stem cell transplantation was evaluated. Despite the spectrum limitations of fluconazole, extension of prophylaxis through the post-engraftment period provided benefit in reduction of IFIs. During prophylaxis, systemic fungal infections occurred in 10 (7%) of 152 fluconazole-treated patients compared with 26 (18%) of 148 placebo-treated patients ($p = 0.004$). The probability of survival at day 110 following transplantation was improved in fluconazole recipients, in whom 31 deaths occurred as compared with 52 deaths in placebo recipients ($p = 0.004$) (28). Eight year follow up of these patients found that extension of fluconazole prophylaxis (400 mg PO qd) for IC for 75 days following allogeneic stem cell transplantation not only decreased subsequent IC (30 of 148 placebo vs. 4 of 152 fluconazole, $p < 0.001$) but also had a positive impact on eight year mortality (68 of 152 fluconazole vs. 41 of 148 placebo, $p = 0.0001$). This decrease in mortality was likely as a result of decreased development of gastrointestinal GVHD (20 of 143 placebo vs. 8 of 145 fluconazole, $p = 0.02$) in addition to decreased IC (29). As a result of this trial, many centers have instituted prolonged fluconazole prophylaxis (through day +75) for allogeneic stem cell transplant recipients.

In addition to IC, invasive mold infections remain a predominant cause of morbidity and mortality in HSCT recipients. Given the limitations of fluconazole coverage, multiple trials have evaluated antifungal agents with activity against yeasts and molds as prophylaxis in hematopoietic stem cell transplant recipients. Risk in autologous transplant recipients is low, particularly compared to allogeneic transplant recipients, due to decreased length of neutropenia and absence of GVHD. Many antifungal doses and agents have been studied as prophylactic agents in HSCT recipients. Current published studies evaluating fungal prophylaxis in HSCT compare itraconazole to placebo (30), itraconazole to fluconazole (31–33), amphotericin B products to placebo (34), and low-dose amphotericin B products to fluconazole (35,36). Results from a comparator trial of fluconazole versus voriconazole are currently pending. The results of a double-blind, randomized controlled trial evaluating posaconazole versus oral fluconazole for prophylaxis against fungal infections during the period of severe acute GVHD in allogeneic HSCT recipients (24) has positioned posaconazole to become a first line agent for prophylaxis against IFI in allogeneic stem cell transplant recipients with GVHD.

In this study of 600 allogeneic stem cell transplant recipients with GVHD, patients were randomized to receive posaconazole, 200 mg thrice daily ($n = 301$) or fluconazole, 400 mg once daily ($n = 299$) for up to 16 weeks (24). Although the incidence of total IFI during the 16-week study period was similar in the posaconazole and fluconazole groups (5% vs. 9%; $p = 0.0740$), there were fewer total breakthrough IFIs in the posaconazole arm (2% vs. 8%; $p = 0.0038$). Notably, *Aspergillus* infections were significantly reduced among patients receiving posaconazole during the study period (2% vs. 7%; $p = 0.0059$). The overall mortality rates were similar in the two arms (25% in the posaconazole arm vs. 28% in the fluconazole arm). Mortality due to IFI was lower in the posaconazole group (1%) versus 4% in the fluconazole group ($p = 0.046$). The side-effect profiles of the two agents were similar.

Itraconazole (oral solution and intravenous injection) can be used in patients undergoing allogeneic HSCT who can tolerate the drug and who are not at increased risk for significant drug interactions (31,33). Based on the insufficient power of available studies and the risk of toxicity, amphotericin B (in any formulation) is not recommended for antifungal prophylaxis (18).

Many options exist for IFI prophylaxis in high-risk stem cell transplant recipients. Comparison of efficacy, toxicity, and cost can be used to determine the appropriate prophylaxis program for individual patients and centers. As prophylaxis regimens become broader spectrum in scope, clinicians must consider new strategies for empiric therapy when patients develop febrile episodes or pulmonary infiltrates while taking prophylaxis. Many experts believe that empiric regimens for febrile neutropenia will evolve to more “watchful waiting” as prophylactic regimens broaden (37), although trials evaluating this strategy are indicated.

OTHER SITUATIONS

Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS)

Based on overall CD4 count or percentage, persons infected with the HIV virus are at risk for a myriad of fungal infections, including *Pneumocystis jirovecii* pneumonia and oro-esophageal

Table 2 Fungal Prophylaxis Strategies in Patients with AIDS

Fungal pathogen	Timing of prophylaxis	Endemic area	Prophylactic agents	Other issues	Key literature
<i>Pneumocystis jirovecii</i>	CD4 cell count <200/ μ L OR oropharyngeal candidiasis	N/A	TMP-SMX DS tablet qd or qMWF; second line: dapsone, atovaquone, inhaled pentamidine	Can discontinue prophylaxis if CD4 count is >200 cells/ μ L for 3 mo and person on stable antiretroviral therapy	38–43
<i>Candida</i> species (oro-esophageal candidiasis)	Typically CD4 < 200 cells/ μ L	N/A	Fluconazole	Not typically recommended as primary prophylaxis due to risk of resistance; may be considered as secondary prophylaxis in patients with recurrent infections	44
<i>Histoplasma capsulatum</i>	CD4 lymphocyte counts of <100 cells/ μ L who live in areas of hyperendemicity (defined by \geq 10 cases per 100 patient-years) or who have high-risk occupations that involve frequent exposure to soil	Ohio River Valley, Puerto Rico	Itraconazole (first line); fluconazole (second line)	Guidelines on discontinuation of prophylaxis not developed; also prevents development of cryptococcal disease	45–47
<i>Penicillium marneffeii</i>	Patients with CD4 count <100 cells/ μ L who live in endemic area	Chiang-Mai province of Thailand	Itraconazole 200 mg PO qd	Discontinuation of prophylaxis reasonable in persons on HAART with CD4 counts of >100 cells/ μ L for >6 mo; also provides prophylaxis against cryptococcal disease	48–50

candidiasis. Geographic location imparts additional risk for infections such as cryptococcosis, histoplasmosis, coccidioidomycosis, and infection with *Penicillium marneffeii*. Recommendations for prophylaxis against fungal infections in HIV-infected persons are thus based on both degree of immune suppression and geography. Major prophylactic recommendations are summarized in Table 2.

CONCLUSIONS

Given the significant morbidity and mortality associated with invasive fungal infections, many situations are suitable for the use of antifungal prophylaxis. While prophylactic regimens have

many goals, the foremost is to prevent invasive fungal infections. Preventing colonization, avoiding drug resistance, and avoiding toxicity are secondary goals to consider. As diagnostics for fungal infections improve, the role of prophylaxis will undoubtedly evolve.

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6 Preemptive Antifungal Therapy: Do Diagnostics Help?

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INTRODUCTION

Despite the availability of broad spectrum antifungal drugs, invasive fungal infection (IFI) remains a major cause of morbidity and mortality in immunosuppressed patients as well as among critically ill patients in the intensive care unit (ICU). Early initiation of appropriate antifungal therapy appears to improve outcomes, especially in neutropenic patients (1,2). Unfortunately, clinical risk assessment in conjunction with physical examination and radiography is often neither sensitive, nor specific enough to make a rapid diagnosis of IFI. The clinical utility of fungal culture is also limited. Cultures frequently remain negative or only become positive in the advanced stages of infection. Furthermore, deciphering colonization from invasive infection can be extremely difficult when samples are obtained from nonsterile sites. Histopathologic examination of infected tissue has historically been the diagnostic gold standard, but invasive testing may not be feasible in critically ill patients or in those with underlying coagulopathy.

Given the potentially devastating effects of IFI, prevention of overt disease is preferable. Current strategies for the prevention and management of IFI include (i) antifungal prophylaxis, (ii) preemptive therapy, (iii) empiric treatment, and (iv) treatment of established infection. Definitions in addition to the advantages and disadvantages of each approach are outlined in Table 1.

Improved diagnostic tests including the galactomannan assay, (1,3)- β -D-glucan test, and the direct detection of fungal DNA have the potential to facilitate identification of IFI and to better inform early antifungal treatment decisions. These tests can be useful as adjuncts for the diagnosis of IFI, but their effectiveness as a trigger for preemptive antifungal therapy in at-risk patients has not been clearly defined. Preemptive antifungal therapy guided by noninvasive laboratory markers is an attractive way to direct early antifungal treatment to those patients most likely to benefit from the intervention. This chapter reviews the primary, nonculture-based, fungal diagnostic techniques and examines the evidence for their use in clinical practice.

GALACTOMANNAN

Assay Principles

Galactomannan (GM) is a polysaccharide cell-wall component released by the growing hyphae of *Aspergillus* and *Penicillium* species (spp.). The GM molecule comprises a nonimmunogenic mannan core with immunoreactive galactofuranosyl (galf) containing side chains of varying lengths (3). GM was first identified as a potential biomarker of invasive aspergillosis (IA) by Reiss and Lehman (4). Commercially available GM assays utilize a rat monoclonal antibody (EB-A2) directed against β (1,5)-linked galactofuranoside side chain residues (5). Four or more epitopes are typically required for antibody binding and multiple immunoreactive epitopes are present on each GM molecule (6). In addition to GM, other fungal glycoproteins including phospholipase C and phytase have been shown to react with EB-A2 antibodies (3,7). It is likely that the so-called "GM antigen" is really a family of molecules whose expression is modulated by the localized fungal microenvironment (8). Interestingly, the actual galf antigens that circulate in vivo have not been fully characterized.

A sandwich enzyme-linked immunosorbent assay (EIA) (Platelia™ BioRad, Marnes-La-Coquette, France) and a latex agglutination test (Pastorex Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) for the detection of GM in vivo are commercially available. The EIA has

Table 1 Strategies for the Prevention and Treatment of Invasive Fungal Infection (IFI)

Strategy	Definition	Advantages	Disadvantages
Universal prophylaxis	Administration of antifungal therapy during a defined period to prevent IFI	Effective and logistically easy	<ol style="list-style-type: none"> 1. Drug toxicity 2. Development of antimicrobial resistance 3. Cost incurred by patients who would never develop IFI
Preemptive therapy	Initiation of therapy based on serial monitoring with sensitive laboratory markers, radiographic studies, or both, to treat early IFI	<ol style="list-style-type: none"> 1. Targets those patients most likely to benefit from antifungal therapy 2. Facilitates early initiation of antifungal therapy which may improve outcomes 3. Monitoring is noninvasive 	<ol style="list-style-type: none"> 1. Effectiveness is based on the performance of the screening strategy in different patient populations 2. Difficult to incorporate into outpatient management 3. Cost of the screening test(s) 4. Not yet shown to improve IFI morbidity or mortality
Empiric treatment	Initiation of therapy to treat suspected IFI based on clinical features (Example: Antifungal therapy for patients with persistent fever in the setting of neutropenia, without a known source, and despite appropriate antibiotic therapy.)	Improved outcomes documented in the setting of neutropenic fever of unknown origin	Waiting until signs or symptoms of IFI are present delays potentially effective therapy
Treatment of established IFI	Treatment of patients who meet criteria for proven or probable IFI	Currently available antifungal agents are effective in some patients	Waiting until signs or symptoms of IFI are present delays potentially effective therapy

a reported limit of detection of 0.5 to 1.0 ng/mL, which is 10 to 15 times lower than the latex agglutination test (6,9). For this reason, the EIA has become the method of choice in most clinical laboratories that perform GM testing and will be the focus of this review. The Platelia assay (Fig. 1) has been available in Europe for over a decade and was approved by the US Food and Drug Administration in May 2003 for diagnostic use in cancer patients. Laboratory test turnaround time is approximately 3 to 3.5 hours, which marks a significant advance over standard culture techniques.

Kinetics

Despite many years of clinical and laboratory investigation, the kinetics of GM production, release, and systemic circulation in vivo are incompletely understood. It is likely that many factors including fungal species, fungal growth rate, localized tissue microenvironment, and characteristics unique to the host all influence detectable GM concentrations (10). Multiple groups have shown that GM production is proportional to the tissue fungal burden using both animal models and models of the human alveolus (11–13). GM levels also appear to have prognostic value, with persistently high or rising concentrations portending a poor prognosis (14–16). Administration of effective antifungal therapy typically reduces circulating GM levels (11,12,17,18); however, clinical improvement without a concomitant decline in GM has been described in the setting of protracted neutropenia and echinocandin therapy (19–21). Similarly, GM has been reported to remain elevated in successfully treated patients with underlying renal failure (22). GM is thought to be metabolized by a combination of renal excretion,

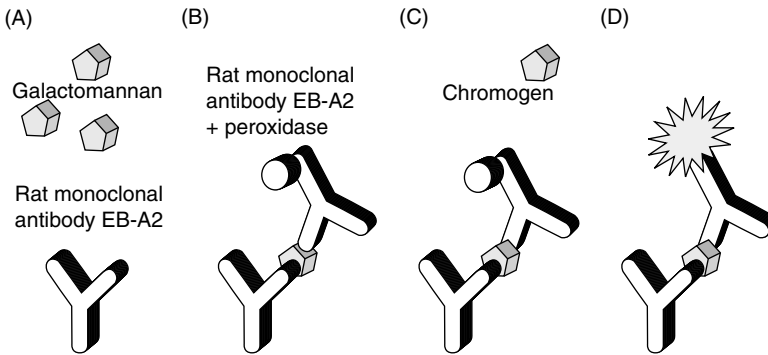


Figure 1 (See color insert) Platelia galactomannan EIA Test. The Sassy uses rat EB-A2 monoclonal antibodies directed against *Aspergillus* galactomannan. (A) Monoclonal antibodies are used to coat the wells of a microplate, (B) monoclonal antibodies capture the galactomannan antigens and a peroxidase-labeled conjugate is added to bind the antigen–antibody complex, (C) a substrate solution is added that reacts with peroxidase, and (D) antigen detection via enzymatic color reaction. Source: Adapted from Ref. 10.

hepatic clearance, and uptake by macrophage mannose receptors (23,24). A feature lending to the potential utility of GM as a screening test is that circulating GM can be detected 1 to 2 weeks before the development of clinical signs or symptoms of IA in some patients (16,25,26). Furthermore, GM may also precede abnormalities on high-resolution CT scan in individuals with suspected pulmonary IA (27). Not all studies, however, have demonstrated the development of GM antigenemia before a conventional diagnosis of IA was made (26,28–30).

Defining a Positive Result

Definition of the optimal optical density (OD) index to define a positive GM EIA result remains a matter of some debate. The OD index is defined as the OD value of the specimen divided by the mean OD of the wells containing control serum. The manufacturer recommends interpreting an index of ≥ 1.5 as a positive test, with < 1.0 defining a negative result, and the 1.0 to 1.5 range being intermediate. A reduced threshold for negative ($OD \leq 0.8$) and for positive samples ($OD > 1.0$) was suggested in the original evaluation of the inter-laboratory reproducibility of the test (30). In practice, many European centers actually implemented lower OD thresholds to classify positive results (31,32) and an index of ≥ 0.5 has been adopted as the positive test cutoff in the United States (33).

Lower OD cutoffs improve the sensitivity of the test, but also lead to some reduction in specificity. In a multicenter study of 124 adult patients at high risk for IA due to prolonged neutropenia, lowering the positive cutoff from 1.5 to 0.5 increased sensitivity by 14% (from 83% to 97%) but also decreased the specificity by 15% (from 100% to 85%) (32). A second retrospective analysis demonstrated a sensitivity increase of 21% (from 76% to 97%) with a decrease in specificity by 7% (from 98% to 91%) when the threshold was lowered from 1.5 to ≥ 0.5 (34). Similarly, in a cohort of stem cell transplant recipients who were receiving empiric or prophylactic antifungal therapy, decreasing the cutoff from ≥ 1.0 to ≥ 0.5 improved the sensitivity significantly (increase from 18% to 82%) while only modestly impacting specificity (decrease from 100% to 77%) (12).

The OD threshold also influences the timing of positive test results in relation to the development of signs and/or symptoms of IA. GM antigenemia was detected in the week preceding or coinciding with a conventional diagnosis of IA in 72% of cases of proven or probable disease ($n = 29$) when a cutoff ≥ 0.5 was applied as compared to 41% of the time using the conventional cutoff of 1.5 (32). This finding was reproduced in 78% of episodes of proven or probable IA when the lower cutoff was used to define a positive test (34). The primary benefit of the lower threshold, therefore, may actually be the earlier detection of cases of IA.

To maximize accuracy, the manufacturer recommends retesting a second fresh aliquot from all GM positive specimens in addition to collecting a new sample for confirmatory testing from all GM positive patients. Requiring two consecutive samples with an OD threshold ≥ 0.5

to define a positive test has been demonstrated to improve test accuracy in some studies (32,34). However, even with consecutive positive samples, the false-positive rate has remained problematic (rate as high as 23%) in some studies (25,35–39).

Frequency of Sample Collection

The optimal sample collection strategy for IA surveillance has not been rigorously defined. Once or twice weekly determination has generally been used in the published reports to date. In theory, the frequency of monitoring may affect sensitivity, with sporadic testing missing periods of transient antigenemia. Some experts have also recommended assessing GM levels immediately in patients with clinical features suggestive of IA to assist in making a definitive diagnosis (40). The test performance of GM in a nonsurveillance setting, however, is less certain than it is for monitoring high-risk patients.

Test Performance

For the purposes of evaluating a novel diagnostic test, it is essential that a correct diagnosis be made by the comparator or gold standard method. Unfortunately, establishing a diagnosis of IFI remains a major obstacle in studies evaluating fungal diagnostics (41). The European Organization for Research on the Treatment of Cancer/Mycoses Study Group (EORTC/MSG) has developed standardized definitions for IFI that are intended for use in the context of research (42). The system employs a combination of clinical and microbiological criteria for the classification of “proven,” “probable,” or “possible” cases of IFI. Diagnoses other than proven disease, however, are often subject to considerable debate. Furthermore, the EORTC/MSG criteria categorize a positive GM *Aspergillus* EIA as microbiologic evidence for probable IA even in the absence of culture confirmation. Inclusion of GM results in the definition of IFI is a potential source of bias in clinical studies assessing the utility of the GM test (43).

The majority of studies evaluating the role of GM monitoring has been conducted in patients receiving cancer chemotherapy or following stem cell transplantation (SCT) with a few investigations focusing specifically on pediatric populations or solid organ transplant (SOT) recipients. Several of the larger prospective surveillance studies providing patient level data are reviewed below and are also summarized in Table 2.

Studies Involving Hematology–Oncology and Stem Cell Transplant Patients

OD Cutoff ≥ 1.5

In theory, studies incorporating an OD cutoff of >1.5 should reflect optimal assessments of specificity and positive predictive value (PPV). Herbrecht et al. followed 728 adult and pediatric patients with either neutropenic fever ($n = 261$), suspected pulmonary infection ($n = 297$), nonpulmonary aspergillosis ($n = 28$), or those undergoing routine surveillance after SCT ($n = 211$) (31). Patients had sequential samples collected for the GM assay at predefined intervals depending on the clinical setting. Overall the test was 35% sensitive and 93% specific for proven or probable IA. Three additional studies reported more favorable sensitivities using the conventional cutoff. The first was a large prospective study of 347 pediatric hematology patients and in 450 adult and pediatric SCT recipients (25). In the pediatric hematology group, the test was 100% sensitive and 90% specific. In the SCT cohort, the sensitivity was 89% with a specificity of 94%. Rovira et al. evaluated 74 SCT recipients and observed relatively few cases of proven or probable IA (53). In this analysis, GM had a sensitivity of 67% with a specificity of 97%. The third study conducted by Pazos et al. included 40 adult neutropenic patients (27). In this small group, the sensitivity and specificity for proven or probable IA were 88% and 90%, respectively.

Cutoff ≥ 1.0

Studies employing a 1.0 OD cutoff have been divided on the clinical efficacy of GM surveillance. Maertens et al. screened 186 consecutive adult hematology patients who were receiving itraconazole prophylaxis (37). Based on the evaluation of 27 patients with autopsy-verified IA, the sensitivity and specificity both exceeded 90% and more than half of the time GM antigenemia was detected before clinical suspicion of IA arose (median, six days before). The same

Table 2 Efficacy of Serum Galactomannan Surveillance for the Diagnosis of Proven or Probable Invasive Aspergillosis in Immunosuppressed Patient Populations

Study	Frequency of sampling	OD	No. of samples required for positivity	Proven cases	Probable cases	Prev (%)	SN	SP	PPV	NPV
(31)	V	1.5	1	31	67	13.46	0.32	0.93	0.38	0.91
(25)	2/wk	1.5	2	27	26	6.65	0.91	0.94	0.52	0.99
(38)	2/wk	1.5	1	1	5	8.11	0.67	0.97	0.67	0.97
(27)	2/wk	1.5	2	5	3	20.00	0.88	0.90	0.70	0.96
(44)	V	1.5	2	8	3	6.67	0.33	0.99	0.75	0.97
(45)	1/wk	1.5	2	33	0	27.04	0.58	0.97	0.86	0.87
(37)	2/wk	1	2	27	0	14.51	0.93	0.95	0.93	0.95
(16)	2/wk	1	2	5	8	13.00	0.85	0.92	0.93	0.99
(26)	2/wk	1	1	7	9	15.38	0.75	0.93	0.86	0.96
(15)	1/wk	1	2	3	3	12.00	1.00	0.77	0.38	1.0
(36)	V	1	2	3	31	4.21	0.50	0.98	0.50	0.98
(12)	1/wk	1	1	13	11	35.82	0.54	0.74	0.93	0.99
(46)	NR	1	1	NR	NR	5.83	0.56	0.94	0.71	0.89
(47)	NR	0.5	2	20	26	...	0.70	0.88	0.52	0.94
(32)	2/wk	0.5	2	16	13	27.88	0.97	0.98	0.93	0.99
(48)	2/wk	0.5	2	2	12	10.9	0.86	0.78	0.32	0.98
(49)	2/wk	0.5	1	0	1	1.56	1.00	0.87	0.11	1.00
(50)	2/wk	0.5	1	9	3	17.14	0.25	0.75	0.17	0.84
(51)	2/wk	0.5	1	0	1	0.65	0.00	0.87	0.00	0.99
(52)	1/wk	0.5	2	9	2	11.46	1.00	0.84	0.35	1.00

Abbreviations: V, variable; NR, not reported; OD, optical density; Prev, prevalence; SN, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.

monitoring strategy was used to evaluate 100 consecutive adult myeloablative SCT recipients on mold-active antifungal prophylaxis (35). Eighteen patients with proven disease were identified when autopsy findings were incorporated into the final determination of IA. In this group, the GM assay had a sensitivity and specificity of 94% and 99%, respectively. When only antemortum data were considered, the test performed less well (85% sensitive and 98% specific). Antigenemia again preceded radiographic findings or culture confirmation in the majority of patients and was also more reliable than unexplained fever, new pulmonary infiltrates, or isolation of *Aspergillus* species in culture for the diagnosis of IA.

Two additional investigations found that GM did not have meaningful impact on clinical decision making in real-time. Williamson et al. followed 104 pediatric and adult bone marrow transplant (BMT) recipients, who were receiving mold-active antifungal prophylaxis (26). In this study, GM was 75% sensitive in patients who died with proven or suspected IA. GM did not contribute to the management of the majority (69%) of patients, either because the test was never positive (4 of 16), or because the diagnosis had been obtained by other means (7 of 16). Bretagne et al. monitored 50 neutropenic adult and pediatric hematology and reported an overall sensitivity of 100% with a specificity of 77% for proven or probable disease; however, a positive GM did not anticipate the initiation of antifungal therapy in any case already based on clinical suspicion alone. Furthermore, a relatively high number of false-positive tests (23%) were observed.

Lastly, Pinel et al. monitored 807 adult and pediatric hematology and ICU patients with GM either once or twice a week (36). The calculated sensitivity for proven or probable IA was a meager 50%. In an attempt to explain this, the authors noted that 4 of 31 patients classified as having probable IA met criteria for that diagnosis based solely on a positive GM result. In addition, the average number of samples collected from patients with probable IA was lower in the subset of patients with negative GM results compared to those that had GM antigenemia detected. The difference (four vs. five serum samples) was minimal, however, and does not explain the false-negative results observed in three of three patients with proven IA.

Cutoff ≥ 0.5

The Platelia *Aspergillus* EIA test package insert summarizes the prospective studies leading to FDA approval of the test (47). Adult data were collected from 143 SCT and leukemic patients across three centers in North America. For the combined diagnosis of proven or probable IA, the test was 70% sensitive (95% CI = 62–90%) and 89% specific (95% CI = 83–93%). The control group in this study included hematology and BMT patients without IA. Similar test performance was documented by Yoo et al., who evaluated 128 patients with hematologic diseases and/or status-post SCT during periods of neutropenic fever (48). Maertens et al. obtained an even better sensitivity (97%) and specificity (98%) using the lower cutoff in 104 adult hematology–oncology patients (32).

Studies Involving Pediatric Patients

Multiple GM studies have included children, but only a few have reported pediatric specific data. Early reports described poorer specificities in pediatric as compared to adult populations (25,31); however, this observation has not been reproduced in subsequent pediatric evaluations. In the pediatric portion of the study leading to licensing in the United States, GM was 53% sensitive (95% CI = 31–74%) and 98% specific (95% CI = 83–93%) for proven or probable IA (47). Of the 17 children with proven or probable disease in this analysis, nine were GM negative. The false-negative results were ascribed to mold-active antifungal therapy administered around the time GM testing was performed. A second, more recent, study involved 64 pediatric SCT recipients (49). Of the 63 patients without IA, eight (13%) had at least one positive GM result with four of these coinciding directly with piperacillin–tazobactam therapy. Overall, specificity was moderate to good (87%; 95% CI = 77–93%).

Studies Involving Solid Organ Transplant Recipients

Three studies have evaluated the utility of serum GM surveillance in SOT recipients. Husain et al. assessed biweekly monitoring in 70 consecutive lung transplant recipients, the majority of whom were receiving fluconazole prophylaxis (50). GM was detected in 3 of 12 patients with proven or probable IA, for a patient-based sensitivity of 25%. Two of the three patients with a positive GM had invasive lung disease and the third had systemic IA. There were also four cases of tracheobronchitis caused by *Aspergillus*, but none of these subjects had a positive GM test. Fourteen patients without IA had 36 false-positive tests. Colonization with *Aspergillus* was not associated with false-positive test results in this study. The same group of investigators evaluated an identical surveillance strategy in 154 liver transplant recipients (51). A single case of probable invasive pulmonary disease developed during the study period, with GM detected in three samples on initial, but not on repeat testing. Twenty-one patients without IA had 23 false-positive tests (patient-based specificity 86%). Fortun et al. conducted a retrospective case-control study of IA in 240 liver transplant recipients using stored frozen serum obtained during the posttransplant period (46). Fourteen cases of proven or probable IA were identified. Using an OD threshold of ≥ 1.0 that was confirmed on repeat testing, the sensitivity of the test was 56% with a specificity of 94%.

Factors Associated with False-Positive Test Results

False-positive GM results have been observed in all of the published clinical studies, but the prevalence of this finding has varied significantly. As discussed previously, requirements for persistent GM detection in sequential specimens may impact the specificity. Other host factors such as age and underlying disease in conjunction with the administration of certain medications, or infection with microbial species known to cross-react with the rat monoclonal antibody (EB-A2) used in the commercial EIA, may also lead to false-positive results in some patients.

Host Factors

Host characteristics appear to be an influential factor in GM test performance. Herbrecht et al. observed high false-positive rates in children with neutropenic fever [11 of 25 patients (44%)] and in pediatric SCT recipients [9 of 12 patients (75%)] (31). In a separate study of critically ill premature infants, five of six (83%) had false-positive GM results documented (54). It has been suggested that GM present in certain milk formulas could cause false-positive test

results in children owing to immature and/or impaired gut integrity in the setting of mucositis (55). Heavy colonization with bifidobacteria, which is also observed in some neonates and young infants, has also been implicated as a possible cause of false-positive tests in young patients (56).

Herbrecht et al. also observed a lower specificity in adult allogeneic SCT recipients (93%) as compared to autologous SCT or nontransplant patients (99%); with the majority of false-positive tests occurring at first month posttransplant (31). Several other groups have also observed false-positive reactions more frequently within the first two weeks following cytotoxic chemotherapy (6,25,37) or after lung transplantation (50) as well as during periods of graft-versus-host disease (GVHD) (57,58). Potential explanations for these observations have included dietary absorption of GM (59) due to impaired mucosal barriers after cytotoxic chemotherapy or from GVHD (58) and the presence of interfering substances such as cyclophosphamide metabolites (60), antibiotics, or auto-antibodies (51,57) that are present during these periods.

β-Lactam Antibiotics

GM reactivity has been observed in some, but not all, patients receiving β-lactam antibiotics. The administration of piperacillin–tazobactam (61–65), amoxicillin–clavulonate (62,66), amoxicillin (62), ampicillin (62), and phenoxymethylpenicillin (62) has all been associated with false-positive GM tests. Since *Penicillium* is used in the production of these drugs, and this organism is known to release GM antigens, it is not surprising that these compounds would cross-react with the GM EIA. Several groups have reported direct detection of GM in batches of reconstituted β-lactams by EIA testing (62,65,67,68), but were unable to either culture or amplify *Aspergillus* DNA from the antibiotics themselves (67).

The kinetics of GM following the administration of various β-lactams has been analyzed using serum samples collected from hematology patients (62,67). Bart-Delabesse et al. described three patterns of GM reactivity in patients receiving GM-positive batches (62). The first group (66%) had persistently elevated OD indexes >2.0 during treatment, the second (26%) had indexes between 0.5 and 1.5, and the third group had variable GM levels over time. In this study, GM concentration in the antibiotic failed to predict GM titers in all patients, and serum sampling during the antibiotic trough period did not necessarily allow the GM level to fall below the positive cutoff in patients. The average time to a negative antigen after antibiotic discontinuation was calculated to be 5.5 days (95% CI = 4.1–7.0) using regression modeling (67).

Cross-Reacting Microorganisms

Fungi

Multiple fungi including several pathogenic species affecting immunocompromised hosts, common human commensals, and potential laboratory contaminants have been shown to cross-react with the antibodies used in the licensed GM assays (Table 3). The magnitude of GM release by fungi other than *Aspergillus* or *Penicillium*, however, is significantly lower in vitro than is seen with either of the two primary genera (39,69). One concern has been that patients colonized with *Aspergillus* or *Penicillium* would have false-positive serum GM tests. This has not been observed in the studies reporting on colonization among hematology–oncology patients (16), lung transplant recipients (50), and children with cystic fibrosis (38).

Bacteria

False-positive GM tests have been reported in neutropenic patients with bacteremia (27,39). When the bacterial isolates from these patients were tested directly, however, no reactivity with the GM EIA was seen (39). Others have failed to confirm the development of false-positive tests in bacteremic patients (37), which suggest that the false positives may actually be related to antibiotic usage or another confounder. In vitro, only Bifidobacteria (except *B. infantis* and *B. adolescentis*) and *Eggerthella lenta* have been shown to possess reactivity with the GM EIA by virtue of cross-reactive lipoglycan epitopes in the cell wall (56). Translocation of these organisms into the systemic circulation, therefore, remains a feasible explanation for false-positive tests in some patients.

Table 3 Non-*Aspergillus* Species that Cross-React with the Galactomannan EIA Test

Fungus

<i>Acremonium</i> species (69)
<i>Alternaria alternaria</i> (69)
<i>Botrytis tuliae</i> (5)
<i>Cladosporium cladosporioides</i> (5)
<i>Cladosporium herbarum</i> (69)
<i>Cryptococcus neoformans</i> (70)
<i>Fusarium oxysporum</i> (6,69) (not <i>F. solani</i>)
<i>Geotrichum capitatum</i> (71)
<i>Paecilomyces variotii</i> (39)
<i>Penicillium chrysogenum</i> (5,69)
<i>Penicillium digitatum</i> (5,39)
<i>Penicillium marneffeii</i> (5)
<i>Rhodotorula rubra</i> (5,69)
<i>Phialophora americana</i> (15)
<i>Trichophyton interdigitalis</i> (5)
<i>Trichophyton rubrum</i> (5)
<i>Wallemia sebi</i> (5)
<i>Wangiella dermatitidis</i> (69)

Factors Associated with False-Negative Tests

The reported sensitivity of the GM immunoassay as an early diagnostic tool for IA has been widely variable, ranging from 22% to 100% overall (9,72). The primary factors influencing sensitivity are selection of the positive cutoff value in addition to receipt of mold-active antifungal compounds and potentially the patients underlying disease.

Impact of Antifungal Therapy

Marr et al. reported a GM EIA sensitivity of 54% with a specificity of 98% in 67 adult and pediatric SCT patients with proven ($n = 13$) or probable ($n = 11$) IA using a positive cutoff of ≥ 1.0 and repeated testing of the same sample to verify positive results (12). Test performance was then recalculated after stratifying for receipt of mold-active antifungal therapy in the two weeks preceding a diagnosis of IA. The sensitivity was significantly lower in those receiving antifungal compounds (18%) as compared to those not receiving empiric or prophylactic treatment (85%) (12). In a separate per-test analysis that used a GM cutoff value of 0.5 in hematology patients with proven ($n = 20$) or probable ($n = 26$) IA, serum samples obtained from patients who were not on mold-active antifungal agents showed a GM sensitivity of 89% (95% CI = 65–97%) as compared to 52% (95% CI = 41–71%) among samples obtained during antifungal treatment or prophylaxis (73).

Underlying Disease

False-negative GM results were reported in a four-year-old boy with chronic granulomatous disease (CGD) and progressive pulmonary aspergillosis despite appropriate antifungal therapy (74). Similarly, Walsh et al. reported reduced expression of GM antigenemia in patients with IA and CGD or Job's syndrome (75). It was hypothesized that angioinvasion might be lower in this host group, thus limiting the amount of circulating GM.

Anti-*Aspergillus* antibodies

One study evaluated the impact that anti-*Aspergillus* antibodies may have on the sensitivity of the GM EIA (31). Serologic testing for antibodies was performed using an in-house developed assay at the onset of infection in 150 episodes of IA. Anti-*Aspergillus* antibodies were detected in 36% of patients with IA despite their immunocompromised status (31), which is similar to separate study of hematology patients with proven IA (33% seroprevalence) (76). Sensitivity of

the GM test was lower in patients with detectable anti-*Aspergillus* antibodies as compared to patients with negative *Aspergillus* serology ($P = 0.0001$) (31).

Galactomannan Testing Using Specimens Other Than Serum

The GM Immunoassay has primarily been validated for use on serum samples. Because the molecule is a water-soluble carbohydrate, it can also be detected in other body fluids as well as in tissue specimens (77,78). There has been particular interest in performing GM testing directly on respiratory specimens for the diagnosis of invasive pulmonary aspergillosis (IPA). Kauffman et al. showed that antigenic determinants released by *Aspergillus* conidia are negative to only weakly positive by immunologic assays, while the components released from growing hyphae are strongly immunogenic (79). It has been extrapolated that the presence of GM in bronchi-alveolar lavage (BAL) fluid might be a better indicator of hyphal growth in the respiratory tract as compared to standard culture for the organism.

Hematology–Oncology Patients

Becker et al. systematically performed BAL in 160 neutropenic cancer patients with radiographic abnormalities on chest CT, who did not have contraindications to the procedure (80). Using standard definitions for proven, probable, or suspected IPA with a positive GM BAL cutoff of >1.0 , the sensitivity, specificity, positive, and negative predictive values of the test were all 100% (80). The sensitivity and specificity of GM detection in serially collected serum from the same cohort were significantly lower (47% and 93%, respectively) than was observed using the BAL specimens. The authors suggest that early initiation of antifungal therapy, based on CT findings, may have impacted the sensitivity of weekly serum GM surveillance. A second investigation assessed the utility of BAL GM in hematology patients (81). Twenty patients with proven or probable IPA in addition to 20 without aspergillosis were included in the analysis. Overall, the sensitivity and specificity were again, both 100% using an OD cutoff value of ≥ 1.5 (81).

Not all studies have found such impressive results using BAL fluid. A relatively large retrospective analysis compared results from 50 HSCT recipients with proven or probable IPA to 50 control patients without IPA (82). The calculated sensitivity of GM in BAL fluid was 61% using an OD cutoff of 1.0 and 76% when a threshold of 0.5 was applied. The corresponding specificities were 98% and 94%, respectively. In addition, Verweij et al. reported a 7% false-positive rate when evaluating BAL fluids from nonneutropenic cancer patients with fever and pulmonary infiltrates visualized on chest X-ray (83).

Solid Organ Transplant Recipients

A recent single-center review of 81 SOT recipients, who underwent bronchoscopy for a variety of reasons, retrospectively evaluated the utility of GM testing of BAL fluid (84). The investigators reported a sensitivity, specificity, positive, and negative predictive value of 100%, 91%, 42%, and 100%, respectively using a GM cutoff of ≥ 1.0 . The sensitivity of GM testing on BAL fluid in this analysis was better than that of serum GM or BAL fungal culture for the diagnosis of invasive IPA. An important observation was that lung transplant recipients (3 of 19 patients) accounted for a significant proportion of the false-positive results in this study (three of seven). The absence of proven or probable cases of IPA in lung recipients, however, precluded a full assessment of the diagnostic utility of the test in this subgroup. The authors hypothesized that the false-positive test results reflected a high rate of airway colonization in the lung transplant population. In a separate evaluation of 116 consecutive lung transplant recipients undergoing bronchoscopy for routine surveillance or suspected rejection, a BAL GM index of ≥ 1.0 had a sensitivity of 60% with a specificity of 98% (85). Seven of the 116 patients (6%) in this study had at least one false-positive test result, with six of the seven patients being colonized by either *Aspergillus* or *Penicillium* spp.

Factors Associated with False-Positive Tests

Airway colonization with *Aspergillus* or other fungal species known to cross-react with the GM EIA is a source of false-positive test results when BAL samples are analyzed (84,85). Plasmalyte (Baxter International Inc, Deerfield, IL), an isotonic solution used to perform the bronchi-alveolar lavage procedure itself, has also been shown to cross-react with the GM EIA (86).

Lastly, Dalle et al. reported false-positive GM results when testing a brain biopsy specimen wrapped in cotton (87). The authors hypothesized that epitopes cross-reactive with the GM *Aspergillus* EIA may be present in this material.

Summary

Individual studies examining the utility of repeated serum GM monitoring for IA in high-risk patients have reported inconsistent results. The observed variability in test performance is likely related to multiple factors including heterogeneity in study design in addition to inherent differences in the populations being studied, plus or minus prior receipt of antifungal therapy in some patients. Such discrepancies make it difficult to generalize results across studies. A recent meta-analysis synthesized the existing English and Spanish literature on serum GM screening, with a total of 27 studies meeting the investigator's inclusion criteria (72). The pooled mean sensitivity was 71% (95% CI = 0.68–0.74) and specificity 89% (95% CI = 0.88–0.90) for proven cases of IA. For proven or probable disease, the mean sensitivity was 61% (95% CI = 0.59–0.63) and specificity 93% (95% CI = 0.92–0.94). The test appeared to perform best in patients with hematological malignancies and following SCT while studies that used strict EORTC/MSG criteria for proven or probable IA tended to report lower sensitivities.

In the majority of hematology–oncology studies performed to date, the negative predictive value of serial serum GM testing has been good, which suggests that the test can be useful to help exclude the possibility of IA in this patient population. False-negative tests do occur, however, and have been associated with anti-*Aspergillus* antibodies, mold-active antifungal therapy, and minimally invasive disease. The reasons for false-negative results are not always readily apparent and future studies must strive to better define confounding factors. False-positive tests are also problematic. Sequential positive results with a rising OD index are typically more suggestive of true infection than are single or isolated determinations.

The clinical utility of GM antigen detection in specimens other than serum is also not well established. Detection in other body fluids may provide corroborative evidence of IA as well as help to exclude the possibility of a false-positive or false-negative serum test. A recent review of the literature concluded that, at best, GM detection in urine, cerebrospinal fluid, BAL, or tissue is a promising diagnostic tool to be used in conjunction with serum monitoring (88). Looking across studies of BAL fluids specifically, the sensitivity has ranged from 60% to 100% for the diagnosis of IPA.

(1,3)- β -D-GLUCAN

Assay Principles

(1,3)- β -D-Glucan (BDG) is a cell wall component found in high concentrations in a wide variety of fungi; the notable exceptions are *Cryptococcus* and the *Zygomycetes* (89–91). Several assays have been developed that are capable of measuring glucan levels in the serum of patients with suspected IFI, but a positive reaction does not yield a genus-specific diagnosis. All of the currently available BDG tests are predicated on the ability of the glucan molecule to induce clot formation in the hemolymph of horseshoe crabs (Fig. 2). Fungi containing BDG within their cell wall structure activate Factor G, a serine protease present in the horseshoe crab coagulation cascade (92,93). Upon contact with trace amounts of BDG (analytical sensitivity approximately 1 pg/mL), crab amebocytes degranulate and release the zymogens that become activated Factor G. Activated factor G then converts inactive proclotting enzyme to the activated form, which in turn cleaves an artificial substrate used for detection via chemiluminescence or turbidity. The commercially available chromogenic assays include FungiTec-G (Seikagaku Kogyo Corporation, Tokyo, Japan), and Fungitell (Associates of Cape Cod, Falmouth, MA) (40). Fungitell, previously called Glucatell, is FDA cleared for the diagnosis of IFI in the United States. The Wako test is a turbidimetric-based assay manufactured by Wako Pure Chemical Industries (Osaka, Japan) (40).

Kinetics

Little is known about the release and metabolism of BDG in vivo. Like galf antigens, BDG is released during the logarithmic growth phase of *Aspergillus fumigatus* in vitro (95). In culture,

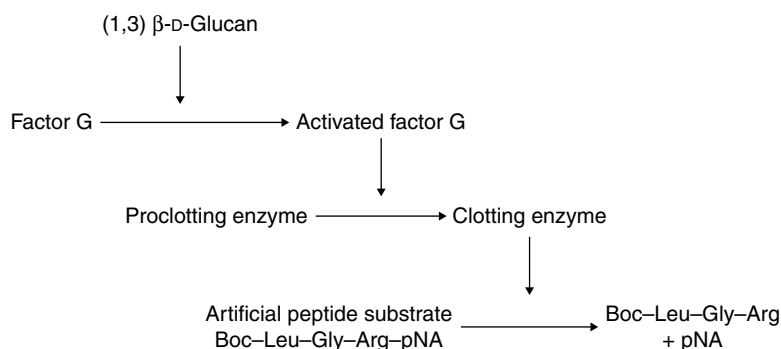


Figure 2 Fungitell (1,3)- β -D-glucan assay. (1,3)- β -D-Glucan activates factor G, a serine protease zymogen in the *Limulus* (horseshoe crab) coagulation cascade. Activated factor G converts the inactive proclotting enzyme to the active form, which in turn cleaves pNA from the chromogenic peptide substrate Boc-Leu-Gly-Arg-pNA, creating a chromophore that absorbs at 405 nm. *Source:* Adapted from Ref. 94.

BDG concentration begins to decrease after 24 hours, which is thought to be related to the activity of *Aspergillus* cell wall-associated glucanases (95). Similar evaluations with other medically important species (e.g., *Candida* spp., *Fusarium* spp., *Pneumocystis*) have not been published.

Defining a Positive Result

The BDG level defining a positive test varies by assay. A provisional cutoff for the Fungitell test was determined using stored serum samples collected from 30 nonneutropenic subjects with symptomatic candidemia (96). These results were then compared to samples collected from 30 healthy adults. Sera obtained from the candidemic subjects contained a mean BDG concentration of 2999 pg/mL (range, 36–22,263 pg/mL) as compared to 17 pg/mL (range, 0–86 pg/mL) in blood of healthy volunteers. A positive cutoff value of ≥ 60 pg/mL was selected in this study because it appeared to optimize both the sensitivity (97%) and specificity (93%) of the test (96). The FDA-approved protocol defines values ≥ 80 pg/mL as positive, and levels from 60 to 79 pg/mL as “equivocal” results (94). The ≥ 80 pg/mL was established in a multicenter validation study reviewed in the following section (97).

The Fungitell cutoff is higher than the values established for the Fungitec-G and Wako assays. A 20 pg/mL threshold was set for Fungitec-G test based on the observation that plasma BDG levels in 60 healthy adult controls were all < 10 pg/mL and 37 of 41 patients with autopsy-verified or microbiologically documented fungal infections had concentrations above this level [sensitivity 90% (95% CI = 77–97%)] (98). At a concentration > 20 pg/mL, the Fungitec-G test also showed 100% specificity when analyzing 59 samples from subjects with nonfungal infection or fever of noninfectious origin (98). The dissimilarity of cutoff values among chromogenic test kits may be related to differences in the affinity/reactivity of reagents used in each assay. Reagents utilized in the test Fungitell are extracted from *Limulus polyphemus*, a different genera of horseshoe crab than is used in Fungitec-G (*Tachypleus tridentatus*) (96).

Test Performance

The majority of studies that have evaluated the clinical performance of BDG testing have been conducted using selected patients. There is relatively little experience using the assay for IFI surveillance. The current literature is summarized below and is displayed in Table 4.

Studies Using Samples Collected from Cases and Controls

Results from the multicenter study that contributed to the FDA approval of the Fungitell assay were recently published (97). Single serum samples were collected from patients within 72 hours of a diagnosis of proven or probable IFI (based on EORTC/MSG criteria). Results from the IFI cases ($n = 170$) were then compared to unmatched control specimens ($n = 163$) obtained from healthy volunteers, outpatients, or hospitalized patients with medical problems other than IFI. The majority of fungal infections in this analysis were due to *Candida* (65%), followed

Table 4 Performance of Serum (1,3)- β -Glucan for the Diagnosis of Proven or Probable Invasive Fungal Infection in Immunosuppressed Patient Populations

Study	Assay	Cutoff (pg/mL)	Frequency of sampling	No. of samples required for positivity	Proven cases	Probable cases	Prev (%)	SN	SP	PPV	NPV
(27)	Fungitell	120	2/wk	1	5	3	5.19	0.88	0.90	0.70	0.96
(99)	Fungitell	120	2/wk	1	3	3	3.90	0.83	0.90	0.63	0.96
(94)	Fungitell	80	NR	1	NR	NR	NR	0.65	0.81	0.81	0.77
(96)	Fungitell	60	NR	1	16	4	7.07	1.00	0.90	0.43	1.00
(100)	FungiTec	20	1/wk	1	16	NR	7.44	0.63	0.76	0.16	0.96
(45)	FungiTec	20	1/wk	1	33	NR	27.05	0.67	0.84	0.61	0.87
(52)	Wako	11	1/wk	1	9	2	11.46	0.55	0.98	0.67	0.96

Abbreviations: NR, not reported; Prev, prevalence; SN, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.

by *Aspergillus* (13%), and *Cryptococcus* (7%). At a cutoff of 60 pg/mL, the overall sensitivity, specificity, PPV, and NPV of the test were 70%, 87%, 84%, and 75%, respectively. Using the 80 pg/mL threshold, the test was less sensitive but more specific (sensitivity 64%, specificity 92%, PPV 89%, and NPV 73%). In the subset of 107 patients with proven invasive candidiasis (IC), the test was 81% sensitive at the 60 pg/mL level. Interestingly, differences in sensitivity were noted among *Candida* species, with the assay detecting *C. parapsilosis* less often than other species. The authors correlate this observation with the fact that the echinocandin class of antifungal agents, which act through the inhibition of BDG synthesis, tend to have higher MICs for *C. parapsilosis*. Additional sub-group analyses of other fungi showed a sensitivity of 80% for the detection of *Aspergillus* spp. ($n = 22$) and 100% for *Fusarium* spp. ($n = 3$). Only 3 of 12 subjects with cryptococcosis had a positive test, while none of the subjects infected with *Mucor* ($n = 2$) or *Rhizopus* ($n = 1$) had BDG levels above the 60 pg/mL threshold. Lastly, the investigators sought to assess the impact of antifungal therapy on the sensitivity of the BDG test. Of the 142 subjects with proven IFI, sensitivity was higher in those not receiving antifungal therapy [83% (20 of 24 subjects with a positive test)] as compared to those receiving antifungal drugs [73% (86 of 118) with a positive test]. This difference did not reach statistical significance ($P = 0.69$), but may be clinically relevant.

A second smaller study evaluated the Fungitell assay using residual serum and plasma obtained from patients who had blood cultures growing with yeast or bacteria (101). Sera that remained after testing for *Histoplasma* antigen or GM *Aspergillus* EIA were also analyzed in conjunction with blood obtained from healthy donors. A threshold of >80 pg/mL was used to define a positive BDG result in this study. None of the healthy volunteers had a positive BDG test. Of the 39 samples obtained from 15 patients with fungemia, 30 were positive (77%) and two were in the "indeterminate" range (60–79 pg/mL). The impact of antifungal therapy, especially in samples that were collected after the blood culture result had been reported to the care providers, was not assessed. Twenty-two of 36 samples drawn from 15 patients with gram-positive bacteremia also had a positive BDG test and 11 of these patients had ≥ 1 positive samples. On review of the medical record, six patients with gram-positive bacteremia had received a β -lactam antibiotic and one had received intravenous immunoglobulin. Three of 10 patients with gram-negative bacteremia also developed false-positive BDG tests, one of whom was treated with a β -lactam drug. All 6 patients with a positive *Histoplasma* antigen and 31 of 32 patients with a GM antigenemia were also BDG positive.

Digby et al. collected single serum samples from 53 patients admitted to the ICU as well as from eight healthy control subjects (102). The ICU patients were stratified as either being infected ($n = 46$) or uninfected ($n = 7$). Surprisingly, definitions for what constituted an "infection" were not specifically stated in the study methods. Using the Fungitec-G assay and a positive threshold of 20 pg/mL, none of the healthy volunteers had BDG detected but two uninfected ICU subjects had a positive test. Thirty-five of the 47 "infected" ICU patients

(74%) had a positive BDG test. Unfortunately, no breakdown of BDG results by underlying infection (i.e., causative pathogen and/or site) or by treatments known to be associated with false-positive BDG tests was provided. Overall, there was no statistical difference in the mean BDG level observed in patients with fungal infections as compared to those with bacterial infections.

Studies Using Samples Prospectively Collected from Selected Patients

Pazos et al. collected serum twice weekly for IFI monitoring in addition to obtaining weekly surveillance cultures for yeasts (27,99). Two separate analyses of adult hematology patients ($n = 154$) considered at high-risk for IA were performed. In the first study, 40 selected neutropenic patients (5 with proven IA, 3 with probable IA, 3 with possible IA, and 29 without IA) were identified (51). Patients were judged to be positive by Fungitell if their BDG level was ≥ 120 pg/mL in a single sample. All of the proven IA cases and two of three probable IA cases had at least one positive BDG test. Ten percent of patients (3 of 29) without IA also had BDG detected at least once. The first patient had *Escherichia coli* bacteremia, the second had low level *Candida* colonization of a single site, and third had high levels of *Candida* cultured from multiple sites. The number of patients colonized with yeasts who had negative BDG tests was not described. Analysis of BDG kinetics was useful for interpreting the validity of positive tests in this study. In the patients with proven disease, BDG levels rose consistently before clinical or microbiologic evidence of IA existed. Levels then decreased if the patient improved with antifungal therapy. Patients with false-positive results tended to have abrupt rises and falls in BDG levels in the absence of antifungal treatment. In the second analysis, banked serum samples from another 35 selected patients (three with proven IC, three probable IC, and 29 without IC) were evaluated to determine the ability of the Fungitell assay to identify patients with candidiasis (99). A ≥ 120 pg/mL cutoff in a single sample was again employed. All three patients with proven IC had a positive test, two of three with probable disease had BDG detected, as did three of the control subjects. Similar to their previous analysis, patients with proven IC had consistent rises in BDG which preceded clinical or microbiologic evidence of infection. BDG levels then declined in response to effective antifungal therapy.

Evaluations of Serial Monitoring

The efficacy of twice weekly BDG monitoring was studied in 283 consecutive neutropenic adult patients with newly diagnosed AML or MDS, who were receiving mold-active antifungal prophylaxis (96). Using a Fungitell cutoff value of 60 pg/mL, all of the patients with proven ($n = 16$) or probable ($n = 4$) IFI had at least one serum sample positive for BDG. The proven and probable infections were due to *Candida* spp. ($n = 11$), *Aspergillus* spp. ($n = 4$), *Trichosporon asahii* ($n = 3$), and *Fusarium* spp. ($n = 2$). Ten patients (4%) without IFI had at least one positive BDG test. The specificity of the test increased from 90% for one positive sample to 96% and 99% when two and three sequential samples, respectively, were required to define a positive result. The sensitivity declined correspondingly when consecutive positive samples were required (i.e., sensitivity 100% for one positive sample, 65% for ≥ 2 sequential positive samples, and 60% for ≥ 3 sequential positive samples), suggesting that glucan antigenemia may be transient in some patients. The potential impact of antifungal therapy in these cases, however, was not explored. On average, BDG was positive 10 days before the clinical diagnosis (range, 32 days before to 2 days after) in this analysis.

Once a week monitoring has also been assessed using the Fungitec-G assay (100). This study included 215 consecutive hematology–oncology patients undergoing cytotoxic chemotherapy with systemic antifungal prophylaxis. Sixteen subjects went on to develop “definite” IA and 14 had “suspected” IA. Using a >20 pg/mL cutoff, the sensitivity and specificity of the test for definite IPA was 63% and 76%, respectively. For definite or suspected IPA, the test was 60% sensitive. BDG typically became positive after the onset of fever (mean 24 days later, range 0–53) and later than CT findings (mean 12 days later, range –7 to 40) in patients with definite lung disease. Other IFIs included four cases of IC and one zygomycosis. All four patients with IC had at least one positive BDG test and the results for the patient with zygomycosis were not specifically described. False-positive tests occurred in 40 patients (19%) without evidence of IFI, with a disproportionate number of these observed during periods of neutropenia

($P < 0.001$). Potential explanations for the high false-positive rate in this study were not explicitly explored.

(1,3)- β -D-Glucan as a Predictor of Response to Empiric Fluconazole Therapy

Takesue et al. enrolled surgical patients with risk factors for IC, documented *Candida* colonization, and fever despite antibiotic therapy (103). Patients with documented IFI and those who had received antifungal therapy in the preceding month were excluded. Before starting empiric fluconazole therapy in these high-risk patients, plasma BDG concentration was measured using the Fungitec-G assay. A total of 64 patients were included in the analysis, 18 of whom responded clinically to empiric fluconazole. Thirty-two patients (50%) had a BDG level >20 pg/mL. Half of these (16 of 32) responded serologically to empiric antifungal therapy (6 normalized, 10 improved) and 15 (47%) improved clinically. Patients with a positive BDG test were more likely to respond to fluconazole treatment [15 of 32 (47%)] than were those who had a negative BDG test [3 of 32 (9%)] ($P < 0.01$). Additionally, there was a significant difference in BDG levels between patients who showed a positive response to treatment as compared to those who did not respond (105.0 pg/mL \pm 127.5 for responders vs. 26.7 pg/mL \pm 51.1 for nonresponders; $P = 0.02$). These findings suggest that BDG assessment could also be useful as a trigger for initiation of antifungal therapy in high-risk surgical patients.

(1,3)- β -D-Glucan as a Predictor of Catheter-Associated Candida Infection

Nett et al. have proposed using BDG as a surrogate marker of biofilm formation by *Candida* (104). Biofilms are microbial communities growing on prosthetic material such as intravenous catheters. Nearly all device-associated infections involve microbial growth in a biofilm and *Candida* cells growing in this way are up to 1000-fold more resistant to antifungal agents than are their free-floating counterparts (105,106). Nett et al. used the Fungitell assay to quantify BDG in the supernatants from planktonic and biofilm cultures and assessed serum obtained from rats with catheter-related disseminated candidiasis (104). They observed increased BDG content in the cell walls of *C. albicans* growing in biofilm as compared to planktonic organisms both in vitro and in the rat model. The authors suggest that the high levels of BDG secreted from biofilms may be a useful diagnostic tool to identify the development of device-associated infections and, in theory, identify patients that would benefit most from catheter removal. Additional studies are needed to determine whether this assay can reliably identify *Candida* biofilms and/or device-associated infections in humans.

(1,3)- β -D-Glucan for the Diagnosis of Pneumocystis Pneumonia

Pneumocystis ribosomal RNA phylogenetically resembles that of fungi and glucan can be detected in the organism's cyst wall (107,108). Several groups have reported on the utility of BDG testing as a noninvasive diagnostic for *Pneumocystis jirovecii* pneumonia (PCP). Studies to date have largely been retrospective analyses of single center experiences, each using a different BDG assay. Marty et al. identified 16 patients who had serum BDG measurements performed around the time PCP was diagnosed by immunofluorescent staining of respiratory samples (109). BDG was positive (≥ 80 pg/mL by Fungitell) in 15 of 16 (94%) patients with a median value of >500 pg/mL (range, 141 to >500 pg/mL). The BDG concentration decreased but remained elevated in five patients who had subsequent measurements performed on treatment. Tasaka et al. evaluated 57 patients with PCP diagnosed by Grocott-Gomori methenamine and Calcofluor white staining of BAL fluid (110). Serum BDG levels were significantly increased in PCP-positive patients ($P < 0.0001$). Using receiver-operator characteristic curves and the Wako BDG test, a cutoff of 31.1 pg/mL yielded a sensitivity, specificity, PPV, and NPV of 92%, 86%, 61%, and 98%, respectively. Compared to other nonspecific markers such as elevated LDH or C reactive protein, BDG was the most reliable noninvasive indicator of PCP in this cohort. Fuji et al. also used the Wako assay to measure serum BDG in 28 AIDS patients with PCP (111). All but one patient had a positive test (threshold > 5 pg/mL), with BDG levels ranging from 5 pg/mL to 6920 pg/mL. Another group used the Fungitec-G test to analyze BAL fluid and serum from AIDS patients with and without PCP (112). The mean level of BDG in BAL fluid from PCP patients ($n = 4$) was significantly higher than in BAL fluids from patients with other lung diseases ($n = 4$) [7268 pg/mL (range, 1355–15,500) in patients with PCP vs. 243 pg/mL

(range, 17–615)]. Elevated serum BDG levels were also observed in six of seven patients with PCP [494 pg/mL (8.5–1135)].

Factors Associated with False-Positive Tests

Laboratory contamination of samples is possible if glucan-free glassware and/or plastics are not used when performing the test. False-positive BDG results have also been associated with immunoglobulin therapy, albumin supplementation, and blood products filtered through cellulose filters (113–116). Likewise, cellulose-containing dialysis membranes are known to cause false-positive BDG tests in some patients (117). Awareness of a patient's surgical history is also essential, as serosal exposure to glucan-containing gauze has resulted in positive BDG tests in the immediate postoperative period (118).

Given the potential for fungal cell wall components or residua from cellulose-containing material to be present in antibiotic preparations, Marty et al. tested 44 different antimicrobials for the presence of BDG (119). Colistin, ertapenem, cefazolin, trimethoprim-sulfamethoxazole, cefotaxime, cefepime, and ampicillin-sulbactam all tested positive for BDG at the reconstituted vial concentrations, but not when diluted to maximal plasma concentrations. Interestingly, none of the lots of piperacillin-tazobactam tested in this study were positive for BDG. Other investigators have correlated false-positive BDG results obtained from patients receiving amoxicillin-clavulanic acid with direct detection of the molecule in the batches of antibiotic used for treatment (120).

Summary

Although BDG testing has been widely used in Japan, experience with the FDA-cleared Fungitell assay remains somewhat limited. Many of the methodological weaknesses that have plagued evaluations of GM have also impaired assessment of (1,3)- β -D-glucan utility. Study design issues including the use of heterogeneous patient populations with a variable prevalence of IFI, use of inappropriate control subjects (i.e., healthy volunteers), use of different criteria to diagnose IFI and/or a positive BDG test, all make generalization of results across studies difficult. Moreover, false-positive tests have been reported in up to 19% of patients and are thought to be due to cross-reacting substances in certain medications, materials, or possibly in bacteria. *Candida* colonization alone, however, has not convincingly been shown to cause BDG positivity (121).

Based on the favorable negative predictive value observed in most of the prospective studies, BDG may be most useful for excluding IFI in high-risk patients. It also has potential as a surrogate marker of PCP in the appropriate clinical setting and could be useful as a guide for empiric antifungal therapy in surgical patients at high risk for IC. The combination of BDG testing with other circulating markers of IFI may help to identify false-positive tests, and BDG kinetics might assist in the differentiation of true and false-positive results. More clinical research is needed, however, to better define the optimal testing strategy and to further elucidate the role the assay could have in preemptive treatment strategies.

MOLECULAR DIAGNOSTICS

Given the significant limitations of culture, histological, and serological techniques, considerable effort has been made toward the development of robust tests that amplify and detect fungal nucleic acid directly in clinical specimens. A few groups have described their experience with isothermal mRNA nucleic-acid sequence-based amplification (NASBA) (48,122–124), but the primary body of literature is accumulating in support of DNA detection by polymerase chain reaction (PCR). PCR technology is discussed here.

PCR is rapid, can be performed quantitatively, and is applicable to a variety of specimens including blood, body fluid, and tissue. Assays have been designed to detect a broad range of medically important fungi as well specific genera and/or species. The application of fungal PCR in clinical practice, however, has been severely limited by a lack of standardization across laboratories as well as by the need for relatively expensive equipment and specialized expertise. There are currently no well-validated, commercially available, fungal PCR assays.

Kinetics

Two groups have assessed the kinetics of fungal DNA release using experimental models. The first team analyzed filtrates from *A. fumigatus* cultures using a standard PCR assay (95). DNA was not detected in culture supernatants during logarithmic growth but was found during the lytic phase, which corresponded with autolysis of fungus due to lack of nutrients. Kasai et al. measured *C. albicans* DNA with a quantitative real-time PCR (qPCR) using culture supernatants and the blood of rabbits with disseminated candidiasis (125). Significant rises in extracellular, or free DNA, was observed as early as two to four hours after culture setup. Rabbits with IC had increasing DNA levels detected in both whole blood as well as in plasma as disease progressed. The clinical significance of these observed differences in the timing of DNA release by *A. fumigatus* as compared to *C. albicans* in vitro is not readily apparent at this point.

Specimens

PCR analysis of blood has become the favored approach for IFI surveillance. The optimal blood fraction (serum, plasma, whole blood, or the white cell fraction), however, is uncertain. Costa et al. performed qPCR on blood samples spiked with 10 ng of *A. fumigatus* and showed that the DNA yield from serum and the white blood cell pellet were similar, but plasma contained significantly less detectable DNA (126). These observations were supported in a separate analysis of 14 serum, plasma, and white cell pellets collected from three patients with either confirmed or probable IA (127). A second group tested plasma and whole blood specimens from three BMT patients with proven IA (128). Nineteen specimens were PCR positive in both fractions, whereas an additional 22 had DNA detected only in whole blood. Comparisons of plasma versus whole blood (120) and serum versus whole blood (129) have also been done using candidemic rabbit models. Serum or plasma PCR was equivalent to whole blood PCR using culture positive samples, but serum was more often PCR positive than whole blood when culture negative samples were assayed (27% vs. 7% positive; $P < 0.05$) (129). Serum PCR was shown to be in full agreement with culture in 12 candidemic patients, but 9 of 16 patients with suspected candidemia and negative blood cultures had *Candida* DNA detected in serum (130). Based on these limited data, serum may be the optimal fraction for the detection of both *Aspergillus* and *Candida* because it does not require cell lysis or separation steps, is less likely to contain PCR inhibitors such as heparin (131), and can be used for simultaneous antigen testing. Others might argue that because circulating leukocytes are known to phagocytose fungal hyphae, whole blood should result in highest DNA yields, but this has not been reproducibly established.

DNA amplification in BAL specimens has been used to help establish a diagnosis of IPA (81–83,52,132,133). Concern has been raised, however, about the potential for inhaled *Aspergillus* spores, conidia ubiquitous in the environment, and/or uncomplicated airway colonization to cause a positive PCR reaction. The advent of qPCR allows for measurement of the fungal burden in the airways (52,82,133), but no definitive threshold that is predictive of invasive disease has been identified (134). Additional considerations include quality of the specimen; for example, if the lavage does not sample the area of the lung that is most involved, inadequate quantitation, or even false-negative results may occur (134).

Technical Features

Multiple technical issues, in addition to sample selection, have hampered the generalizability of fungal PCR results across studies. The nucleic acid extraction technique, the gene target exploited, and the platform selected for amplification and detection will all impact test performance.

Nucleic Acid Extraction Methods

The most significant impediment to efficient nucleic acid extraction from clinical specimens is the fungal cell wall, which is not readily susceptible to lysis. All extraction methods, therefore, involve: (i) lysis of red and/or white blood cells (if a cellular fraction of blood is used), (ii) breakdown of the fungal cell wall either by enzymatic, chemical, or mechanical means, (iii) disruption of the cell membrane to release DNA, and (iv) DNA purification followed by isolation. Multiple protocols have been described and compared, but no single method is optimal for all fungal pathogens (135–139). In general, commercially available reagents and kits have the

advantage of promoting standardization, and automated methods are desirable for laboratory workflow. Unfortunately, contamination of commercial reagents with fungal DNA has been described (140), and this must carefully be controlled for in the quality assurance process.

Fungal Targets and Primer Design

Selection of the gene target sequence is an essential step in assay design. The decision to target multi- or single-copy genes will influence the performance of the assay. Multi-copy genes can bolster the sensitivity of an assay because a larger number of molecular targets will be present in each sample, while single-copy genes can be highly species specific. The majority of studies to date have utilized multi-copy genes including the ribosomal DNA (rDNA) gene cluster, *Aspergillus* or *Candida* mitochondrial genes, the *Aspergillus* alkaline proteinase gene, or the *Candida*-secreted aspartic proteinase gene.

The rDNA complex has been the most widely exploited fungal PCR target. This region comprises three highly conserved subunits, the small subunit (SSU) 18S gene, the 5.8S gene, and the large subunit (LSU) 28S gene. Intergenic transcribed spacer (ITS) sequences, ITS1 and ITS2, are positioned between the 18S and 5.8S and the 5.8S and 28S genes, respectively, and vary markedly across various fungal species. Amplification and detection within the rDNA complex allows for the “panfungal” detection of a wide range of fungal genera and/or species simultaneously. Species level discrimination can then be accomplished using either species-specific hybridization probes (141–143), restriction fragment length polymorphism (RFLP) analysis (144), single-strand conformational polymorphism detection (145), or by sequencing the amplicons (146,147). The latter method is particularly promising given its high specificity, reproducibility, and easy automation.

Amplification Techniques

Multiple amplification and detection formats have been used in the assays published to date. Many groups have elected to use a nested PCR design, mainly to improve sensitivity. Nested PCR involves two sets of primers and two rounds of DNA amplification. The first set of primers is used for the initial 15 to 30 cycles of PCR. Products from this reaction are then re-amplified using a second set of primers designed to anneal to sequence within the product generated by the first primer pair. Amplification by the second primer pair increases the DNA yield and also verifies the specificity of the first PCR reaction. The major disadvantage of nested PCR is the high risk of carryover contamination that occurs during the transfer of amplicons generated in the first round of PCR to a reaction second tube.

In recent years, real-time PCR has become the preferred PCR platform in many clinical microbiology laboratories and reports of its utility for the diagnosis of IFI are emerging (52,121,135,136,148–157). Real-time PCR means that DNA amplification and detection take place simultaneously in the same reaction tube. Postamplification handling is not required, which greatly reduces the potential for contamination. An additional advantage is that real-time assays can be quantitative. Quantification of fungal burden is theoretically useful for predicting the development of disease or monitoring treatment response. Real-time PCR typically yields a high degree of sensitivity in conjunction with a broad dynamic range of quantitative linearity; however, these assays may be less sensitive than nested designs (44,133,158).

Prospective Surveillance Studies

Hematology–Oncology and Stem Cell Transplant Patients

Fluorent et al. collected serum twice weekly from patients with hematological malignancies for IA surveillance using a PCR method that amplified a fragment of the *Aspergillus* mitochondrial gene common to *A. fumigatus* and *A. flavus* (159). During the study period, 33 proven ($n = 4$) or probable ($n = 29$) cases of IA were observed among 201 patients. Most of those with proven or probable IA (88%) had at least one positive PCR test and a significant proportion of patients in the possible (72%) as well as no IA groups (45%) had single positives. Of note, all of the patients with proven disease had consecutive positive PCRs, as did 59% of probable cases, 28% of possible cases, and 10% of patients without IA. In the majority of subjects, PCR positivity preceded or was simultaneous with clinical indicators of infection. A second study of acute

leukemics and SCT patients also used a nested PCR to target both *A. fumigatus* and *A. flavus* (160). Twelve (71%) of 17 patients had *Aspergillus* nucleic acid detected in whole blood as a part of weekly surveillance (two of three with probable IA, four of four with possible IA, and six of 10 with no IA). Consistently positive results were observed in just one patient with probable IA and intermittent positivity did not predict disease. In contrast, a third study failed to observe the same degree of "false positivity" when using a single positive PCR to define a positive test result (161). Hebart et al. monitored the whole blood of 92 patients after SCT and/or during periods of neutropenia once a week. Samples were analyzed using a panfungal PCR specific for *Aspergillus* and *Candida* species. PCR positivity was documented in 34 of 92 (37%) patients. Of those with a positive PCR, eight had a previous history of IFI, four developed proven IFI, five developed probable disease, 11 had neutropenic fever despite antibiotics and received empiric antifungal therapy, and the remaining six (18%) neither developed clinical signs of IFI nor received empiric antifungal therapy. PCR was the earliest indicator of IFI preceding clinical evidence of infection by an average of six days (range, 0–14 days). The number of patients with consecutive and/or intermittent positive PCR results was not described.

Two additional studies required consecutive PCR positive whole blood samples to define a positive test. White et al. monitored a group of 203 at-risk hematology patients twice weekly (162). Using an *Aspergillus*-specific nested PCR, the positivity rate for patients with proven/probable IA was 92%, compared with 15% for possible IA, and 5% for patients with no IA. The second study also involved twice weekly monitoring in 78 hematology–oncology patients (163). A real-time panfungal PCR detecting both *Aspergillus* and *Candida* species was employed. Sequential positive PCR results were observed in four of the five (80%) cases of proven IFI, two of the three (67%) cases of probable disease, and six of 11 (55%) possible cases. All of the proven disease was due to *Candida* and the PCR became positive before empiric antifungal therapy was initiated based on clinical suspicion in the majority of cases. Consecutive positive results were also obtained during 29 of 106 (27%) episodes where no IFI was suspected, but five of these patients did subsequently go on to develop IFI at a later time point.

Solid Organ Transplant Recipients

A single study evaluated the utility of PCR screening for IFI following SOT (164). Whole blood specimens collected at weekly intervals from 48 liver transplant recipients were analyzed using a panfungal assay that was capable of detecting and differentiating multiple *Aspergillus* and *Candida* species. Ten patients developed proven ($n = 3$) or probable ($n = 7$) disease, with all but one infection due to *C. albicans*. A single positive PCR result had a sensitivity, specificity, PPV, and NPV of 83%, 92%, 77%, and 94%, respectively. PCR became positive an average of 21 days (range, 7–70 days) before clinical signs of IFI was present. The number of patients with sequential or intermittent positive results was not described.

Patients with Febrile Neutropenia or Suspected IFI

Several investigators have speculated that PCR screening could be particularly valuable in patients with neutropenic fever and/or in those with suspected IFI. In a subgroup of 51 neutropenic patients without a history of IFI, a single positive panfungal PCR performed within 72 hours of fever onset had a 100% sensitivity, 73% specificity, 37% PPV, and 100% NPV for proven or probable IFI (161). Similar test performance was reported in an analysis of 65 patients with neutropenic fever using whole blood assayed twice weekly with a nested *Aspergillus* PCR (165). When two, not necessarily consecutive, positive PCRs were used to define a positive test, the sensitivity, specificity, positive, and negative predictive values were 100%, 75%, 46%, and 100%, respectively for proven or probable IA ($n = 13$). Median time between positive results was 10 days (IQR, 5–18 days) in patients with proven/probable IA versus 21 days (IQR, 13–32 days) for the no IA group. Consecutive positives occurred in 62% of proven/probable cases. If preemptive antifungal therapy were initiated in response to two positive PCR results, the use of empiric antifungal treatment would have been reduced by up to 23% in patients with no IA at this institution. Williamson et al. collected at least two serum samples from 18 BMT patients with suspected IA (166). Samples were also obtained from 19 additional matched patients without IA. A nested *Aspergillus* PCR was used for sample analysis. All of the patients with IA (six proven, nine probable, three possible, two suspected) had positive PCR results, as did four of

the 19 patients without IA. If two positive PCR results were required to define a positive test, excluding the cases of suspected IA, the sensitivity of their PCR was 81%, specificity 100%, PPV 100%, and NPV 90%.

Impact of Antifungal Therapy on the Detection of Fungal DNA

A single study attempted to determine the impact of antifungal therapy on the detection of *Aspergillus* DNA (167). The investigators evaluated patients with hematological malignancy or SOT who were receiving treatment due to clinical and radiographic findings suggestive of IA. Of the 36 patients identified over a three year period, 15 had proven, 9 had probable, and 12 had possible IA. Only 40% of patients with proven disease had fungal DNA detected in whole blood, while 100% had a positive test using BAL fluids who were receiving therapy. The clearance of fungal DNA from blood was associated with clinical improvement in six of nine patients and repeated positive results were associated with a fatal outcome.

***Aspergillus* PCR using BAL Specimens**

Multiple groups have applied PCR to BAL washings for the diagnosis of IPA. Taking the studies conducted for the evaluation of pneumonia in patients with underlying malignancy as a whole, 74% (89/121) of patients with proven or probable IPA had *Aspergillus* DNA detected in BAL fluid (81–83,132,133,168). Studies varied in their definitions of proven/probable IPA, PCR assay design, and whether or not patients were on mold-active antifungal therapy at the time bronchoscopy was performed. Each investigation also included a control group, but descriptions of these subjects were not provided in all of the reports. Overall, 5% (20/370) of patients not suspected of having IPA had PCR positive BAL results. Higher false-positive rates (22%) have been observed in studies that included a broader range of immunosuppressed with a lower pretest probability for IPA (e.g., HIV positive patients) (169). PCR methods proved to be more sensitive than culture and equivalent or less sensitive than antigen detection in BAL (81–83). In the studies that performed qPCR, culture-positive samples tended to have higher levels of fungal DNA than culture-negative specimens (81,82).

Summary

Caution must be exercised when comparing the results of in-house PCR assays, but in general, well-designed fungal PCRs are highly sensitive and specific. The analytic sensitivity (i.e., lower limit of detection) of published assays has consistently been high, on the order of 1 to 10 colony-forming units per mL blood or as little as 1 to 10 fg DNA required for a positive PCR reaction. As a result, it is often difficult to know whether a positive PCR reaction obtained from a patient with no clinically apparent IFI reflects the development of early or subclinical disease, transient DNAemia possibly related to colonization and/or circulation of nonviable hyphae, or, alternatively, is a false-positive test. Nested PCR assays in particular have the potential to generate carryover contamination and commercial reagents have been shown to contain fungal nucleic acids on occasion. Care must be taken to control and monitor for laboratory contamination as a source of false-positive PCR reactions.

One approach to improve clinical specificity of fungal PCR has been to rely on consecutive or intermittently positive reactions obtained in close temporal proximity to define a positive test. The trade-off is that this strategy may also decrease clinical sensitivity in some cases of IFI. Concomitant GM or BDG assessment might also facilitate the interpretation of positive PCR results in patients without signs or symptoms of IFI and future studies correlating fungal DNA load with the likelihood of disease will be useful to determine whether genome copy number could also be used to define a positive PCR test.

At the present time, the absence of fungal DNA appears to have the most clinical value. Most studies have demonstrated an excellent negative predictive value of PCR, particularly in patients with neutropenic fever that is unresponsive to broad-spectrum antibiotic therapy. When a negative fungal result is obtained in these patients, an alternative diagnosis should be sought. One caveat to this general rule may be patients receiving empiric antifungal therapy, where the sensitivity for detecting fungal DNA in blood has been shown to be significantly reduced.

STUDIES COMPARING OR COMBINING DIAGNOSTIC METHODOLOGIES

A few studies have directly compared fungal diagnostics. All have focused on *Aspergillus* specifically and no single test or method has emerged as being superior. Pazos et al. compared the Glucatell BDG test with the Platelia *Aspergillus* EIA for twice weekly IA surveillance (27). The two tests performed identically in hematology patients. The false-positive rate was the same, but false positives were observed in different patients suggesting that combined testing could be useful. Using a combination of the two assays, the specificity and PPV was increased to 100%. Kawazu et al. compared GM, the Wako BDG test, and an in-house *Aspergillus* PCR in patients with hematological disorders (52). Using ROC analyses, the BC test and PCR performed identically. The area under the ROC was greatest for GM, which correlated with a superior sensitivity and fewer false positives in this study. Lastly, two additional groups have reported that a single positive PCR was more sensitive than two sequential GM tests (OD >1.5) for the diagnosis of IA, but that PCR also generated more false-positive test results (44,45). When ≥ 2 positive PCR results were required to define a positive test, the two sensitivities became nearly equivalent and the specificity of PCR improved (44).

STUDIES OF PREEMPTIVE ANTIFUNGAL THERAPY

Only one report has assessed the feasibility and safety of preemptive antifungal treatment (170). In this study, 88 adult patients with acute leukemia or undergoing myeloablative SCT were enrolled. All received fluconazole prophylaxis and were monitored daily during periods of neutropenia using the GM assay. Batched GM testing was then performed thrice a week. A positive GM result was defined as an OD index ≥ 0.5 in two sequential samples and a standardized algorithm to guide the evaluation and management of study patients was employed. Only seropositive patients or those with positive microbiologic test results plus supportive radiographic findings received preemptive antifungal therapy. The primary endpoint was a comparison of the number of patients treated under a preemptive strategy versus a standard fever-driven empiric approach. Seven cases of proven and 12 cases of probable IA were identified and all met criteria for preemptive therapy. Forty-one of 117 episodes (35%) satisfied standard criteria for empiric antifungal therapy versus 8% for the protocol-driven preemptive approach. The protocol prompted initiation of antifungal therapy in 10 episodes (7%) that were not clinically suspected of being related to IFI. Overall, a preemptive approach incorporating daily GM and high-resolution thoracic CT spared patients exposure to expensive and potentially toxic drugs (a 78% reduction) while effectively controlling for the development of IA. Other IFIs including one case of zygomycosis and two cases of breakthrough *C. glabrata* fungemia were identified by traditional methods.

CONCLUSIONS AND FUTURE DIRECTIONS

Current IFI management is limited by our inability to identify these infections in the early stages. Difficulty with diagnosis in combination with the high associated morbidity and mortality has promoted the routine use of universal antifungal prophylaxis and empiric therapy. Preemptive treatment strategies that incorporate sensitive diagnostic tests are an attractive alternative because they offer an opportunity to serially monitor at-risk patients and identify infection at a point where treatment may be more effective. In addition, patients without surrogate markers of infection could then be spared the potential toxicity and cost associated with empiric antifungal treatment. At this time, however, the impact of routine monitoring on patient morbidity and mortality is uncertain and the cost effectiveness of IFI surveillance is not known.

The GM and β -glucan tests have been cleared by the FDA, but molecular diagnostics remain poorly standardized and have not been prospectively evaluated for clinical care. Many important questions regarding the optimal use of these fungal diagnostics remain. For instance: What cutoff most accurately defines a positive test? Should the positive test threshold vary by patient population and/or sample type? Does fungal load, as determined by qPCR, predict invasive disease? How often should patients be monitored? Should the tests routinely be combined for surveillance purposes? Finally, what impact does antifungal therapy have on the reliability of the test results? To improve our antifungal treatment strategies for future patients, well-designed prospective multicenter trials are needed to determine the feasibility and efficacy of a targeted preemptive approach. In addressing the key issues outlined here, we must also

rigorously investigate the etiologies and impact of false-positive and false-negative test results on patient care.

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7 | The Immune Response to Fungal Challenge

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INTRODUCTION

Fungal infections are a leading cause of morbidity and mortality in immunocompromised patients. Nearly 40 years ago, Bodey et al. made the critical observation that profound and prolonged neutropenia increases the risk for disseminated fungal infection (1). Since this critical observation, understanding for the mammalian immune response in defending the host against fungal disease has dramatically increased (2). Along with an enhanced understanding for fungal host defense has been the expansion in synthetic immunomodulatory and antifungal agents (3), which have altered the ability to treat invasive fungal infections (IFIs) in immunocompromised patients. Despite pharmaceutical advances in antifungal therapy, the fundamental requirement for surviving an IFI in the context of immunosuppression remains recovery in host immune function (4) (Fig. 1). Thus, the quest continues to define the host immune response to fungal pathogen and to understand the ability of fungi to evade immune detection and elimination.

This chapter defines the known mammalian immune response to fungal disease and how deficiencies in host immunity predispose to fungal infections with the following caveats. First, review of fungal immunity is limited to providing an overview of immune responses with specific examples of certain pathogens, as a comprehensive review of immunity relevant to all fungal pathogens is beyond the scope of this chapter. Second, immunomodulatory agents and their influence on fungal immunity and immune restoration are introduced, but more detailed explanation for their use as treatment for fungal disease is reserved for the chapter dedicated to antifungal immunotherapy. Lastly, contributions to the antifungal response beyond immune effector cells (5) are not addressed in this chapter.

OVERVIEW OF THE MAMMALIAN IMMUNE RESPONSE TO FUNGAL CHALLENGE

Immunity is a coordinated and redundant response designed to discriminate between self and nonself. The overall goal of the immune response is host preservation, particularly with respect to infectious challenge. To this end, the immune response has classically been divided into the innate and adaptive effector arms. These distinct, but not mutually exclusive, cellular responses are complemented by production of soluble factors including cytokines, chemokines, and complement, which serve to eliminate (i.e., antimicrobial) and modify (i.e., immunomodulatory) the immune response to pathogen. Furthermore, the microenvironment comprising these soluble factors directly influences both innate and adaptive immune cell activation, differentiation, and function. The immune response also establishes memory to the pathogen in order to respond rapidly to future infectious challenges. Finally, regulation and tolerance (nonresponse to self) are critical to prevent aberrant autoimmune damage to the host.

Pathogen Recognition Receptors

Fungi are eukaryotic cells and thus share similar features with mammalian cells. The major distinguishing feature between fungi and mammalian cells is that fungi possess a rigid cell wall, containing unique pathogen-associated molecular patterns (PAMPs) including β -glucans, chitins, and mannoproteins. Initiation of the immune response to fungal challenge involves recognition of these PAMPs by pathogen recognition receptors (PRRs) such as Toll-like receptors (TLRs), mannose receptors, and β -glucan receptors (6,7).

Toll-like receptors (TLRs) are type I integral membrane glycoproteins that belong to the TIR (Toll/interleukin-1 receptor) superfamily. The majority of TLRs are expressed on the cellular surface of immune cells (TLR 1,2,4–6,11), while TLR 3,7/8, and 9 are located in endosomal

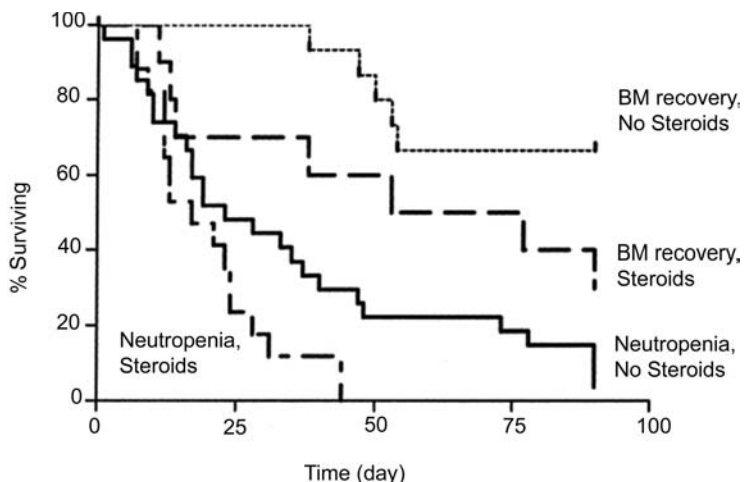


Figure 1 Recovery in immune function is imperative to surviving an invasive fungal infection. Percent survival of 84 patients with hematologic malignancies and *Fusarium* infection are shown. The highest survival rate was seen in those patients with bone marrow (BM) recovery and who were no longer receiving immunosuppressive therapy (No steroids). Source: Adapted from Ref. 4.

compartments (8). TLRs use a conserved TIR domain in the cytosolic region to activate one of four adaptor proteins: the death-domain containing myeloid differentiation factor 88 (MyD88), TIRAP (TIR-adaptor containing adaptor protein, also known as MyD88-adaptor-like protein, MAL), TRIF (TIR-domain containing adaptor protein inducing IFN- β), and TRAM (TRIF-adaptor molecule). Differential use of these adaptor proteins confers specificity to the TLR signaling pathways (9). The majority of TLRs utilize MyD88 signal adaptor proteins to activate IRAKs and TRAF6, which ultimately activate NF- κ B and mitogen-activated protein (MAP) kinases to synthesize inflammatory cytokines like IL-6 and TNF- α (10). In contrast, TLR3-mediated signaling utilizes TRIF and IRF3 in producing type I interferons in a MyD88-independent manner (11). TLR4 activation uniquely leads to both MyD88-dependent, early phase NF- κ B transcription of proinflammatory cytokines (IL-1 β , TNF- α , and IL-6) and MyD88-independent, late phase NF- κ B transcription of IFN- β (10).

Plasticity and redundancy in innate-mediated cytokine responses directly reflect TLR expression and signaling utilized by effector cells (12). For example, plasmacytoid dendritic cells (pDCs) are the principal producers of type I IFN following viral (TLR-7) and bacterial (TLR-9) challenge (13), while myeloid DCs produce smaller amounts of IFN- α in response to viral challenge (TLR-3) (14). Finally, tight regulation of TLR signaling cascades is needed to avoid detrimental allo- and autoimmune inflammatory responses (15,16).

Additional PRRs relevant to fungal pathogens include β -glucan (Dectin-1) (17,18), mannose receptors (MRs) (19), and complement receptors (CRs). Like TLRs, these PRRs are located on the surface of phagocytes including macrophages, DCs, and neutrophils and can modulate immune cell function (20). Specifically, ligation of Dectin-1 and MRs initiates phagocytosis in the absence of opsonization (see sections on phagocytosis and complement below), whereas dual ligation of complement receptors like CR3 (CD11b/CD18 or Mac-1) with receptors for the Fc portion of immunoglobulins (FcRs) dramatically enhance microbial phagocytosis (21). In summary, PRRs and their ligands initiate the detection phase of the fungal immune response.

Innate Immunity

Key features of innate immunity include PRR activation via recognition of PAMPs (22), induction of antimicrobial effector cell cytokines and chemokines (23), and modulation of adaptive immunity (24). Innate immune cells functioning as phagocytes in the antifungal immune response include DCs, macrophages/monocytes, and polymorphonuclear (PMN) cells. These phagocytes are primarily responsible for eliminating fungal pathogens via oxidative and nonoxidative intracellular killing. Specifically, PMN cells ingest and package fungi into phagosomes to

which intracellular granules fuse and then discharge their antimicrobial contents (25). Nonoxidative killing is mediated largely through the content of specific and gelatinase granules, which release lactoferrin, lysozyme, gelatinase, and peroxidase-positive granules including α -defensins. Oxygen-dependent mechanisms include generation of reactive oxygen species (ROS) via the NADPH-oxidase complex in combination with superoxide dismutase and myeloperoxidase. Of note, soluble factors including complement and antibodies promote phagocytosis, enhancing intracellular elimination of fungal pathogens. Finally, PMNs also eliminate fungi via extracellular mechanisms such as neutrophil extracellular traps (NETs), which bind and kill fungal pathogens, particularly *Candida albicans* (26). Composed of DNA and associated histones, NETs also contain granule proteins from azurophilic, specific, and gelatinase granules. These processes literally grab and capture fungal elements, concentrating and preventing their spreading from the site of infection.

Innate immune cells also serve as antigen-presenting cells (APCs) to adaptive immune cells, namely, T cells. In so doing, innate APCs provide two critical signals to activate T-cells—antigen in the context of MHC (major histocompatibility complex) class I or II molecules and costimulation. Antigen processing differs depending upon the location of antigen (27). Intracellular proteins (e.g., viral peptides) in the cytosol are degraded into peptides in proteasomes and presented with class I MHC to CD8⁺ T-cells. In contrast, extracellular peptides (e.g., fungal proteins) are taken up by endocytosis, sequestered into endosomes, and degraded by lysosomal enzymes and presented with class II MHC to CD4⁺ T-cells. In addition to endocytosis, other intracellular pathways exist to deliver antigen for lysosomal degradation and MHC II presentation, through a process known as autophagy (reviewed in Ref. 28).

The physical location where cellular exchange of information and molecular interaction among innate APCs and T-cells occurs is known as the “immunologic synapse” (29). Here, T-cell receptor (TCR)-MHC-peptide cognate interactions in the context of costimulation (CD28-CD80/86, CD40-CD40L) activate receptor signaling cascades in T-cells resulting in their activation and cytokine production. Given its critical role in T-cell activation, the immunologic synapse and its associated molecules are ripe targets for immunotherapy directed at modulating T-cell function (30).

Innate APCs function to recruit adaptive cells through production of soluble immunomodulatory factors, including cytokines and chemokines. For example, TLR-stimulated phagocytes produce IL-23, which then expands the IL-17-producing Th-17 population (discussed further in the section on adaptive immunity) (31). IL-17, in turn, induces proinflammatory cytokines and chemokines (32) as well as matures and recruits phagocytes to the site of infection (33).

Dendritic cells are the most potent APC for naïve T-cell activation and are critically poised to bridge innate and adaptive immune responses following PRR activation by fungal pathogens (34). For example, activation of TLR4 causes maturation of peripheral DCs, increasing their surface expression of adhesion and costimulatory molecules, altering their function from antigen-capturing to antigen-processing cells, and promoting their interaction with naïve T-cells by enhancing expression of CCR7 and migration to secondary lymph nodes (35). In addition DC crosstalk with other innate effector cells, particularly NK cells (36), is common and such exchange modulates function in each effector cell (37,38). Finally, DCs are highly plastic effector cells (39), a reflection of the TLRs they possess (as discussed above) as well as the pathogens they encounter (40).

Human DCs include plasmacytoid (pDC) and myeloid (mDC) subtypes, whereas mice have an additional lymphoid DC phenotype (41). Whether from man or mouse, DC subtypes have distinct surface markers (42), unique TLR (43), chemokine (44), and cytokine (45) profiles, and diverse effects on the immune response (46,47). In humans, mDCs are the primary producers of IL-12 (48), and pDCs are the chief producers of type I interferon (49), a key mediator of antiviral (50) and antitumor (51) immunity and of immunomodulation (52,53).

Adaptive Immunity

B- and T-cell lymphocytes comprise the adaptive immune response to fungal pathogens. In general, B-cells produce antibodies, while T-cells produce immunomodulatory and antimicrobial cytokines in response to fungal challenge. Both lymphoid effector cells have memory subsets that are activated during fungal re-challenge.

The humoral immune response to fungal pathogens is multifunctional. First, B-cells serve as critical APCs to T-cells, and the latter also provides a reciprocal helper function to promote antibody production [though antibody production can occur in the absence of T-helper cells (54)]. Of note, B-cells themselves can also influence innate cells (55). Once activated, differentiated B-cells (plasma cells) produce antibodies that have direct antifungal and immunomodulatory effects. For example, IgG-coated fungal pathogens bind to Fc γ R on phagocytes to initiate antibody-dependent cellular cytotoxicity (ADCC) (56). Cellular processes such as phagocytosis and soluble process like the classic pathway of complement (antibody-dependent complement opsonization) are also activated by antibodies. Finally, antibodies are involved in the memory response to fungal infection (57). Given these roles in antifungal immunity, antibodies are the focus of intense study for successful immunotherapy against fungi (58).

T-cell phenotypes are classically divided into CD4⁺ and CD8⁺ subsets, and each is responsible for different immune functions. Within the CD4⁺ genre are T-regulatory (T_{reg}) cells, Th-17 cells, Th-1 and Th-2 cells, each with its own unique ontogeny and immune function. For example, Th cells originate from peripheral naïve CD4⁺ T-cell precursors. In contrast, T-regulatory cell ontogeny is more complex, as subsets arise from both peripheral Th-1 precursors, including induced T_{reg} cells (CD4⁺CD25⁺FoxP3⁺), Tr1 cells and Th3 cells, and directly from thymic precursors (naturally occurring T_{reg} cells). Differentiation of CD4⁺ subsets is mediated by cytokines inducing transcription factor activation within T-cell precursors. For example, TGF- β alone induces FoxP3 expression in naïve T-cells to promote inducible T_{reg} cells (59), while TGF- β in combination with IL-6 results in Th-17 cell differentiation (60). Finally, CD8⁺ T-cells can be divided into effector (primarily cytolytic or cytokine producing in function) and memory subsets (61). Sustained CD8⁺ T-cells memory requires priming from CD4⁺ T-cells (62,63).

Despite their common ontogeny from the CD4⁺ precursor, Th-17 and T_{reg} cells have divergent functions in the context of inflammation. Th-17 cells promote inflammation via IL-17 production (64,65), while T_{reg} cells counteract inflammation through IL-10 and TGF- β production (66,67) in order to prevent deleterious chronic inflammation. Like DCs, CD4⁺ cells regulate the balance between autoimmunity and tolerance within the host (68).

Soluble Factors: Complement, Cytokines, and Chemokines

Complement activation has typically been associated with the innate immune response, but complement pathways also function to influence adaptive immune responses (21). For example, complement augments antibody responses and enhances immunologic memory, in addition to enhancing phagocytosis (opsonization) and mediating immune cell activation and migration. Three activation pathways culminate to activate C3 convertase, which is instrumental in initiating development of the terminal membrane attack complex, whose formation is usually blocked by the fungal cell wall (69). The classical pathway is initiated by immune complexes, specifically C1 complex binding to antigen-antibody complexes on the surface of pathogens. The alternative pathway is initiated by C3b binding to various hydroxyl groups on proteins and carbohydrates on cell surfaces. Finally, the mannose-binding lectin (MBL) pathway is activated by the binding of the MBL-MASP (MBL-associated serine protease) complex to mannose groups contained within pathogens such as *C. albicans* (70,71). Interestingly, low levels of circulating MBL have been associated with increased susceptibility to fungal infections (72,73), so recombinant MBL could potentially be used as immunotherapy against IFI (74).

Inducible cytokine profiles in response to fungal challenge are a direct reflection of the form of fungal element encountered as well as the types of PRR and intracellular signaling pathways activated (reviewed in following section). In addition, cytokines function as direct fungicidal agents and also mediate immune cell activation and modulate immune cell function. Proinflammatory (Th-1) cytokines including IL-6, IL-12, TNF- α , and IFN- γ provide antifungal immunity, while antiinflammatory (Th-2) cytokines including IL-4, IL-5, IL-10, and TGF- β confer susceptibility to and progression of fungal disease (2,75,76). However, the distinction between pro- and antifungal cytokines is ambiguous for several reasons. First, without antiinflammatory cytokines, proinflammation following fungal challenge is deleterious to the host (77). Second, antiinflammatory cytokines can protect the host against fungal infections in certain settings (78). Thus, effects of cytokines like IL-4 (79), IL-10, and TGF- β (74) are highly context-dependent, much like effects of suppressor cell populations themselves (80). In addition to these pro- and

antiinflammatory cytokines, cytokine growth factors including granulocyte-stimulating factor (G-CSF) and granulocyte-macrophage-stimulating factor (GM-CSF) have dual roles as stimulators of myeloid proliferation and differentiation and as immunomodulatory agents, enhancing phagocyte fungicidal activity and antigen presenting capacity and potentially regulating Th-1 responses (reviewed in Ref. 81).

Like cytokines, chemokines have critical roles in immune cell activation and recruitment in the context of fungal infection (82) and inflammation (83). For example, macrophage inflammatory protein-1 alpha (MIP-1 α)/CCL3 and monocyte chemoattractant protein-1 (MCP-1)/CCL2 mediate phagocyte recruitment to sites of infection (84). Likewise, chemokines such as Epstein-Barr I1 ligand chemokine (ELC)/CCL19 and secondary lymphoid-tissue chemokine (SLC)/CCL21 form gradients to facilitate DC trafficking and antigen presentation within secondary lymph nodes and link innate and adaptive responses (85). Cytokines like TNF- α can also induce chemokine production from immune cells, further driving effector cell recruitment to sites of infection and inflammation (86). Finally, chemokines like thymus and activation-regulated chemokine (TARC)/CCL17 directly modulate antifungal responses (87).

Other soluble factors relevant to the fungal immune response include collectins, defensins, and heat shock proteins (HSPs). In general, these factors function to enhance phagocytosis (collectins) or to mediate direct antimicrobial effects (defensins). Specifically, HSPs are intracellular molecular chaperones, which normally shuttle peptides during steady-state hemostasis, and also function as danger signals during cell stress responses (88). Interestingly, antibodies to HSP90 protect against *C. albicans* (89), enhance effects of antifungal agents (90), and potentially decrease resistance of fungal pathogens (91,92).

Regulation

Intracellular signaling cascades of pathogen-recognition receptors like TLRs (10) ultimately converge to activate nuclear factor κ B (NF- κ B), which mediates gene transcription of proinflammatory factors that holistically comprise the protective antifungal immune response. In contrast, cytokine receptors signal through either Janus kinase (JAK)-signal transducers and activators of transcription (STAT) pathways (93) or mitogen-activated protein (MAP) kinase cascades (94). Left unchecked, acute inflammation progresses to chronic inflammation and causes host damage (95). Therefore, the inflammatory response is regulated to preserve host integrity (96). Such regulation occurs at multiple levels, including at the level of TLR (16), and cytokine (97) receptor activation and signaling, at the level of NF- κ B gene transcription (98), and at the level of MAP kinase activation (99). In addition to these signaling regulators, cytokines (as reviewed above) and regulator cell populations down-modulate the host immune response. Examples of suppressor populations include hematopoietic [e.g., myeloid suppressor cells (80), regulatory T-cells (100), NKT cells (101)] and nonhematopoietic [e.g., mesenchymal stem cells (102)] cells. Roles for these regulatory signaling and cellular factors in modulating the immune response to fungal challenge remain largely undefined. Furthermore, these soluble and cellular factors are also likely involved in immune evasion by fungal pathogens, and so may be important targets to enhance antifungal immune responses (103).

PUTTING IT ALL TOGETHER: IMMUNE RESPONSES TO *CANDIDA ALBICANS* AND *ASPERGILLUS FUMIGATUS*

The host response to fungal pathogens is a complex and coordinated interaction among innate, adaptive, and complement effector arms and their associated soluble factors that combine to eliminate the pathogen and create long-lasting immunity against the fungal pathogen encountered (Fig. 2). The antifungal response to *C. albicans* and *A. fumigatus* will be highlighted as representative immune responses against yeasts and molds, respectively. Table 1 provides a summary to the key elements of the host immune response to these clinically important fungi, while the following text provides more details highlighting the complex interactions among immune cells and soluble factors responding to fungal challenge.

Detection, Activation, Elimination, and Regulation

Toll-like receptors (TLRs) 2 and 4 have established roles in detecting fungal elements (104), whether alone or in combination with other PRRs such as dectin-1 (105,106). Interestingly,

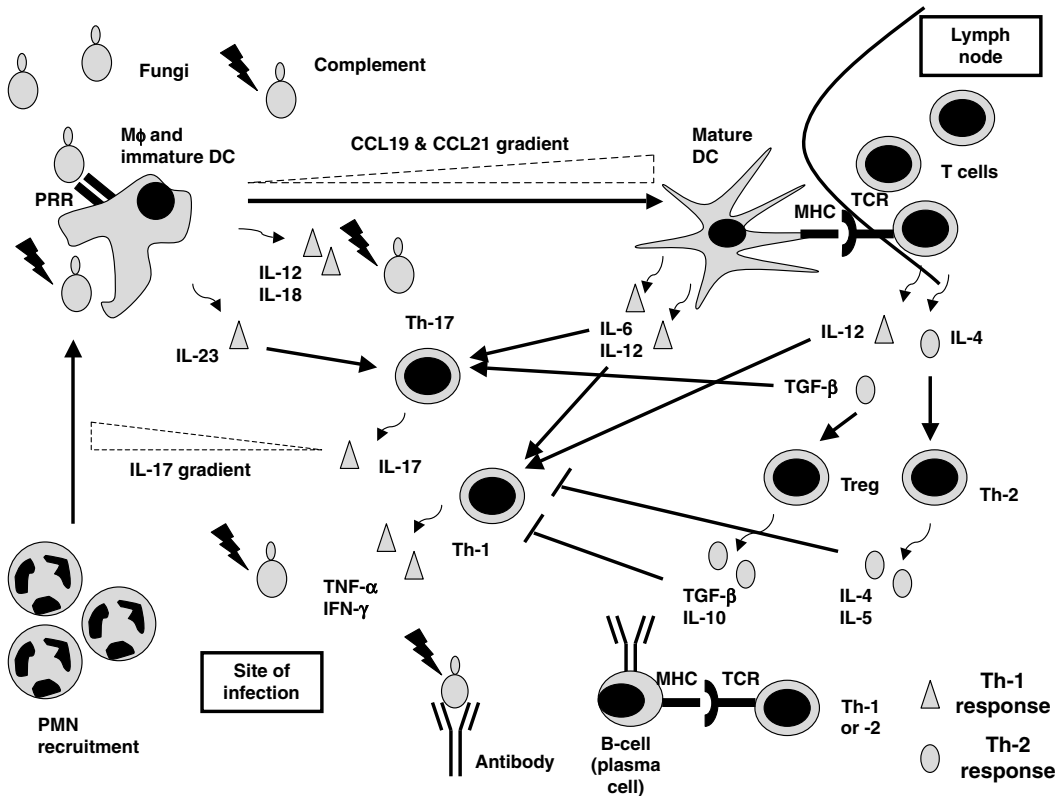


Figure 2 The immune response to fungal challenge. The host immune response to fungal challenge involves coordination among the innate and adaptive effector arms as well as activation of complement cascades. In brief, phagocytes (macrophages, M ϕ , and dendritic cells, DCs) are activated through pathogen recognition receptors (PRR) to produce antimicrobial and immunomodulatory cytokines and chemokines. In addition, phagocytes serve as antigen-presenting cells, processing antigen in the context of major histocompatibility complex (MHC) class I and class II molecules, which are recognized by the T-cell receptor (TCR) on naïve T-cells in lymph nodes. Activation of T-cells requires antigen-presentation and costimulation through cognate interactions (CD28-CD80/86) and soluble factors (IL-12, IL-4). Activation then drives proliferation of distinct CD4 subsets, including IL-17 producing Th-17 cells, inducible T regulatory cells (T_{reg}), proinflammatory Th-1 cells, and antiinflammatory Th-2 cells. Th-2 cells are also important for humoral immunity, including B-cell production of antibodies. Cytokine and chemokine gradients drive immune cell differentiation and expansion, migration to secondary lymph nodes, and recruitment to the site of infection. Secreted soluble factors are shown with curved arrows, while effects on immune cell activation, differentiation, and migration are shown with straight arrows.

fungal dimorphism results in distinct TLR activation, ultimately leading to contrasting cellular and cytokine responses (107,108). For example, *A. fumigatus* conidia and hyphae as well as *C. albicans* yeasts activate TLR2, while only hyphae from either *A. fumigatus* or *C. albicans* activate TLR4 (109). Furthermore, differential activation of TLRs during germination of *A. fumigatus* from conidia (TLR2 and 4) to hyphae (TLR2 only) results in IL-10 induction and thus may contribute to the mold's ability to escape immune surveillance (110,111). Finally, multiple elements of the same fungi can activate different PRRs, resulting in different downstream effects. For example, *Candida* mannan activates TLR4, resulting in proinflammatory cytokine and chemokine release and PMN recruitment (protective response) (112), while *Candida* glucan activates TLR2 and induces IL-10 (susceptibility response) (113).

Similar to PRR activation, phagocytosis is complex and is affected by different recognition receptors, resulting in unique fungicidal and immunomodulatory responses (114,115). The different forms of phagocytosis likely reflect the plasticity in phagocyte function conferred by possessing different PRRs. For example, *Candida* yeasts and *Aspergillus* conidia undergo 'coiling' phagocytosis and induce IL-12 production, resulting in protective Th-1 responses.

Table 1 Host Immune Responses to *Candida albicans* and *Aspergillus fumigatus*

	<i>Candida albicans</i>	<i>Aspergillus fumigatus</i>
Acquisition	Colonization (breach in integrity of intestine mucosa)	Inhalation (breach in pulmonary host defense)
Detection	Yeast and PLM (TLR2), Pseudohyphae and hyphae (TLR4/CD14), β -glucan (Dectin-1/TLR2), mannose (MR)	Conidia (TLR2) Hyphae (TLR2 and TLR4/CD14)
Cellular activation and migration	TLR-inducible cytokine production; PMN and DC migration via chemokine gradients	TLR-inducible cytokine production; PMN and DC migration via chemokine gradients
Cellular phagocytosis	PMN, DC, macrophage; Oxidative and nonoxidative killing	Alveolar macrophages (conidia), PMN (hyphae), and DC (conidia and hyphae); Oxidative and nonoxidative killing
Chemokines	IL-8, KC, MCP-1, MIP-1 α/β , MIP-2, RANTES	IL-8, MCP-1, MIP-2
Fungicidal cytokines	IL-1 β , IL-6, IL-12, IL-15, IL-18, IFN- γ , TNF- α	IL-1 β , IL-6, IL-12, IFN- γ , TNF- α
Regulatory cytokines	IL-4, IL-10, TGF- β	IL-4, IL-10
Memory	Antibody	Antibody
Immune risk factors	T-cell dysfunction (predominant), neutropenia	Neutropenia or aberrant PMN function (predominant)
Acquired risk factors	Broad-spectrum antibiotics, CVAD, recent GI surgery, HAL, extreme ages, prolonged ICU stay, steroids	Steroids, broad-spectrum antibiotics, infliximab therapy
Associated diseases	AIDS, HSCT/SOT, malignancy	Chronic granulomatous disease, GVHD, HSCT/SOT

Abbreviations: CVAD, central venous access device; DCs, dendritic cells; HAL, hyperalimentation; HSCT/SOT, hematopoietic stem cell/solid organ transplant; ICU, intensive care unit; KC, keratinocyte derived; MIP, macrophage inhibitory protein; MCP, monocyte chemoattractant protein; MR, mannose receptor; PLM, phospholipomannan; PMN, polymorphonuclear; RANTES, regulated upon activation, normal T-cell expressed, and secreted; TLR, Toll-like receptor.

In contrast, *Candida* and *Aspergillus* hyphae are internalized by 'zipper-type' phagocytosis and induce IL-4 and IL-10 production, resulting in nonprotective Th-2 responses (reviewed in Ref. 2). Furthermore, coiling and zipper-type phagocytosis are TLR-independent but involve different PRRs, in particular MRs and CR3-Fc γ R cooperation, respectively. In similar fashion, chemokine receptor expression (116) and chemokine induction profiles (84,117) differ depending upon the internalized fungal form, which may ultimately impact immune cell migration and function.

Once initiated, the proinflammatory Th-1 response undergoes down-modulation. Established mechanisms responsible for immune attenuation in the context of fungal infections include myeloid suppressor cells and regulatory cytokines (both reviewed in previous sections). Interestingly, the role of suppressor cells such as T_{reg} cells is still being defined, as these cells are likely involved in regulating the proinflammatory response and in suppressing the immune response at the site of infection [similar to their proposed effects in tumor beds (118)].

Immune Escape Mechanisms

Despite the redundancy and complexity of the antifungal immune response, fungal pathogens cause infection, even in the immunocompetent host. Fungal pathogenesis directly reflects both virulence factors (119) and immune escape mechanisms. The very nature of dimorphism is perhaps the most obvious tool used by fungi to obviate the immune response (120). In addition to undergoing morphologic changes, fungi possess virulent structures (capsule of *Cryptococcus neoformans*) and toxins (gliotoxin of *A. fumigatus*), which inhibit immune cell activation and function and induce immune cell apoptosis (121,122). Other forms of fungal immune evasion include PRR escape (108), loss of TLR signaling (110), preferential PRR ligation leading to intracellular survival within phagocytes (107), and induction of suppressor cell populations and soluble factors as previously discussed. Finally, the intrinsic ability of fungi to evade

Table 2 Synthetic Immunomodulatory Agents

Class	Agent	Effect
Corticosteroids Antiproliferation	Prednisone, methylprednisolone	General immune suppression
	Azathioprine	Induction of thioguanine derivatives, inhibit DNA synthesis
Calcineurin inhibitors	Mycophenolate mofetil	Blocks purine synthesis, preventing B/T cell proliferation
	Cyclosporin A, Tacrolimus (FK506)	Inhibit IL-2 production, T-cell activation
Target of rapamycin (TOR) inhibitors	Sirolimus, Everolimus	Inhibit T-cell cell cycle, proliferation
Cell depletion	Rituxumab (anti-CD20)	B-cell depletion
	Gemtuzumab (anti-CD33)	Myeloid cell depletion
	Alemtuzumab (anti-CD52)	Mononuclear and B/T-cell depletion
	OKT3 (anti-CD3)	T-cell depletion
Soluble factor blockade	Daclizumab (human), Basiliximab (chimeric)	Soluble IL-2 receptor blockade
	Infliximab	TNF- α receptor blockade
	Etanercept	TNF- α blockade
	Adalimumab	TNF- α blockade
	Fontolizumab	IFN- γ blockade
	Anti-p40	IL-12 and IL-23 blockade
Activation blockade	Adaptacept	CD28-B7 blockade
	Belatacept	CTLA-4-Ig blocking CD28-B7

and/or suppress immunosurveillance is often complemented by iatrogenic suppression in host immune function, as synthetic agents targeting immune cells and soluble factors have dual roles in ameliorating deleterious allo- or auto-immunity and in inhibiting protective antifungal immunity (30,123) (Table 2).

HOST DEFENSE: AN IMMUNOMODULATORY INTERFACE BETWEEN HOST AND PATHOGEN

Host defense is an immunomodulatory interface between host and fungal pathogen. The potential for fungal infection reflects the immune status of the host and the cumulative attributes of the pathogen to cause infection (Fig. 3). With respect to the patient, the more immunosuppressed, the higher the incidence of clinical infection and the risk for disseminated disease. In contrast, immune restoration within the patient dramatically decreases the risk for disseminated disease and improves the likelihood for complete eradication of fungal pathogen. With respect to the fungal pathogen, its overall prevalence, its intrinsic and acquired resistance, and its virulence factors directly influence the type of host it will infect and the type of disease it will cause (colonization, infection, or dissemination).

Several factors influence the net immunosuppressive state of the host, including the presence of comorbid conditions such as diabetes mellitus, prematurity or advanced age, and concomitant infection, particularly with immunomodulatory viruses like HIV, EBV, CMV, and HHV-6 (124). Primary underlying disease and its treatment also affect the immune status of the host. For example, cancer and its associated therapies (chemotherapy and radiation) affect all arms of the immune system (125,126), whereas chronic granulomatous disease is a selective qualitative deficiency in neutrophil function (127). Finally, exposure to hospital environments, to antifungal chemotherapy, and to high glucose (hyperalimentation) affects the susceptibility of the host to fungal disease (128).

Hematopoietic stem cell transplantation (HSCT) serves as the extreme clinical example in which host defense is severely compromised. Thus, HSCT patients often develop (129) and succumb (130) to IFI due to innate and adaptive cytopenia and dysfunction. HSCT involves replacement of malignant or nonmalignant disease in the transplant host with normal donor-derived hematopoiesis and immunity (Fig. 4). However, donor-derived HSC engraftment and immune reconstitution are influenced by a variety of factors, which upset their precarious

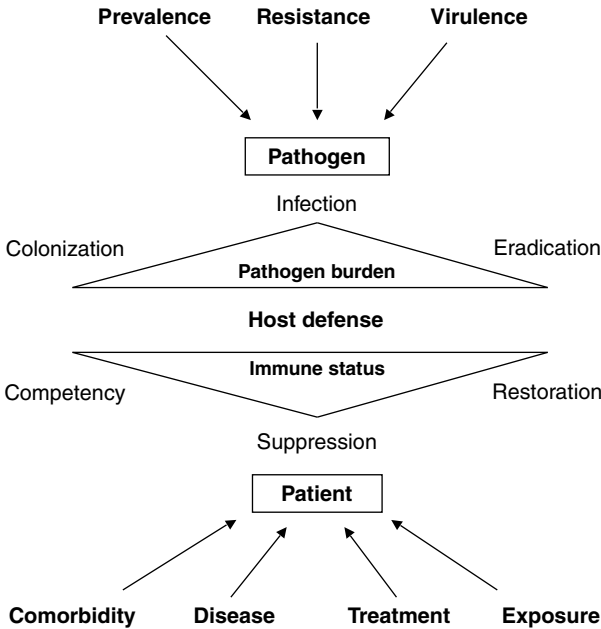


Figure 3 Host defense is the immunomodulatory interface between fungal pathogen and host (patient). Factors that affect the ability of fungal pathogen to overcome host defense and to cause infection include its overall prevalence, its resistance, and its virulence factors. With respect to the patient, factors that influence level of immunosuppression include the presence of comorbid states (e.g., age extremes, diabetes mellitus, infection), the patient’s underlying disease (e.g., malignancy, HIV/AIDS) and its associated treatment (e.g., chemotherapy, radiation, antibiotics), and patient exposure (e.g., nosocomial exposure, environmental exposure). Increases in pathogen burden associate with level of immunosuppression, and resolution in pathogen burden associates with immune restoration.

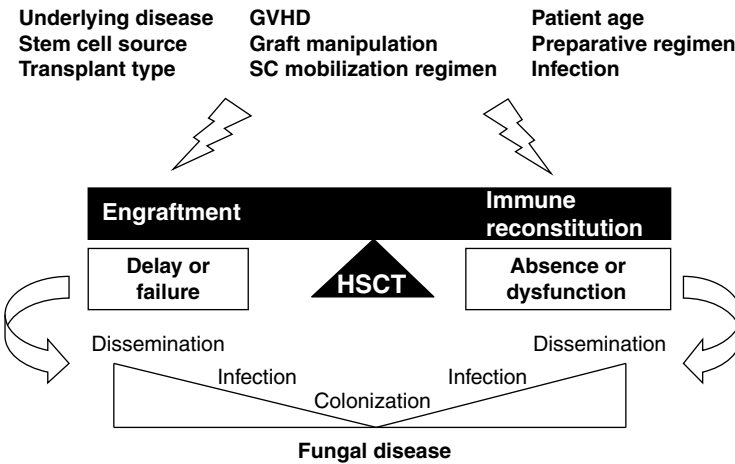


Figure 4 Successful outcome following hematopoietic stem cell transplantation (HSCT) requires a balance between donor-derived hematopoiesis (engraftment) and immune reconstitution. Delays and/or failures in HSC engraftment result in cytopenias that predispose the HSCT patient to fungal infection and dissemination. Similarly, absent and/or dysfunctional immune reconstitution also predisposes the HSCT patient to fungal disease. Multiple factors summarily upset this precarious balance, most notably graft-versus-host disease (GVHD), but also underlying disease (malignant vs. nonmalignant), stem cell source (bone marrow, peripheral blood, umbilical cord blood), stem cell (SC) mobilization regimen (G/GM-CSF vs. flt3 ligand), graft manipulation (CD34⁺-selection, T-cell depletion, tumor purging), transplant type (matched-sibling donor vs. matched-unrelated donor), patient age (young vs. older patient), preparative regimen (myeloablative vs. nonmyeloablative), and infection (bacterial, fungal, viral).

recovery. For example, prophylactic and empiric antifungal therapies have dramatically changed the epidemiology of fungal pathogens in HSCT recipients (131,132). Furthermore, immunosuppressive agents targeting deleterious alloreactivity in the HSCT patient (i.e., graft-versus-host-disease, GVHD) also increase the risk for IFI (133). Cellular and soluble factor immunotherapies targeting immune restoration in the HSCT host are currently being explored to augment host defense against infection (134).

CONCLUSION

An enhanced understanding of fungal pathogenesis and immunity has occurred over the last 20 years. Paralleling this understanding has been the increase in pharmaceutical antifungal agents as well as *ex vivo* expanded and manipulated cellular and synthetic soluble therapies. Thus, the advent of a “third age of antimicrobial therapy” is truly emerging (135). However, success in using immunotherapy will require a thorough mechanistic understanding for the underlying cellular interactions and soluble mediators involved in the desired clinical response (134).

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8 Immunomodulators: What is the Evidence for Use in Mycoses?

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INTRODUCTION

Because alterations in immunity are the major risk factor for developing invasive mycoses, strategies to augment immune function are being explored as adjunctive therapy in these infections. In neutropenic patients, efforts to increase neutrophil number and function include the use of granulocyte infusions and colony-stimulating factors (CSFs). Investigators have also studied the role of cytokines, such as interleukin-12 and interferon-gamma, in enhancing immunity in immunocompromised patients. The administration of nonspecific immunoglobulin has been used for decades in the treatment of infections; however, recent advances in this technology include the passive administration of specific antibodies directed against fungal pathogens. As the immune status of the patient improves, clinicians must be aware of the immune reconstitution syndrome. Although a sign of clinical improvement, this syndrome may mimic worsening infection.

ALTERED IMMUNITY AND INVASIVE FUNGAL INFECTIONS

In the past few decades, the expanding population of immunosuppressed patients has resulted in a corresponding increased incidence of invasive fungal infections (IFIs). The direct correlation between immunosuppression and systemic mycoses has prompted clinicians to explore adjunctive immune therapy for the treatment of these diseases. If a compromised immune system is the main risk factor for developing IFIs, perhaps therapy directed against these infections should include an attempt to restore or augment the immune response.

Rarely, IFIs occur in patients with innate disorders of immune function. For example, the impaired neutrophil oxidative burst in chronic granulomatous disease places affected patients at risk for invasive aspergillosis (1). Chronic mucocutaneous candidiasis, causing recurrent and disfiguring *Candida* infections, results from various T-cell abnormalities such as mutations in the autoimmune regulator (AIRE) gene (2). Also, *Coccidioides immitis* more frequently disseminates in patients of African or Filipino ethnic backgrounds, presumably due to uncharacterized genetic determinants (3).

However, most IFIs are directly attributable to acquired immune defects such as those resulting from AIDS, solid organ transplantation, and antineoplastic therapy. Immune restoration using antiretroviral therapy in AIDS has resulted in a dramatic improvement in treating and preventing the opportunistic infections associated with this syndrome. Similar strategies are being explored to manipulate the immune system to better treat mycoses in other immunosuppressed patient populations.

GRANULOCYTE INFUSION

Periods of prolonged neutropenia after cytotoxic chemotherapy pose an important time of risk for the development of infections. In 1994, Morrison evaluated 1186 consecutive patients undergoing bone marrow transplantation at a university hospital (4). Of these patients, 10% (123 patients) developed an invasive infection by a fungus other than *Candida*. This high rate of fungal infection was associated with accelerated mortality, with only 17% of infected patients surviving more than 180 days. Among this cohort, risk factors for developing IFIs included age older than 18, prior CMV exposure, graft-versus-host disease, and delayed engraftment. Of these, delayed engraftment carried the greatest relative risk for subsequent fungal infections, underscoring the importance of neutrophil restoration in the prevention and cure of fungal infections.

Granulocyte transfusions would appear to be logical therapy for neutropenic patients with infections, restoring functional neutrophils to patients during this period of highest infectious risk. However, several challenges have limited the widespread use of this therapy. Normal healthy adults make approximately 10^{11} neutrophils each day, and these cells have an active life span of 9 to 10 days (5). Therefore, harvesting and infusing adequate numbers of neutrophils have historically been technical challenges that limited granulocyte infusion therapy. Additionally, multiple granulocyte infusions might be required to support the patient during the entire period of neutropenia.

Early case reports of granulocyte transfusions in neutropenic patients with active infections lead to an initial enthusiasm for this therapy (6–8). However, subsequent studies failed to demonstrate a clinical benefit to neutropenic patients for the routine, prophylactic administration of granulocyte transfusions during periods of neutropenia. For example, Strauss evaluated 102 patients with neutropenia resulting from therapy for acute myelogenous leukemia (9). The patients were randomized to receive daily granulocyte transfusions or placebo. No reduction was observed in the survival or incidence of infection between the two study groups. Moreover, the granulocyte transfusion–treated group had significantly more pulmonary infiltrates than the control patients (9).

Several studies specifically designed to evaluate the role of granulocyte transfusion in neutropenic patients with fungal infections similarly did not demonstrate a measurable benefit from this therapy. A retrospective analysis was performed of 87 patients with IFIs during the first 100 days after bone marrow transplantation (10). Fifty patients received both granulocyte transfusions and appropriate antifungal therapy, and 37 patients received antifungal therapy alone. Although the transfusions were well tolerated, no clinical benefit was observed for those patients who received granulocyte transfusions versus those who did not.

Other studies during this time also demonstrated that patients treated with granulocyte transfusions seemed to develop more pulmonary infiltrates compared to control patients, especially when the neutrophils were coadministered with amphotericin B, resulting in serious pulmonary damage due to alveolar hemorrhage (11). In one such study, acute respiratory decompensation occurred in 64% of patients in which amphotericin B and granulocyte infusions were administered together, as opposed to 6% among patients who only received the cell infusion (11). Subsequent studies failed to confirm this association, including a retrospective analysis of 144 patients in which no excess pulmonary toxicity was attributable to granulocyte transfusions (12). Nonetheless, largely due to concerns for toxicity and unproven efficacy, granulocyte transfusions were rarely performed for several years. Also, improvements in the supportive care of neutropenic patients made life-threatening bacterial and fungal infections less common in this patient group.

Enthusiasm for granulocyte infusions increased with advances in neutrophil harvesting techniques. Because of the concern for inadequate dosing of neutrophils in early studies of granulocyte infusions, newer neutrophil acquisition methods involve pretreating neutrophil donors with G-CSF and steroids. This intervention allows the harvesting and infusion of large numbers of functional granulocytes. Using this strategy, Peters et al. demonstrated that the transfusion of granulocytes in neutropenic patients was well tolerated and resulted in measurable increases in peripheral leukocyte counts (13). In this study, seven granulocyte transfusions were required on average for each patient to support them until bone marrow recovery, and these multiple granulocyte infusions were well tolerated.

A later study used community neutrophil donors stimulated with a single dose of G-CSF plus oral dexamethasone to maximize neutrophil recovery (6). In this study, 19 patients were treated with a mean of 8.6 transfusions. Granulocyte transfusion therapy resulted in restoration of the peripheral neutrophil count to normal values in 17 of 19 patients. Also, function of the infused neutrophils was confirmed by a buccal neutrophil infiltration response. Transfusion-associated symptoms were observed in 7% of patients, but these symptoms were not sufficient to limit therapy. Eight of the 11 patients with bacterial or fungal infection resolved their infections with combination antimicrobial therapy and granulocyte infusions (6).

Several more recent trials have also attempted to study the efficacy of granulocyte transfusions in adult patients using modern neutrophil harvesting techniques. Most of these trials found that multiple granulocyte transfusions were well tolerated. One uncontrolled, observational trial evaluated granulocyte transfusions in 25 neutropenic patients with active infections, concluding

that patients with infections due to fungi or gram-negative bacilli may experience more clinical benefit from this intervention than patients with infections due to gram-positive cocci (14). Three additional trials suggested that granulocyte transfusions may be effective as adjunctive secondary prophylaxis for patients undergoing stem cell transplantation, preventing infections during subsequent periods of neutropenia in patients with recent serious fungal and bacterial infections (15–17).

In 2006, Sachs et al. published one of the most striking studies demonstrating clinical benefit of granulocyte infusion therapy, describing their experience with early granulocyte transfusions in neutropenic children (18). In contrast to prior studies, the 27 patients in this prospective Phase II trial received early granulocyte transfusions, administered a median of 7.5 days after the onset of neutropenia. All patients tolerated the infusions well, and the mean absolute neutrophil count remained greater than 1×10^9 cells/mL for eight days after granulocyte transfusion. Of the 27 total patients in this study, 25 (92.6%) cleared their initial infection, and 81.5% were alive one month after infection. All six children with invasive aspergillosis cleared their infection. The authors concluded that early granulocyte transfusions were feasible and safe in immunocompromised children with neutropenia (18).

Published case reports also describe the potential efficacy of granulocyte infusions in neutropenic patients with life-threatening IFIs. For example, the combination of antifungal therapy and granulocyte transfusions was used in three children undergoing stem cell transplantation with life-threatening fungal infections. The infections in this case series included a cerebral mold infection, disseminated candidiasis, and nasopharyngeal zygomycosis. All three children were cured of the fungal infections, and the anticancer therapy was pursued without delay (19).

In addition to the administration of larger numbers of neutrophils to neutropenic patients, other novel variations of granulocyte transfusions are also being pursued. The coadministration of granulocyte transfusions and interferon-gamma-1b was safely performed in 20 patients with neutropenia and active infections (20). This cytokine enhances the immune response against a number of intracellular pathogens. Also, immortalized phagocytic cells from culture have been administered to neutropenic animals and demonstrated to protect them from otherwise lethal *Candida* infections (21).

Summary

Although theoretically promising, granulocyte infusion therapy has not historically offered major benefits to adult neutropenic patients with IFIs. However, one major limitation of this therapy has been administering sufficient doses of functional neutrophils to offer a sustained antimicrobial effect until patients can make their own granulocytes. This may explain why granulocyte transfusions in pediatric populations appear to be more efficacious than in adults, given a more favorable dose relative to body weight. Modern granulocyte-harvesting techniques have improved the effective number of cells that can be administered (6,13).

Currently, granulocyte transfusions are not recommended for routine administration or prophylaxis in neutropenic patients. They are used most frequently in highly immunocompromised patients with life-threatening infections, even without good clinical data to support this practice. Immune augmentation with granulocyte infusions in nonneutropenic patients with serious mycoses has been suggested but never studied in large clinical trials. Alloimmunization and delayed engraftment are potential side effects of granulocyte infusions (22); however, recent studies suggest that granulocyte transfusions are well tolerated in neutropenic patients (Table 1). Well controlled and adequately powered trials are needed to allow clinicians to evaluate the relative risks and benefits of granulocyte transfusions in neutropenic patients with life-threatening infections.

CYTOKINE THERAPY

Colony-Stimulating Factors

Mature human leukocytes differentiate from precursor cells in response to CSFs. Granulocyte-CSF (G-CSF) increases the number of mature PMNs by increasing the production of these cells and by inhibiting apoptosis (23). Clinically, G-CSF has played a major role in the treatment of many hematological disorders. This cytokine reduces the period of neutropenia after chemotherapy, resulting in reduced hospitalizations and antimicrobial use (24,25). Also, G-CSF

Table 1 Selected Clinical Trials of Granulocyte Transfusions in Neutropenic Patients

Reference	Study description	Patients	Conclusions
(10)	Retrospective study of GTx in patients with neutropenia and fungal infections	87 Patients; 50 received GTx and antifungal therapy; 37 received antifungal therapy alone	GTx was well tolerated, but no clinical benefit was observed with this therapy over antifungals alone
(67)	Pilot study of GTx in patients with hematological malignancies and serious fungal infections	15 Patients	<ol style="list-style-type: none"> 11/15 patients with GTx had a favorable clinical response 8/15 patients were free of infection 3 wk after therapy
(13)	Prospective phase I/II trial of GTx in neutropenic patients with serious bacterial or fungal infections	30 Patients; median of 7 GTx per patient	GTx was well tolerated; 20/30 patients were alive with complete clinical resolution 100 days after GTx
(6)	Prospective phase I/II trial of GTx in neutropenic patients with serious bacterial or fungal infections	19 Patients receiving GTx from G-CSF/dexamethasone-treated community leukocyte donors	<ol style="list-style-type: none"> Restoration of normal neutrophil counts in 17/19 patients Normal buccal neutrophil response in most patients Resolution of infection in 8/11 patients with invasive bacterial or <i>Candida</i> infections
(14)	GTx in neutropenic patients with active infections	25 Patients	Favorable clinical response in 8/11 patients with fungal infections, 9/15 patients with gram-negative bacilli infections, and 5/16 with gram-positive cocci infections
(15)	GTx in patients with neutropenia-related infections; evaluating this therapy as treatment as well as secondary prophylaxis for infections	42 Patients received GTx, 18 with active infection (treatment group) and 8 with severe prior infection (prophylaxis group)	<ol style="list-style-type: none"> 12/18 patients with improved or resolved active infection 0/8 patients with recurrence of severe prior infection
(16)	GTx in patients with prior invasive aspergillosis (IA) or high risk for developing IA	8 Patients with IA (or high risk for IA), 18 controls (similar conditioning regimens but no prior IA)	Compared to controls, GTx-treated patients had decreased duration of fevers and fewer days of neutropenia; 4/7 GTx-treated patients had improved chest radiographs
(17)	Observational trial of GTx in patients undergoing stem cell transplantation with active infections or recent serious fungal infections	67 Patients; 44 with active infections, and 23 with recent serious fungal infections	<ol style="list-style-type: none"> No reactivation of fungal infections (0/23) in GTx-treated patients Control of active infections in 36/44 patients
(18)	Prospective Phase II trial of early-onset GTx in neutropenic children with severe infections	27 Pediatric patients with neutropenia-related infected refractory to standard therapy	<ol style="list-style-type: none"> 92.6% of patients resolved initial infection 81.5% of patients alive without signs of infection at 1 mo 6/6 children with IA resolved their infection

Abbreviations: GTx, granulocyte transfusion; IA, invasive aspergillosis.

therapy has allowed the transplantation of hematopoietic stem cells instead of bone marrow cells for recovery after marrow ablative therapy. Similarly, GM-CSF (granulocyte-macrophage colony-stimulating factor) and M-CSF (macrophage colony-stimulating factor) promote the maturation of granulocytes/macrophages or macrophages, respectively (26).

Numerous in vitro studies have suggested that G-CSF may enhance fungal killing by antimicrobials and leukocytes (27–29). For example, G-CSF enhances the antifungal effect of voriconazole and neutrophils against *Candida albicans* in vitro (30). This cytokine was also demonstrated to increase the antifungal effects of neutrophils by promoting an increased oxidative burst (31,32). G-CSF treatments in neutropenic AIDS patients resulted in an increased number of circulating neutrophils as well as an augmentation of the in vitro fungicidal effects of these cells (33).

In other studies, the administration of CSFs protected animals from death due to experimental infections with *Aspergillus*, *Candida*, and *Cryptococcus* (34–37). For example, although G-CSF had no beneficial effect alone in a murine neutropenic model of disseminated candidiasis, it did improve the survival of fluconazole-treated animals more than drug therapy alone (38). Also, in mouse models of systemic aspergillosis, the addition of G-CSF to various combinations of antifungal drugs improved survival in neutropenic animals (39,40).

Despite the effectiveness of CSFs in promoting hematopoietic cell recovery, clinical trials have been less consistent in demonstrating a clear survival benefit for the routine use of G-CSF or GM-CSF as prophylactic therapy in neutropenic patients with infections. For example, Crawford et al. demonstrated that prophylactic use of G-CSF in neutropenic patients receiving therapy for small-cell lung cancer resulted in reduced duration of neutropenia and fewer episodes of fever. However, no reduction in mortality was demonstrated in the G-CSF-treated patients (25). Similarly, in a randomized, double-blind, placebo-controlled trial, G-CSF administration for chemotherapy-induced neutropenia resulted in reduced median days of neutropenia and time to resolution of febrile neutropenia; the trial was not designed to evaluate a mortality difference (41). There were insufficient numbers of fungal infections in these trials to determine whether this cytokine contributed to the prevention of invasive mycoses.

Cytokine therapy in neutropenic patients was associated with increased survival in one study in which 124 patients aged 55 to 70 years were randomized to receive either GM-CSF or placebo after induction therapy for AML. Compared to the placebo-arm, the GM-CSF-treated patients experienced reduced periods of neutropenia, improved survival, and decreased mortality due to IFIs (26). Therefore, in this neutropenic subpopulation of patients, prophylactic therapy with GM-CSF, and not G-CSF, has been demonstrated to offer a survival benefit. The authors postulate that the macrophage-stimulatory activity of GM-CSF may be important in preventing the growth of pathogenic microorganisms in the lungs and on mucosal surfaces.

It is important to note that GM-CSF therapy is often associated with more patient-reported symptoms (myalgias, fatigue) than treatment with G-CSF. G-CSF is rarely associated with allergic reactions, and the most commonly reported side effect is mild bone pain (20–30% of patients) (42,43). Most patients tolerate this medication well. In contrast, the most frequent side effect associated with GM-CSF is fever (20% of patients), which can complicate the clinical assessment of neutropenic patients. Also, flu-like symptoms have been associated with GM-CSF administration, and high doses may contribute to a capillary-leak syndrome (43).

Because of the enhancement of leukocyte antifungal effects by G-CSF, this cytokine has also been used in nonneutropenic patients with various infections. For example, a phase II trial of recombinant G-CSF in hospitalized patients with pneumonia failed to demonstrate a survival benefit in patients treated with G-CSF compared to placebo-treated patients. However, adverse secondary end points, such as development of empyema and progression to ARDS, were less common in patients in the G-CSF arm of the study (44).

Interferon-Gamma

Interferon-gamma activates macrophages and other immune cells. Although it induces only a modest antiviral activity, its effect on macrophages and T-cells is associated with a marked increase in the antifungal action of these cells.

Interferon-gamma therapy has been studied extensively in patients with chronic granulomatous disease (CGD). The neutrophils of these patients have impaired respiratory burst and a resulting inability to produce antimicrobial reactive oxygen species. Therefore, patients with

CGD are at risk for developing a number of bacterial and fungal diseases, often presenting with recurrent staphylococcal infections as well as invasive aspergillosis.

In 1991, a large clinical trial in patients with CGD demonstrated that interferon-gamma therapy resulted in substantially fewer serious infections compared to placebo (45). Interestingly, there was no measurable effect of this therapy on superoxide production of the immune cells. Among treated patients, INF-gamma therapy was well tolerated (45). The combination of early recognition of CGD, aggressive management of infections, antimicrobial prophylaxis, and interferon-gamma therapy in selected patients has resulted in significant improvement in the clinical outcomes of this disease.

In addition to its use as prophylactic therapy, case reports have demonstrated that interferon-gamma may be helpful in the treatment of IFIs in patients with CGD (46,47). However, no large clinical trials have yet indicated that routine use of interferon-gamma is beneficial in other immunosuppressed patient populations. Of note, increased levels of endogenous interferon-gamma are associated with increased risk of rejection in transplant patients (48,49). Therefore, careful study of this agent in clinical trials is warranted before its more general use can be recommended in immunosuppressed patients.

INF-gamma has been studied as adjunctive therapy in cryptococcosis. A phase II, double-blind trial of INF-gamma versus placebo in AIDS-associated cryptococcal meningitis revealed that this intervention was well tolerated. Also, the patients in this study treated with INF-gamma had a trend toward improved clinical and microbiological outcomes (50). INF-gamma has also been used in patients with *C. neoformans* meningitis refractory to aggressive medical therapy (49). A trial of INF-gamma in aspergillosis was planned but terminated prior to patient recruitment.

Interleukin-12 (IL-12)

Expression of the interleukin-12 cytokine is associated with a Th-1 immune response. Th-1 immunity is often required for effective clearance of many systemic fungal infections. Therefore, IL-12 therapy has been studied as a way to help resolve IFIs in experimental animals. For example, two studies demonstrated that IL-12 given to mice infected with *C. neoformans* resulted in increased survival and reduced fungal burden (51,52). Similarly, the early administration of IL-12 in a model of disseminated histoplasmosis resulted in improved survival compared to untreated animals (53).

Importantly, high doses of IL-12 in nonneutropenic animals resulted in an excessive immune response and poorer clinical outcomes in an animal model of aspergillosis. It is unclear if the excessive inflammation could be avoided with a reduced dose of IL-12, or if this therapy should only be considered for profoundly immune-deficient subjects (54).

There are numerous clinical trials of IL-12 as adjunctive therapy in treating patients with various malignancies. However, there is no substantial, published clinical experience describing IL-12 therapy in human mycoses.

Interleukin-2

IL-2 plays a major role in promoting the graft-versus-tumor effect after stem cell transplantation. Since this is often a desirable outcome after myeloablative therapy, investigators in Israel conducted a trial to determine whether routine administration of this cytokine would improve clinical outcomes in patients undergoing stem cell transplantation. The investigators noted that two of the first 12 patients treated with IL-2 therapy developed IFIs; this was a much higher incidence of systemic mycoses than would be expected from historic data. Therefore, the trial was terminated prematurely (55). The unexpected increase in IFIs associated with this cytokine therapy underscores the concern that immunomodulation may result in unpredictable clinical outcomes, and that novel therapy should be conducted in the setting of monitored clinical trials that are designed to identify adverse events.

PASSIVE ANTIBODY THERAPY

The use of nonspecific antibody therapy in immunocompromised patients was studied in a series of patients undergoing bone marrow transplantation (51). Forty-five patients received intravenous immunoglobulin weekly for three months after transplantation. Although the

IVIG-treated patients received less amphotericin B than controls, the untreated controls were less likely to experience fatal veno-occlusive disease of the liver. No difference was noted in other transplant-related complications or in two-year survival between the two groups, suggesting that no clinical benefit is derived from routine IVIG therapy in this patient population (51). In contrast, liver transplant patients receiving IVIG for CMV prophylaxis experienced significantly fewer fungal infections than untreated controls (56).

Pathogen-specific passive antibody therapy has demonstrated efficacy in models of human fungal infections, even when humoral immunity does not play a major role in the clearance of natural infections. For example, a monoclonal antibody directed against the polysaccharide capsule of *C. neoformans* helped to prevent death due to cryptococcosis in experimentally infected mice (57). Such studies have prompted human trials of antibody therapy for cryptococcosis. An anticryptococcal antibody preparation was well tolerated in treated patients, and it was associated with more rapid clearance of serum antigen than untreated controls. However, inadequate sample size prevented clear answers about efficacy (58). Monoclonal antibodies directed against the *C. neoformans* capsule are also in human trials (59).

Similarly, monoclonal antibodies have been developed against the *C. albicans* hsp90 heat shock protein and various surface polysaccharides. Several of these antibodies protect mice in experimental models of lethal candidiasis (60–62). The Mycograb monoclonal antibody directed against *C. albicans* hsp90 is currently being evaluated in clinical trials (63).

IMMUNE RECONSTITUTION SYNDROME (IRS)

The specific restoration of defective immune function offers significant promise in treating and preventing infections in immunocompromised patients. However, this same intervention may also result in unexpected worsening symptoms of infections. This observation has been frequently observed in patients with AIDS, who undergo immune recovery in response to highly active antiretroviral therapy. With increased immune system function, these patients can paradoxically develop symptomatic infections with various pathogens, presumably due to a reawakening immune system that is now able to respond to present pathogens. For example, patients may experience worsening retinal or colonic inflammation due to CMV disease as their CD4 counts increase in response to antiretroviral therapy.

Similarly, other instances of increasing immune function might also paradoxically result in increased symptomatic fungal infection. Pulmonary aspergillosis is frequently identified during neutrophil recovery after periods of prolonged neutropenia, presumably due to the recovered ability of the infected patient to actually mount an appropriate inflammatory response to this pathogen (64).

In a retrospective analysis of immune reconstitution syndrome (IRS) associated with invasive fungal diseases, Singh described that AIDS patients with cryptococcal meningitis developed IRS in 30% to 33% of cases when antiretroviral therapy was initiated soon after diagnosis of the infection (65). IRS was also reported in 5% of solid-organ transplant patients within 5.5 weeks of initiating antifungal therapy for an invasive mycosis (65,66). In a recent review, Singh and Perfect also proposed diagnostic criteria for defining IRS associated with opportunistic fungal infections (Table 2).

Table 2 Suggested Diagnostic Criteria for Immune Reconstitution Syndrome (IRS) Associated with Opportunistic Mycoses

-
1. New appearance or worsening of
 - (a) Clinical or radiographic manifestations consistent with increased inflammation
 - (b) CSF pleocytosis
 - (c) Increased intracranial pressure
 - (d) Histopathology showing granulomatous inflammation
 - (e) Unexplained hypercalcemia
 2. Symptoms occurring during receipt of appropriate antifungal therapy, and symptoms not consistent with newly acquired infection
 3. Negative cultures for initial fungal pathogen, or stable/reduced biomarkers of initial infection
-

For clinicians who care for highly immunosuppressed patients with IFIs, it will be important to develop an acute awareness of the possibility of IRS. As novel strategies for immunotherapy or augmentation of immune function are used to treat systemic mycoses, clinicians will need to be able to distinguish between symptoms of worsening infection and symptoms associated with enhanced immune response to the fungal pathogens.

CONCLUSION

With advances in medical therapies, there is also an increase in the number of immunocompromised patients at risk for IFIs. The specific enhancement of the immune system may offer clinical benefit in treating fungal diseases.

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9 Fungal Biofilms and Catheter-Associated Infections

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INTRODUCTION

The use of indwelling medical devices (e.g., central venous catheters; CVCs) in current therapeutic practice has been found to be responsible for more than 80% to 90% of hospital-acquired bloodstream and deep tissue infections (1). Transplantation procedures, immunosuppression, and prolonged intensive care unit stays have also increased the prevalence of nosocomial, especially fungal infections. *Candida* spp. are the most commonly associated fungal organisms with such nosocomial infections, with *Candida albicans* being the most common species causing both superficial and systemic diseases. Even with current antifungal therapy, mortality associated with invasive candidiasis due to nosocomial infections can be as high as 40% in adults (2,3) and up to 30% in the neonate population (4). In a multicenter study of 427 consecutive patients with candidemia, the mortality rate for patients with catheter-related candidemia was found to be 41% (5). *Candida* infections are often associated with indwelling medical devices such as dental implants, catheters, heart valves, vascular bypass grafts, ocular lenses, artificial joints, and central nervous system shunts, and commonly involve biofilm formation. Forty percent of patients with microbial colonization of intravenous catheters develop occult fungemia, with consequences ranging from focal disease to severe sepsis and death (5,6). The tenacity with which *Candida* infects indwelling biomedical devices necessitates their removal to effect a cure. For candidemia-associated nontunneled CVCs, initial management includes exchanging of catheter and performing semiquantitative or quantitative catheter cultures (7), and the Infectious Diseases Society of America (IDSA) guidelines suggest that antifungal therapy is necessary in all cases of vascular catheter-related candidemia (8). Catheter-related bloodstream infections (CRBSIs) commonly involve colonization of microorganisms on catheter surfaces where they eventually become embedded in a biofilm (9).

Biofilms are defined as extensive communities of sessile organisms irreversibly associated with a surface, encased within a polysaccharide-rich extracellular matrix (ECM), exhibiting enhanced resistance to antimicrobial drugs (9). Since *C. albicans* is the most common fungus associated with CRBSIs, biofilms formed by this fungus can be used as a model to investigate the biology and pathogenesis of biofilm-associated infections. Recent studies have provided revealing insight into the effect of different variables (including growth time, nutrients, and physiological conditions) on fungal biofilm formation, morphology, and architecture. This chapter provides a "state-of-the-biofilm" update and discusses major recent advances achieved in the clinically relevant area of catheter-associated fungal biofilms.

QUANTIFICATION OF BIOFILMS FORMED IN VITRO

Various model systems have been used to investigate the properties of *Candida* biofilms in vitro (10). These range from simple assays with catheter disks to more complex flow systems, such as the perfused biofilm fermentor (11). Subsequent in vitro model systems have included a variety of different plastics, microtiter plates, glass slides, microporous cellulose filters, acrylic strips, voice prostheses, catheter disks, contact lenses, and tissue culture flasks (12–18). Although a variety of substrates support formation of biofilms, those formed on clinically relevant substrates like catheters, denture acrylic strips, and contact lenses under physiological conditions are likely to be closer to the clinical setting than those formed on nonphysiologically relevant substrates.

Initial characterization of *C. albicans* biofilms by Hawser and Douglas (14) involved growing adherent *C. albicans* populations on the surface of small disks cut from a variety of catheters (14), including latex urinary catheters, polyvinyl chloride CVCs, silicone elastomer-coated latex urinary Foley catheters, silicone urinary Foley catheters, and polyurethane CVCs. In this model, growth was quantified using a colorimetric assay on the basis of reduction of a tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)] or incorporation of ^3H -leucine (14). This study showed an increase in MTT values and ^3H -leucine incorporation levels with the maturation of biofilms and showed that both methods resulted in strong correlation with biofilm dry weight (14). Our initial work on fungal biofilms involved development and characterization of *C. albicans* biofilms formed on common bioprosthetic material: silicone elastomer (SE), a model material used for indwelling devices including catheters (13). Measurement of biofilm growth was performed using two quantitative methods: (i) colorimetric assays that involved the reduction of 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl amino) carbonyl]-2H-tetrazolium hydroxide] (XTT) by mitochondrial dehydrogenase in the living cells, into a colored water-soluble product measured spectrophotometrically, and (ii) dry weight determination, in which biofilms were scraped off the substrate surface and filtered through a pre-weighed membrane filter under vacuum (12–14). Dry weight and XTT values increased with the formation of biofilms (13). Garcia-Sanchez et al. (19) utilized disks to form biofilms and quantified biofilm formation using XTT and also showed an increase in XTT values with the formation of biofilm. Ramage et al. (20), using 96-well microtiter plate model, performed a series of experiments to assess the variability between *C. albicans* biofilms formed in independent wells of the same microtiter plate. All biofilms formed on the microtiter plates over a 24-hour time period displayed consistent XTT readings when the intensity of the colorimetric product was measured (20). In general, formation of biofilm is associated with increase in metabolic activity and dry weight (12–14). However, some later studies showed that there were some limitations of the XTT assay, especially when using metabolic activities to compare biofilm formation between *C. albicans* and *C. parapsilosis* isolates (21,22). Additionally, increase in dry weight of biofilms may not always correlate with increase in metabolic activity with biofilm development, since these assays characterize different properties within the biofilm (biomass and metabolic state, respectively) (21,22). Therefore, careful interpretation is critical while assessing results obtained using these in vitro models. Development of these catheter-related in vitro models has allowed detailed investigation, microscopic evaluation, and gene/protein profiling of *Candida* biofilms.

FUNGAL BIOFILMS EXHIBIT PHASE-DEPENDENT GROWTH AND A HETEROGENEOUS ARCHITECTURE

Various microscopic techniques, including scanning electron microscopy (SEM), fluorescence microscopy (FM), confocal scanning laser microscopy (CSLM), have been used to visualize the structure of biofilms. Fluorescence microscopy analysis allows visualization of gross biofilm morphology and the appearance of extracellular matrix during biofilm formation, while SEM enables evaluation of detailed surface topography and morphology at very high resolutions. However, sample processing for SEM involves fixation and dehydration steps, which degrade the native hydrated structural features. Confocal microscopy is a nondestructive technology that can visualize the intact structure of live biofilms at lower resolutions (23) but allows researchers to overcome some drawbacks of SEM.

Hawser and Douglas (14) used SEM to show that *C. albicans* biofilms comprise a network of yeasts, hyphae, pseudohyphae, and extracellular polymeric material visible on the surface of some of these morphological forms. Similar structures were seen by Chandra et al. (12), where they showed amorphous granular material [Fig. 1(B), arrow] covering yeast and hyphal forms [Fig. 1(A) and 1(B)]. Using a 96-well plate model, Ramage et al. (24) also showed that mature *C. albicans* biofilms consisted of a dense network of yeast cells and hyphal elements embedded within exopolymeric material. Hawser and Douglas (14) showed that after one hour of incubation, cell population consisted of all budding yeast cells adhering to the catheter. After three to six hours, some cells had developed into germ-tubes, and biofilms grown to 24 to 48 hours consisted of a mixed population of yeast, hyphae, pseudohyphae, and extracellular matrix (14).

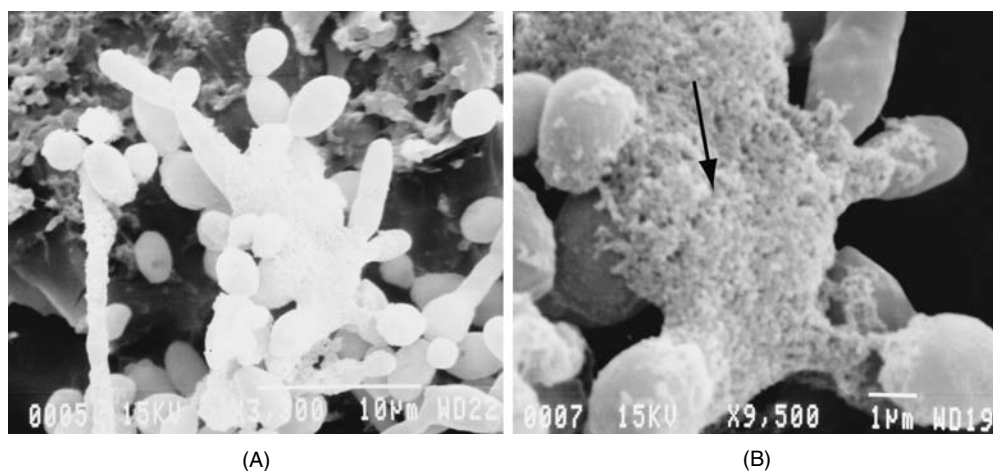


Figure 1 (A) Scanning electron microscopy (SEM) of a *C. albicans* biofilm showing fungal cells covered with biofilm matrix. Some hyphal forms are also seen (magnification 3300 \times). (B) *C. albicans* cells observed embedded in extracellular polymeric material that had an amorphous granular appearance (as shown by arrow) (magnification 9500 \times).

Owing to the limitations of SEM, FM and CSLM were used to visualize intact structures of biofilm and the developmental phases associated with the biofilm growth were identified. FM studies showed that biofilm develops in three distinct phases: early (0–11 hours), intermediate (~12 to 24 hours), and mature phase (24–48 hours) (13). CSLM allows visualization of three-dimensional (3D) architecture at different depths of biofilms and also the measurement of biofilm thickness without distortion of the native biofilm structure. In CSLM studies, two stains were utilized: Concanavalin Alexa Fluor (CON A), which binds polysaccharide and gives a green fluorescence, and FUN-1, which stains metabolic active yeast cells as red fluorescence. In the 3D reconstructed images of *C. albicans* biofilms, at the early phase, only adhered yeast cells were attached to the catheter surface and lacked any extracellular material, while mature biofilms consisted of *C. albicans* cells and hyphae encased in a dense extracellular matrix (13). Our study and other studies showed that biofilms have two distinct morphological layers: a thin, basal yeast layer that anchors the biofilm to the surface and a thicker, but more open hyphal layer surrounded by an extracellular matrix (13,25). These microscopic evaluations of catheter-related *Candida* biofilms also showed that biofilms are formed in three distinct developmental phases and exhibit a highly heterogeneous structure.

BIOFILM GROWTH IS INFLUENCED BY DIFFERENT SUBSTRATES

The type of substrate used to form biofilm can greatly influence architecture, morphology, and thickness of the biofilm. Initial characterization of *C. albicans* biofilms by Hawser and Douglas (14) involved growing *C. albicans* biofilms on different types of catheter materials. The most extensive biofilm formation by *C. albicans* was observed on latex urinary catheter material (14). Moreover, biofilm formation was slightly increased on silicone elastomer (SE) compared with polyvinyl chloride, and decreased substantially on polyurethane or 100% silicone (14). Other studies showed that *C. albicans* cells grown on SE (which have uniformly flat hydrophobic surface) produced a nearly uniform confluent layer of adherent blastospores, which at maturation were several cells thick [approximately 10–12 μm ; Fig. 2(A) and 2(B)] (13). Above this layer of cells, profuse matrix (300–400 μm thick) was apparent which consisted of extracellular material and hyphal elements [Fig. 2(A) and 2(B)] (13). Hyphal elements originating at the base layer pervaded the extracellular material, both in proximity to the blastospores and through the entire thickness [Fig. 2(C) and 2(D)] (13).

To further investigate the effect of substrate surface on biofilm-forming ability of *C. albicans*, it was determined whether surface modifications of polyetherurethane [Elasthane 80A (E80A)], polycarbonateurethane, and poly(ethyleneterephthalate) (PET) can influence fungal

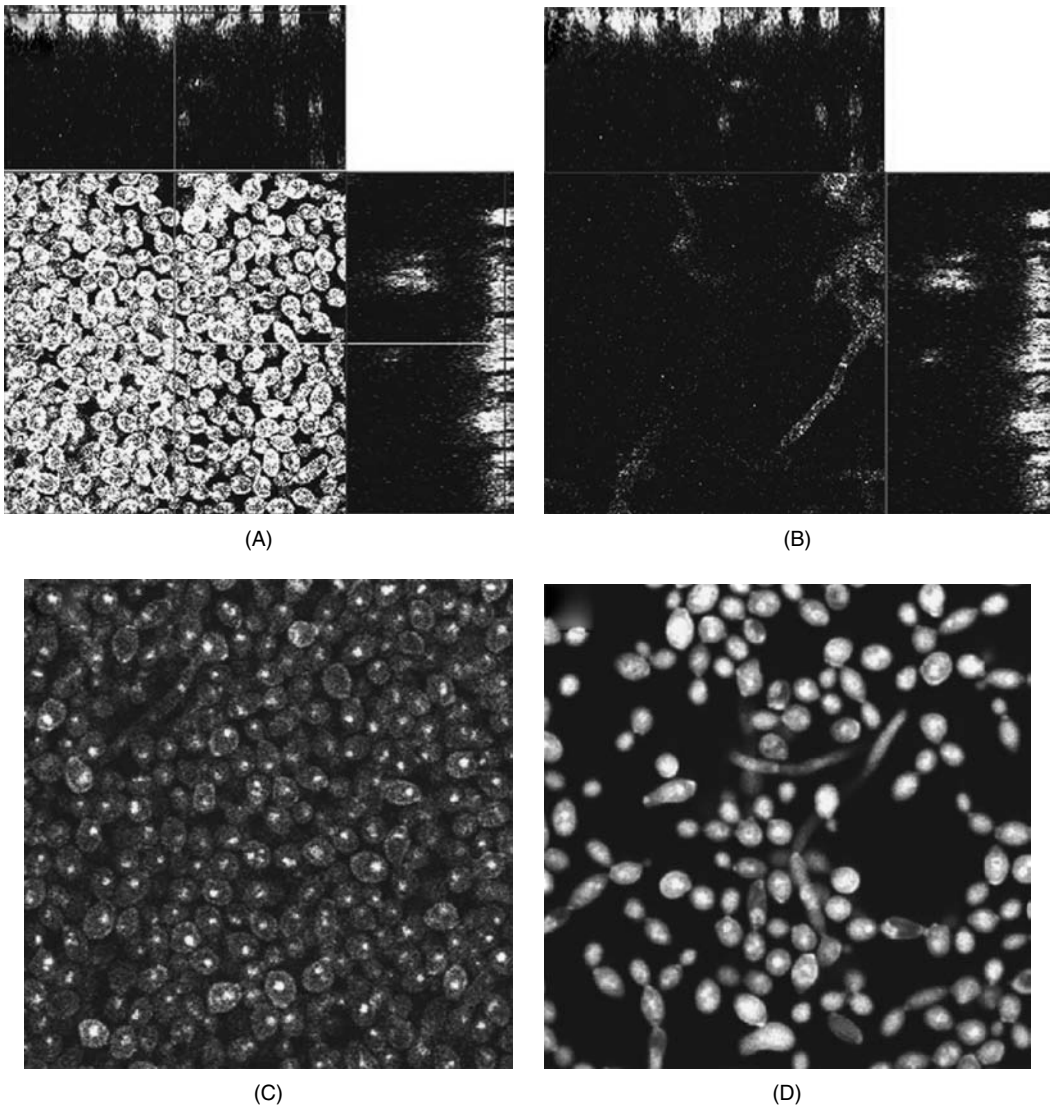


Figure 2 Confocal scanning laser microscopy (CSLM) images of mature *C. albicans* biofilms formed on silicone elastomer surface. Orthogonal images of the basal (10–12 μm thick) (A) and upper layers (450 μm thick) of *C. albicans* mature biofilm (B). The ECM-derived haziness seen in mature biofilm (C) is absent when the extracellular material is removed (D) (magnification 20 \times).

biofilm formation (26). Polyurethanes were modified by adding 6% polyethylene oxide (6PEO), 6% fluorocarbon, or silicone, while the PET surface was modified to generate hydrophilic, hydrophobic, cationic, or anionic surfaces. We found that biofilm formation by *C. albicans* on 6PEO-E80 A was significantly reduced (by 78%) compared to that of biofilm formed on the nonmodified E80 A (optical densities of 0.054–0.020 and 0.24–0.10, respectively; $p = 0.037$) (26). The total biomass of *Candida* biofilm formed on 6PEO-E80 A was 74% lower than that on the nonmodified E80 A surface (0.46–0.15 vs. 1.76–0.32 mg, respectively; $p = 0.003$). In contrast, fungal cells were easily detached from the 6PEO-E80 A surface, and it was unable to detect *C. albicans* biofilm on this surface (26). Taken together, these studies showed that substrate type and surface coatings play an important role in the pathogenesis of device-related fungal biofilms and indicated that surface modification is a viable approach for identifying surfaces that have antibiofilm characteristics.

ANTIFUNGAL RESISTANCE IN BIOFILMS IS MEDIATED BY MULTIPLE MECHANISMS, IN A PHASE-DEPENDENT MANNER

The development of various models has helped detailed characterization of *C. albicans* biofilms and gaining insight into biofilm resistance and underlying mechanisms. Hawser and Douglas (27) initially showed that fungal biofilms grown on catheter material for 48 hours become resistant to a variety of antifungals including amphotericin B, flucytosine (5-fluorocytosine), fluconazole, itraconazole, and ketoconazole. Ramage et al. (20) using 96-well microtiter plate model studied antifungal susceptibility testing of several *C. albicans* strains grown as biofilms against amphotericin B and fluconazole, and the increased resistance of *C. albicans* biofilms against these antifungal agents was demonstrated. Chandra et al. (13) showed a similar resistance pattern with silicone elastomer model where MICs of fluconazole were 1 and >128 $\mu\text{g}/\text{mL}$ for planktonic and biofilm-grown *C. albicans* cultures, respectively. Since it is possible that antifungal resistance evolves as the biofilm grows to maturation, Chandra et al. (13) investigated correlations between biofilm development and antifungal susceptibility. MICs of amphotericin B, nystatin, fluconazole, and chlorhexidine were determined for early, intermediate, or mature biofilm phases. *C. albicans* exhibited low MICs at the early biofilm phase. MICs during this phase were 0.5, 1, 8, and 16 $\mu\text{g}/\text{mL}$ for amphotericin B, fluconazole, nystatin, and chlorhexidine, respectively (Fig. 3). Moreover, as the biofilms developed, MICs progressively increased (Fig. 3). By 72 hours, *C. albicans* cells were highly resistant, with MICs of 8, 128, 32, and 256 $\mu\text{g}/\text{mL}$ for amphotericin B, fluconazole, nystatin, and chlorhexidine, respectively (13) [Fig. 3(A–D)]. The progression of drug resistance was associated with the concomitant increase in metabolic activity of developing biofilms (Fig. 3). This indicated that the observed increase in drug resistance was not simply a reflection of higher metabolic activity of cells in maturing biofilms but that drug resistance develops over time, coincident with biofilm maturation (13).

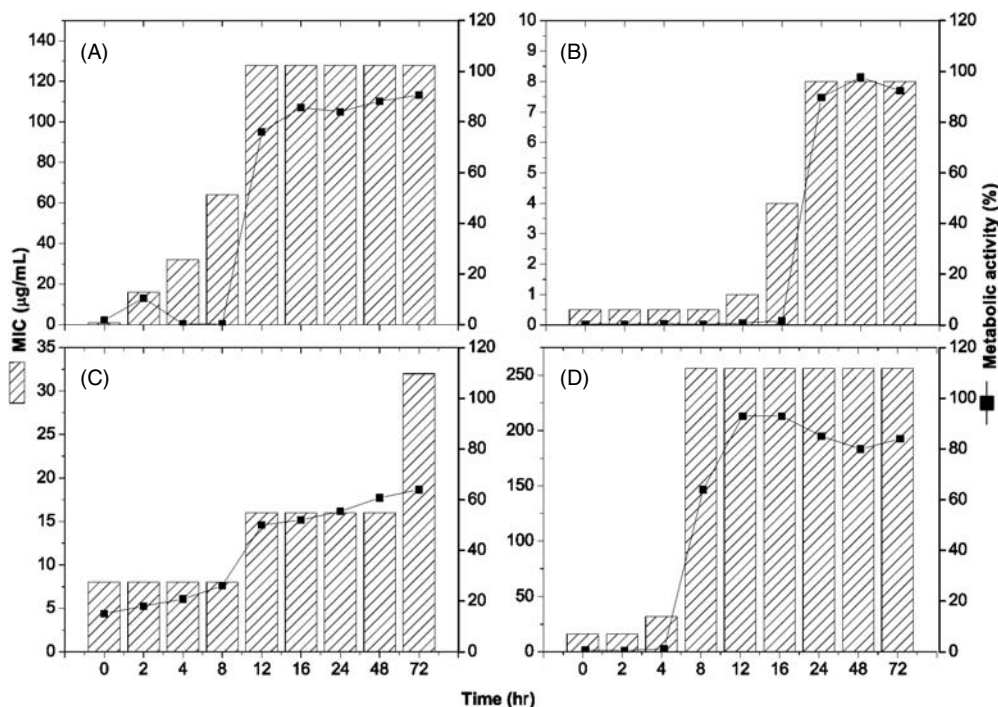


Figure 3 Correlation of biofilm development and metabolic activity with antifungal resistance. The susceptibilities of *C. albicans* at different stages of biofilm development to fluconazole (A), amphotericin B (B), nystatin (C), and chlorhexidine (D) are represented as histograms. The line curves show percent metabolic activity of growing *C. albicans* biofilms exposed to fluconazole (64 $\mu\text{g}/\text{mL}$), amphotericin B (4 $\mu\text{g}/\text{mL}$), nystatin (8 $\mu\text{g}/\text{mL}$), or chlorhexidine (64 $\mu\text{g}/\text{mL}$). Metabolic activity was normalized to the control without drugs, which was taken as 100%.

To further evaluate drug resistance mechanisms involved with *C. albicans* biofilms, studies were conducted at the biochemical and molecular levels. Earlier studies showed that antifungal resistance of planktonically grown *C. albicans* has been linked to the expression of efflux pumps such as Cdr1p, Cdr2p, and Mdr1p (28). In this regard, using a 96-well microtiter plate model of biofilm formation, Ramage et al. (29) reported that efflux pumps including Cdr1p, Cdr2p, and Mdr1p were not involved in drug resistance associated with mature *C. albicans* biofilms. In a subsequent study, Mukherjee et al. (30) compared the mechanism of antifungal resistance in biofilms at early and mature phases. These investigators showed that in early phase biofilms, efflux pumps contributed to antifungal resistance, while in mature phase biofilms, resistance was associated with changes in levels of ergosterol biosynthesis intermediates (30). The role of efflux pumps in biofilm-associated resistance was confirmed in a separate study by Mateus et al. (31) Adherence of *C. albicans* to silicone induces immediate enhanced tolerance to fluconazole (31) and they showed that expression of *MDR1* and *CDR1* genes was significantly lower in daughter cells from 48-hour biofilms than in firmly adherent cells (two hours after attachment), suggesting that efflux pump expression in adherent cultures is transient. These studies clearly demonstrated that antifungal resistance in *Candida* biofilms is due to multiple mechanisms that are phase dependent.

BIOFILM FORMATION IS ASSOCIATED WITH DIFFERENTIAL EXPRESSION OF METABOLIC PATHWAYS

Evidence from Gene Expression and Microarray Studies

Since biofilms are very complex structures and highly resistant to antifungals, it was necessary to identify detailed molecular mechanisms involved with biofilm formation. Initial studies involved investigating biofilm-specific expression profile of genes known to be associated with adhesion (e.g., *ALS* family genes) and germination of *C. albicans* cells. Chandra et al. (13) showed that there was a differential expression of *ALS* family of genes between biofilm and planktonic cultures with additional gene(s) expressed in biofilms. Higher expression of *ALS* genes in biofilms suggested that adhesion phase plays an important role in biofilm formation.

Microarray analyses have been used in three different studies to identify biofilm-specific gene expression patterns in *C. albicans* (19,32,33). In the first such study, Garcia-Sanchez et al. (19) identified a cluster of 325 differentially expressed genes, using different sets of biofilm models. In agreement with the overrepresentation of amino acid biosynthesis genes in this cluster, Gcn4p, a regulator of amino acid metabolism, was shown to be required for normal biofilm growth (19). To identify biofilm-related genes that are independent of mycelial development, Garcia-Sanchez et al. (19) also studied the transcriptome of biofilms produced by a wild-type, hypha-producing strain and a *cph1/cph1 efg1/efg1* strain defective for hypha production. This analysis identified a cluster of 317 genes expressed independently of hypha formation, whereas 86 genes were dependent on mycelial development (19). Both sets revealed the activation of the sulfur-amino acid biosynthesis pathway as a feature of *C. albicans* biofilms (19).

In another study by Murillo et al. (32), the early stage of *C. albicans* biofilm was characterized by the adhesion of single cells to the substratum, followed by the formation of an intricate network of hyphae and the beginning of a dense structure. These authors showed that changes in the transcriptome begin within 30 minutes of contact with the substrate and include expression of genes related to sulfur metabolism, in particular *MET3*, and the equivalent gene homologues of the Ribi regulon in *Saccharomyces cerevisiae* (32). This study showed that some of these changes were initiated early and maintained throughout the process; others were restricted to the earliest stages of biofilm formation (32). They also identified a potential alternative pathway for cysteine metabolism and the biofilm-associated expression of genes involved in glutathione production in *C. albicans* (32).

In a separate study, Yeater et al. (33) used microarrays to identify changes in gene expression patterns associated with different developmental phases of biofilms formed by two different clinical isolates of *C. albicans* (one associated with denture stomatitis, the other with invasive candidiasis) on two different substrates (denture strips and catheter disks, respectively). These investigators showed that 243 genes were differentially expressed over the experimental time-course in either biofilm or planktonic cells, of which the majority (191 genes) was differentially

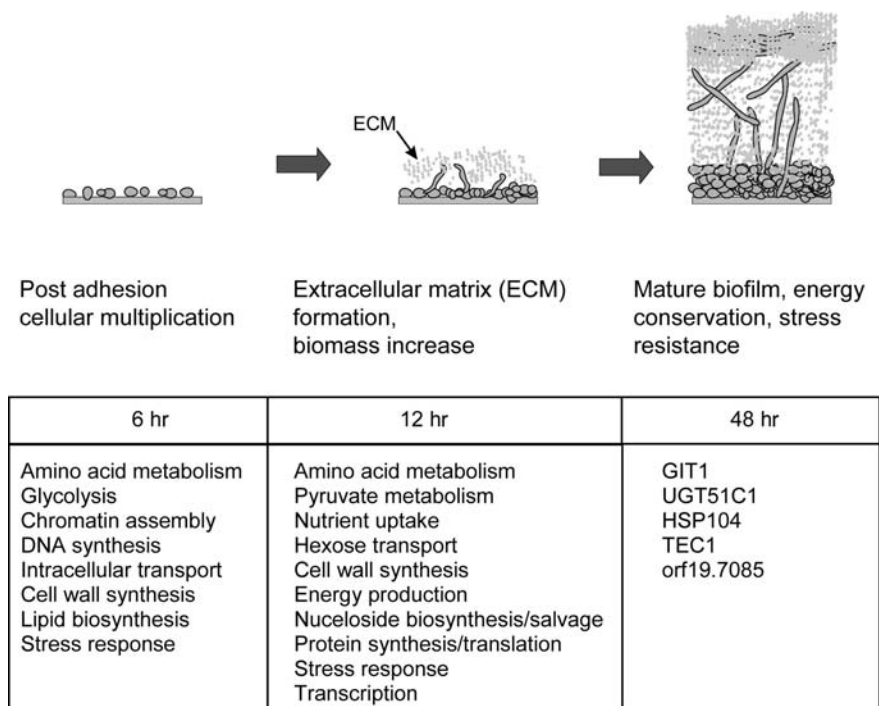


Figure 4 Summary of the cellular processes that are associated with genes upregulated at the different time points during *C. albicans* biofilm development. The time-course of biofilm development is shown highlighting the time points (6, 12, and 48 hours) studied by microarray analysis. Descriptions of cellular processes are summarized. Categories of genes upregulated at 6 hours versus 12 hours are summarized under the 6-hour heading. Data from both the 12 hours versus 6 hours and 12 hours versus 48 hours comparisons are placed under the 12-hour heading. Few genes are upregulated at 48 hours compared to 12 hours, suggesting that initiation of new metabolic activity is relatively low in the mature biofilm. The individual genes upregulated at 48 hours versus 12 hours are listed.

expressed only during biofilm development. Genes involved in cellular processes like glycolytic and nonglycolytic carbohydrate assimilation, amino acid metabolism, and intracellular transport mechanisms were upregulated during the early phase of biofilm formation (Fig. 4). These early events increased intracellular pools of pyruvate, pentoses, and amino acids and upregulated genes involved with these processes. These intermediate processes prepared the biofilm for the large biomass increase that begins around 12 hours of development (33). This developmental stage also demands energy and utilizes specific transporters for amino acids, sugars, ions, oligopeptides, and lactate/pyruvate. At mature phase (48 hours), few genes were differentially expressed compared with the 12-hour time point, suggesting a relative lack of initiation of new metabolic activity (Fig. 4) (33).

Despite differences in experimental design and focus, data from all three microarray studies compared favorably with each other (19,32,33). Yeater et al. (33) and Murillo et al. (32) had similar results with the expression of genes involved in sulfur metabolism and oxidative metabolism as discussed above (33). Additionally, overlap between the published gene sets of Garcia-Sanchez et al. (19) and Yeater et al. (33) occurred primarily for genes identified at 12-hour time point, suggesting that the 48-hour biofilms analyzed by Garcia-Sanchez et al. were still undergoing active growth. Although overlap between lists of differentially expressed genes was somewhat limited, the cellular processes identified in the two studies were similar and included protein synthesis/translation, and amino acid and nucleotide metabolism (19,33).

The fact that similar gene upregulation can be found between disparate datasets suggests that processes fundamental to biofilm development are conserved across various models.

In another study, evaluation of sets of genes or gene families indicated that loss of wild-type *ALS2* (34) or *ALS3* (35) decreases biofilm mass in the catheter model and that deletion of

genes encoding the CFEM (common in several fungal extracellular membrane) domain results in abnormal biofilm formation on a polystyrene surface (36). Assay of transcription factor mutants showed that Efg1p is required for wild-type biofilm formation on plastic surfaces, suggesting a requirement for Efg1p-mediated filamentation or for the product of an Efg1p-regulated gene for biofilm growth (37). Screening of a set of transcription factor mutants revealed that strains lacking Bcr1p could not form wild-type biofilms (38). Further work demonstrated the contributions of Bcr1p targets *ALS3*, *ALS1*, *HWPI*, and *ECE1* to biofilm formation (38,39).

These varied experimental approaches have contributed to a better understanding of gene expression associated with biofilm development. These studies suggested that *C. albicans* biofilms exhibit changes in gene expression to adjust to varying requirements during development, with the expression of specific subsets of genes at different developmental phases. However, no single gene has been found to be the “master-regulator” that controls *Candida* biofilm formation, suggesting that complementary or alternative gene expression exists in *Candida* biofilms.

Evidence from Proteomics and Metabolic Pathway Mapping Studies

Although microarray and other gene expression studies have identified a number of differentially expressed genes in *Candida* biofilms, such expression is not always correlated at the functional protein level. Moreover, redundant gene expression profiles likely compensate for any loss of function in the biofilms. Therefore, it is necessary to evaluate global protein profile of biofilms and identify protein/s that are specifically produced in biofilms, since such proteins represent novel drug targets. Initial studies by Mukherjee et al. (40) involving proteomic screening of early phase *C. albicans* catheter-related biofilms showed differential expression of 24 proteins. One of the proteins that was downregulated was alcohol dehydrogenase (Adh1p) (40). Targeted disruption of *ADH1* or inhibition of the enzyme using specific inhibitors resulted in thicker biofilm in vitro than those of the parent and revertant strains (40). As it is known that Adh1p catalyzes the reversible conversion of acetaldehyde to ethanol, Mukherjee et al. (40) also showed that deletion of the *C. albicans ADH1* gene resulted in a decrease in ethanol and an increase in acetaldehyde levels (40). These results suggested that the effect of Adh1p on biofilm formation was mediated by its enzymatic activity and not by a general change in cellular metabolism. In another study, Crowe et al. (41) used a proteomics-based approach in planktonic *C. albicans* to identify Adh1p as one of eight *C. albicans* cell wall proteins that were able to bind plasminogen and that activated plasmin release. Since plasmin is known to have proteolytic activity, these authors suggested that Adh1p may also contribute to fungal invasion of host tissues (41).

In a recent study, proteomic analyses of the carbohydrate-rich extracellular matrix (ECM) and cell wall proteins of catheter-associated biofilms at early phase (6 hours) and mature phase (48 hours) showed differential expression of 151 proteins (107 proteins to be differentially expressed in ECM, while 44 were differentially expressed in cell walls), compared to planktonically grown cells (42). Among these differentially expressed proteins, 95% (102/107) and 68% (30/44) were upregulated in ECM and cell walls of biofilms, respectively (42). To narrow down the list of targeted proteins, these investigators mapped the differentially expressed proteins based on their putative functions to known pathways. Such pathway mapping analyses revealed that majority of these differentially expressed proteins were associated with metabolic pathways, in a phase-dependent manner (42). Among ECM-associated proteins, proteins within 18 pathways were differentially expressed, with two pathways (glutamate and nitrogen metabolism) unique to early phase, and four pathways (purine, Gly/Ser/Thr, inositol metabolism, and carbon fixation) unique to mature phase biofilms (42) (Table 1). Such differences were also observed in cell wall associated proteins, where proteins associated with 14 specific pathways were differentially regulated (Table 1). Lattif et al. (42) also showed that glycolytic enzymes including the key enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were overexpressed in biofilms at both early and mature phases, compared to planktonic controls (42).

These results suggested that glycolytic pathway and GAPDH play critical roles in *Candida* biofilm formation. These investigators also found that although the same pathways (e.g., glycolysis) may be differentially expressed at both early and mature phases in biofilms, the number and identity of the actual proteins involved in these pathways differed considerably between the

Table 1 Differentially Expressed Pathways in Matrix and Cell Walls Isolated from *C. albicans* Biofilms Grown to Early and Mature Developmental Phases

Protein sample	Early phase (6 hr)	Mature phase (48 hr)	
Matrix	Purine metabolism	Fructose and mannose metabolism	
	Fructose and mannose metabolism	Purine metabolism ^a	
	Pentose phosphate pathway	Glycolysis/gluconeogenesis	
	Glycine, serine, and threonine metabolism	Pentose phosphate pathway	
	Glycolysis/gluconeogenesis	Glycine, serine, and threonine metabolism ^a	
	Biosynthesis of steroids	Carbon fixation ^a	
	Urea cycle and metabolism of amino groups	Inositol metabolism ^a	
	Glutamate metabolism ^a	Biosynthesis of steroids	
	Methionine metabolism	Urea cycle and metabolism of amino groups	
	Valine, leucine, and isoleucine biosynthesis	Methionine metabolism	
	Lysine biosynthesis	Valine, leucine, and isoleucine biosynthesis	
	β -Alanine metabolism	Lysine biosynthesis	
	Glycerolipid metabolism	β -Alanine metabolism	
	Glycerophospholipid metabolism	Glycerolipid metabolism	
	Pantothenate and CoA biosynthesis	Glycerophospholipid metabolism	
	Terpenoid biosynthesis	Pantothenate and CoA biosynthesis	
	Nitrogen metabolism ^a	Terpenoid biosynthesis	
	Calcium signaling pathway	Nitrogen metabolism ^a	
	Cell walls	Glycolysis/gluconeogenesis	Calcium signaling pathway
		Fructose and mannose metabolism	Glycolysis/gluconeogenesis
Inositol metabolism		Fructose and mannose metabolism	
Fatty acid metabolism		Fatty acid metabolism	
Bile acid biosynthesis		Oxidative phosphorylation	
Oxidative phosphorylation		Carbon fixation	
Valine, leucine, and isoleucine degradation		Inositol metabolism	
Valine, leucine, and isoleucine biosynthesis		Aminoacyl-tRNA biosynthesis ^a	
Lysine biosynthesis		PPAR signaling pathway ^a	
Lysine degradation		Citrate cycle (TCA cycle) ^a	
Tyrosine metabolism		Pentose phosphate pathway ^a	
Phenylalanine, tyrosine, and tryptophan biosynthesis		Bile acid biosynthesis	
Glycerolipid metabolism		Glycine, serine, and threonine metabolism ^a	
1- and 2-methylnaphthalene degradation		Methionine metabolism ^a	
3-Chloroacrylic acid degradation		Cysteine metabolism ^a	
Carbon fixation		Valine, leucine, and isoleucine degradation	
Pantothenate and CoA biosynthesis		Valine, leucine, and isoleucine biosynthesis	
		Lysine biosynthesis	
		Arginine and proline metabolism ^a	
		Tyrosine metabolism	
	Phenylalanine, tyrosine, and tryptophan biosynthesis		
	Glycerolipid metabolism		
	α -Linolenic acid metabolism ^a		
	Pyruvate metabolism ^a		
	1- and 2-methylnaphthalene degradation		
	3-Chloroacrylic acid degradation		
	Pantothenate and CoA biosynthesis		
	Metabolism of xenobiotics by cyt-P450 ^a		
	Polyunsaturated fatty acid biosynthesis ^a		
	Proteasome ^a		
	Type III secretion system ^a		
	Calcium signaling pathway ^a		

^aPhase-specific pathways overexpressed specifically in matrix or cell walls of early or mature biofilms.

two phases (42). Such changes in the number and identity of differentially expressed proteins involved in carbohydrate and amino acid metabolism, and in the calcium and PPAR signaling pathways, may account for the dramatic increase observed in metabolic activity and matrix production in mature biofilms.

In a separate study, Thomas et al. (43) reported the use of conventional two-dimensional gel electrophoresis (2D-GE)-based proteomics for the comparative analysis of the protein profiles of surface-associated and secreted proteins obtained from mature biofilm and planktonic cultures of *C. albicans*. These investigators reported that 9 surface-associated and 34 secreted proteins were differentially expressed in biofilms. In contrast, the study involving 2D difference-in-gel electrophoresis (DIGE) analyses revealed much higher number of differentially expressed proteins in mature *C. albicans* biofilms (35 cell wall-associated and 56 extracellular matrix-associated proteins were differentially expressed, of which 24 and 54 were upregulated, respectively) and early phase biofilms (42).

Proteomic studies were in agreement with the overall trend seen in the earlier microarray studies, although the specific genes and proteins did not always correlate (42). Interestingly, in the earlier microarray study, Yeater et al. (33) showed that at mature phase (48 hours), few genes were differentially expressed, suggesting a relative lack of initiation of new metabolic activity at the transcriptional level. However, in the proteomic study Lattif et al. (42) observed active metabolic processes occurring at the mature phase. The difference between microarray and proteomic data (33,42) may be due to the fact that cellular processes in *Candida* cells are known to be regulated differently at the transcriptional and translational levels, and similar differential regulation of pathways involved in cell wall biogenesis, general metabolism, and signaling events is likely to occur in mature *Candida* biofilms.

Studying these pathways and associated proteins helped tremendously in understanding the critical roles played by them in *Candida* biofilm formation. Importantly, these proteins represent potential targets for designing antibiofilm drugs, as well as for early diagnosis of infections associated with *Candida* biofilms.

FUNGAL BIOFILMS DYNAMICALLY INTERACT WITH HOST IMMUNE CELLS

Candida albicans biofilms represent a source of persistent infection on indwelling devices and are associated with high antifungal resistance and evasions of the immune system's defenses. *C. albicans* can influence the innate immune response, mediated by alterations in cytokine production by the major products of innate immune cells. Several fungal factors, including mannan and glycoproteins (mannoprotein constituents of the cell wall), and surface antigens have been shown to have immunomodulatory activity (44–47). Although interactions between the host immune system and *Candida* have been investigated in some detail for bacterial biofilms and planktonically grown *Candida* cultures (48–54), there is not much information available for such interactions in a candidal biofilm environment. In the first study of its kind, the author and colleagues (55) demonstrated that a coculture of *C. albicans* with adherent peripheral blood mononuclear cells (PBMCs) enhances the ability of this pathogen to form biofilms on the silicone elastomer disks. This enhanced activity was mediated by a soluble factor present in the supernatant of *C. albicans* biofilm–PBMC coculture (55). CSLM analyses revealed an asymmetric localization of PBMCs within the biofilm, inside which monocytes were localized mostly to the basal layers and were unable to escape the biofilms. The characteristic heterogeneous bilayer architecture (basal yeast cells and top hyphal elements) was retained even in the presence of monocytes (55). Time lapse real-time microscopy evaluated the real-time interactions between PBMCs and *C. albicans* biofilms. Images of a single area of the *Candida* biofilm–PBMC coculture were obtained beginning with biofilm adhesion and followed at regular intervals up to the mature phase at 48 hours (55). Analyses of these images revealed that PBMCs interact with *C. albicans* biofilms over time. Active PBMC migration/movement was observed within localized areas of the biofilm. In contrast to PBMCs cocultured with planktonic *C. albicans*, where phagocytosis was clearly evident, analyses with *C. albicans* biofilms did not reveal any occurrence of phagocytosis (55). Cytokine profile analysis revealed that the biofilm–PBMC coculture supernatant contained increased levels of the proinflammatory cytokine IL-1 β but decreased levels of IL-6, IL-10, MCP-1, I-309, TNF- α , and the chemokine MIP1- β . The downregulated cytokines included both proinflammatory (e.g., TNF- α) and antiinflammatory (e.g., IL-6 and

IL-10) cytokines, suggesting that *C. albicans* biofilms and PBMCs undergo multiple interactions mediated by different cytokines (55). More in-depth studies investigating the link between biofilms and host immune cells may result in the identification of biomolecules that regulate such interactions and help develop novel strategies to manage and treat biofilm-related infections.

IN VIVO FUNGAL BIOFILMS ARE STRUCTURALLY SIMILAR TO IN VITRO BIOFILMS

Most of the studies involving fungal biofilms are performed using in vitro systems. It is important to validate the in vitro results using in vivo biofilm models that mimic the clinical environment. Use of in vivo models involving *C. albicans* biofilms has been limited to the vascular catheter models. Recently, murine model of subcutaneous catheter-associated *C. albicans* biofilms is also developed (56). There are two models that have been studied involving *C. albicans* catheter biofilms, which mimic vascular catheter infection in patients; these models are (i) catheter-associated *C. albicans* biofilm rabbit model and (ii) *C. albicans* catheter rat model (57,58). Schinabeck et al. (57) for the first time described development of a rabbit model of catheter-associated infection with *C. albicans* biofilms and showed that antifungal lock therapy with liposomal amphotericin B was an effective treatment strategy for these infections (57). These authors used two types of central venous catheters (CVCs), polyurethane and silicone CVCs, and placed them surgically in the jugular vein of female white rabbits (57). They showed by quantitative catheter culture (QCC) and SEM analyses that mature biofilms were formed equally on both catheters with abundant hyphal elements and blastospores embedded in thick extracellular matrix (57) [Fig. 5(A and 5(B)]. Previously, Hawser and Douglas reported that

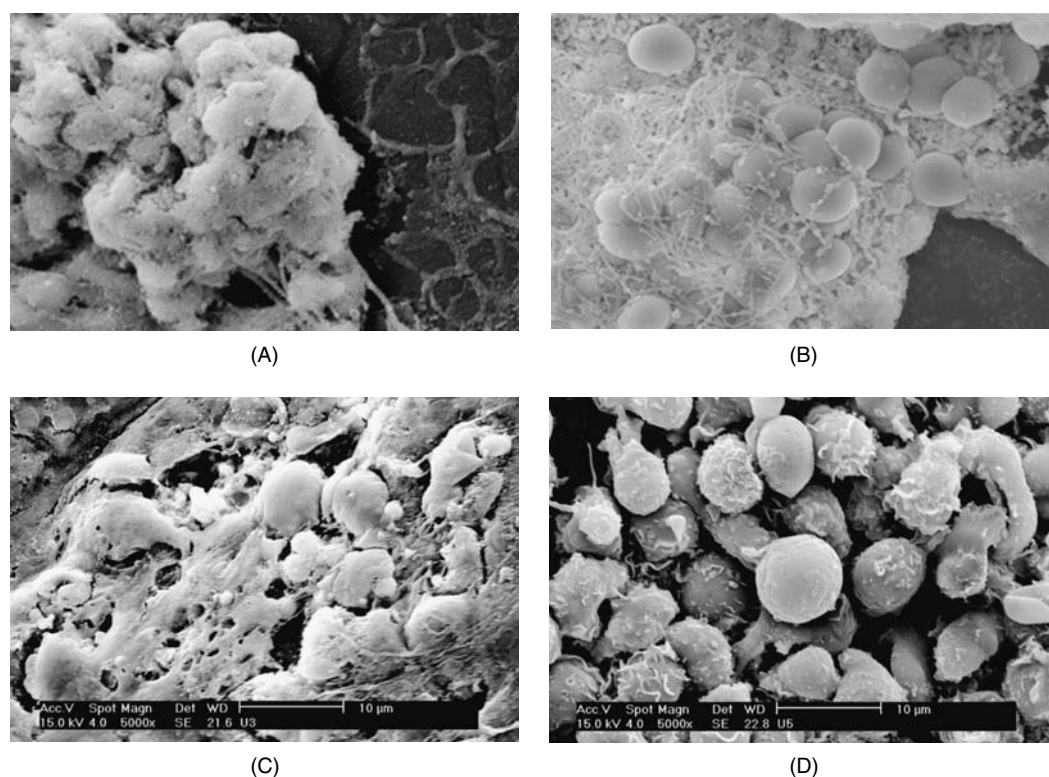


Figure 5 Scanning electron microscopy (SEM) pictures of in vivo *C. albicans* biofilms. (A) Mature in vivo biofilm formation during rabbit model development. SEM of *C. albicans* biofilms adherent to the intraluminal surface of catheters showing no difference in biofilm architecture at seven days postinfection (magnification 6500 \times) and (B) three days postinfection (magnification 2500 \times). (C) *C. albicans* biofilms seen on the surface of subcutaneously inserted silicone catheters removed from the mice. (D) Certain areas of the disks were covered with a large number of *Candida* cells mixed with white blood cells (magnification 5000 \times).

C. albicans biofilm formation in vitro was significantly decreased when grown on polyurethane compared to silicone elastomer disks (14). Schinabeck et al. (57) showed that this difference in biofilm formation on two types of catheters between in vitro and in vivo models may be due to the formation of conditioning films, or fibrin sheaths, on the intraluminal catheter surface in vivo after exposure to blood which may have enhanced the adherence of *C. albicans* and the biofilm formation (57). Nett and Andes in their review also reported that in vivo biofilms are structurally similar to those formed under laboratory conditions with the exception that in vivo biofilms have numerous host cells including red blood cells, macrophages, neutrophils, and platelets that are embedded within the matrix (59). Using a rat central venous catheter model, Andes et al. (58) characterized in vivo *C. albicans* biofilm development. Time-course quantitative culture demonstrated a progressive increase in the burden of viable cells for the first 24 hours of development. Fluorescence and scanning electron microscopy revealed a bilayered architecture on the catheter surface with yeast cells and hyphal forms densely embedded in an extracellular matrix and were similar to those described for in vitro models (58). Andes et al. (58) using their in vivo model determined drug susceptibility and demonstrated a biofilm-associated drug resistance phenotype. They also showed a differential gene expression associated with in vivo biofilm growth and in this regard two fluconazole efflux pumps, *CDR1* and *CDR2*, were upregulated in the in vivo biofilm-associated cells (58). However, transcription of *ERG11* (14- α demethylase) and *MDR1* (major facilitator efflux pump) did not appear to be effected by biofilm growth in vivo when compared to planktonic cells (59).

Recently, the author's group developed murine model of subcutaneous catheter-associated *C. albicans* biofilms (56). In this model, catheters were inserted subcutaneously by making a mid-line incision on BALB/c mice backs, and formation of biofilms on catheters was confirmed using QCC assays and SEM. The data revealed abundant *Candida* hyphal elements and blastospores embedded in thick ECM [Fig. 5(C) and (D)] and showed that 1×10^3 CFU/mL grew on cultured catheters. This data correlates well with clinical catheter-associated infections where growth of 1×10^3 CFU/mL from catheter indicates catheter-associated infection (60).

These in vivo models have provided some understanding of *C. albicans* catheter-associated biofilms, their resistance, and genes involved with these processes.

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10 Polyenes for Prevention and Treatment of Invasive Fungal Infections

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INTRODUCTION

For decades, polyene antifungals have been mainstays for the prevention and treatment of invasive fungal infections (IFIs). However, because nystatin is restricted to topical (nonsystemic) administration, its application as prophylaxis against IFI in selected high-risk patients has largely been replaced by alternate strategies. Similarly, treatment with nystatin is restricted to mucocutaneous forms of candidiasis (such as oropharyngeal, cutaneous, mucocutaneous, and vulvovaginal infections). Investigations with lipid-based formulation of nystatin for systemic use have not yet yielded a commercial product (1). In contrast, amphotericin B has been a mainstay for the prevention and treatment of IFIs since 1957 (2). While less frequently employed today as prophylaxis (given the expanded availability of safer alternatives), amphotericin B [administered intravenously as either amphotericin B deoxycholate (AmBd) or one of three lipid-based formulations (LBFAmB)] is still considered a treatment option in select cases of severe or life-threatening IFIs (2–11).

This chapter reviews the clinical use of polyenes for the prevention and treatment of IFIs. Because of the limited indications for nystatin, the discussion will focus on amphotericin B unless otherwise stated.

MECHANISM OF ACTION

Polyenes interact with sterols in the fungal cell membrane (12,13). The result is the production of pores that allow concentration-dependent leakage of intracellular cations (such as potassium and magnesium) and other cell components (12,13). This leads to loss of membrane potential and subsequent fungal cell collapse (12,13). Other mechanisms proposed include cell damage resulting from oxidative reactions linked to lipoperoxidation of the cell membrane (14). While polyenes demonstrate a greater affinity for ergosterol (the primary sterol of the fungal cell membrane), the nonselective binding to cholesterol (the primary sterol in human cell membranes) may contribute to polyene-related adverse events.

Although *in vitro* activity can vary between test conditions and isolates tested, studies have generally documented that amphotericin B's activity is rapidly fungicidal against susceptible organisms (15–17). Fungicidal concentrations *in vitro* are generally 1 to 3 dilutions higher than that required for inhibition (18).

The immunomodulatory effect of amphotericin B has been examined by numerous investigations (19–22) and summarized in detail elsewhere (23). These effects include stimulation of proinflammatory cytokines: tumor necrosis factor (TNF) α , interleukin (IL)-1 and IL-6, the chemokines IL-8, MCP-1, MIP1 β , nitric oxide, prostaglandins, and ICAM-1 from murine and human immune cells *in vitro* and *in vivo* (19–23). The cytokine release resulting from amphotericin B administration may also be responsible (in part) for infusion-related reactions, and differences may exist between preparations (22–24). Other proposed mechanisms for such reactions include the release of prostaglandins (25). Finally, the antifungal activity of pulmonary alveolar macrophages and polymorphonuclear leukocytes against *A. fumigatus* may be augmented by the administration of amphotericin B (14).

In addition to its rapidly fungicidal activity, amphotericin B has demonstrated a prolonged postantifungal effect (PAFE) against both *Candida* (26,27) and *Cryptococcus* spp. (27). The PAFE for *Aspergillus*, however, may be species-dependent, with significantly shorter PAFEs or lack of PAFE observed for *Aspergillus terreus*, *A. ustus*, and *A. nidulans* (28).

In contrast to their activity against planktonic fungal cells, the *in vitro* activity of antifungals (including polyenes) can vary significantly in biofilms (29–31). While AmBd's activity *in vitro* against *Candida* spp. in biofilms is markedly reduced, both liposomal amphotericin B (LAmB) and amphotericin B lipid complex (ABLC) exhibited similar inhibitory activity *in vitro* against *Candida* biofilms in one report (30). Similar findings were seen for ABLC in a rabbit model (32).

Numerous *in vitro* and animal model studies have been performed to assess the interaction of amphotericin B with other antifungals (such as flucytosine, azoles, and echinocandins) (see section Drug Interactions). Review of such data may be found elsewhere (33–36) and is beyond the scope of this chapter. However, the lack of standards for synergy and antagonism testing may limit the utility of such information, and data may differ with the agents tested, model, test conditions, and endpoint(s). The potential for amphotericin B in combination with nonantifungals has also been explored. Examples include combinations with azithromycin [against *Fusarium* (37) and *Aspergillus* spp. (38)] and rifabutin [for both *Fusarium* and *Aspergillus* (39)]. However, the clinical significance of such interactions has not been established.

MECHANISMS OF FUNGAL RESISTANCE

While infrequently encountered in clinical practice, intrinsic polyene resistance has been demonstrated in *Candida lusitanae*, *A. terreus*, and *Scedosporium* spp. (13,40,41). Likewise, reports of acquired polyene resistance during therapy are rare. Proposed mechanisms of resistance include depletion of ergosterol in the cell membrane (42) or other sterol-independent modifications of the cell membrane (43). Alternate mechanisms include elevations in catalase levels, enhancing resistance against oxidative damage to the fungal cell by amphotericin B (44).

While incomplete, the potential exists for significant *in vitro* cross-resistance in yeasts between amphotericin B and nystatin (45). *In vitro* resistance of molds to nystatin has not been reported (45).

PHARMACODYNAMICS

The majority of studies investigating the pharmacodynamics of polyenes have involved AmBd (46,47). Existing *in vivo* and *in vitro* models for both *Candida* spp. and *Aspergillus* spp. suggest the rate and extent of amphotericin B's activity increases as the concentration of drug is increased (15,17,26,48,49). Similar findings have been observed with LBFamB (49,50). However, the limited drug solubility, reversible binding in tissues, and dose-dependent drug clearance of amphotericin B may limit the benefit of dose-escalation in attempts at increasing the antifungal activity (51–53) (see section Pharmacokinetics).

Data in humans to examine relationship between pharmacodynamics and outcomes are limited (54). Subset analysis of data obtained in 10 pediatric oncology patients treated with liposomal amphotericin B (LAmB) for whom pharmacokinetic and susceptibility data were available suggested that a peak serum concentration:minimum inhibitory concentration (C_{max}/MIC) ratio exceeding 40 were more likely to achieve a complete versus partial clinical response (55). However, while such information might be important in the empiric selection and dosing of antifungal therapy, neither amphotericin B serum concentrations nor *in vitro* susceptibility information is routinely available in clinical situations.

SPECTRUM OF ACTIVITY

Nystatin has demonstrated activity against a variety of fungal isolates in various *in vitro* and *in vivo* models (45). This includes a variety of *Candida* spp., *Cryptococcus neoformans*, *A. fumigatus*, *Histoplasma capsulatum*, and *Coccidioides immitis*. However, the clinical usefulness of such activity is significantly limited by lack of a preparation for systemic administration.

Amphotericin B possesses a broad spectrum of antifungal activity against a variety of yeasts and molds (56). Potency differences *in vitro* are consistently observed between AmBd and the three lipid-based formulations: amphotericin B lipid complex (ABLC), amphotericin B colloidal dispersion (ABCD), and liposomal amphotericin B (LAmB). LBFamB generally exhibits a fivefold reduction in potency *in vitro* (when expressed as mg/kg) relative to AmBd (49). The relevance of these *in vitro* differences remains uncertain, since higher MICs for LBFamB may not account for release from the lipid carrier. In contrast, recent data from the European

Committee on Antimicrobial Susceptibility Testing (EUCAST) reported increased potency for LAmB (relative to AmBd) for several strains of *Candida* spp. (18). The reasons for such findings, however, were not clear.

While Clinical Laboratory Standards Institute (CLSI) standards exist for susceptibility testing of amphotericin B against yeasts by both macrodilution and microdilution methods, no breakpoints have been approved (57). CLSI-recommended disk diffusion methods for susceptibility testing of yeasts do not include amphotericin B (57). In addition, the utility of *in vitro* testing may be limited by the general lack of correlation between *in vitro* susceptibility and treatment outcome in patients with IFIs (54,58).

Amphotericin B is highly active *in vitro* against most *Candida* spp. (including *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis*), with MICs generally ranging between 0.5 and 1 $\mu\text{g}/\text{mL}$ (59). More recently, MICs from a variety of *Candida* spp. tested according to methodology described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) ranged from 0.06 to 2 $\mu\text{g}/\text{mL}$ (18). While *C. lusitaniae* is thought to be intrinsically resistant to amphotericin B, *in vitro* data are often conflicting, and the clinical significance of this resistance is questionable (18,60,61). Amphotericin B also displays favorable activity *in vitro* against *Cryptococcus* spp. (62), *Malassezia* spp., and *Saccharomyces* spp. (63). However, *Trichosporon beigelii* displays variable *in vitro* susceptibility to amphotericin B (64).

Amphotericin B is highly active *in vitro* against fungi responsible for endemic mycoses, such as *Coccidioides* spp., *Blastomyces* spp., *H. capsulatum*, and *Paracoccidioides brasiliensis* (65,66). It also exhibits activity *in vitro* against most *Sporothrix schenckii*, although strain-dependent resistance has been reported (67).

In addition to its activity against yeasts, amphotericin B has also demonstrated significant activity against a variety of molds, including most *Aspergillus* spp. (with the exception of *A. terreus*) (18,56,68,69). Amphotericin B is active *in vitro* against the *Zygomycetes* (70). While *Scedosporium apiospermum* may be susceptible to polyenes, but *Scedosporium prolificans* is generally resistant (4,13). Although amphotericin B may be active against *Fusarium* spp., MICs of 0.25 to 8 $\mu\text{g}/\text{mL}$ have been reported for *F. solani* and therefore some strains of this species are considered resistant (13). *Cladophialophora* isolates have demonstrated variable susceptibility to amphotericin B. Finally, intrinsic resistance has been reported in both *Malassezia furfur* (4) and *Paecilomyces lilacinus* (13).

PHARMACOKINETICS

Nystatin is not significantly absorbed following oral or topical administration (45). Therefore, metabolism, distribution, and elimination of nystatin are largely unknown.

Amphotericin B demonstrates limited and erratic absorption following oral exposure. Low (yet detectable) serum concentrations of amphotericin B have been reported following oral administration (71,72). While such absorption may be altered in the setting of mucositis, it is generally considered insufficient to treat systemic infections.

The dose, frequency, and infusion rate of amphotericin B can influence plasma concentrations. Peak plasma concentrations following IV infusion of 1 mg/kg of AmBd have been reported to be approximately 2 $\mu\text{g}/\text{mL}$ (5). The high protein binding of amphotericin B (in excess of 90%) to serum albumin and α -1-acid glycoprotein is directly related to concentration (52,53). Distribution has been described using a three-compartmental model (52,53,73) with the resulting volume of distribution approximately 4 L/kg (73). Penetration of amphotericin B into the central nervous system is thought to be minimal, with cerebrospinal fluid (CSF) concentrations being 0% to 4% of simultaneous serum concentrations (74). However, such CSF concentrations are not likely to reflect higher concentrations in the meninges.

Metabolism plays a minor role in elimination of amphotericin B (52,53). Elimination of amphotericin B is also poorly understood. For example, only 3% of the total dose is excreted as unchanged drug (52,53). Clearance from plasma is slow and dose-dependent, with a terminal half-life of >15 days (52,53). Concentrations in blood have been detected up to four weeks after an amphotericin B treatment course and in urine for four to eight weeks following completion of therapy.

The pharmacokinetic differences between preparations of amphotericin B have been reviewed in detail elsewhere (50,52,53,75–77). In general, LBFAmB exhibits a range of serum concentrations, with ABLC and ABCD demonstrating similar C_{max} values to AmBd when given at recommended dosages. In contrast, LAmB exhibits both a higher C_{max} and area under the time–concentration curve (AUC) relative to AmBd, ABLC, and ABCD at comparable doses likely due to significant reduction in LAmB's volume of distribution and total body clearance (77). Reductions in unbound amphotericin B have been reported with LBFAmB relative to AmBd (52,53). LAmB may produce lower tissue concentrations in liver, spleen, lung, and kidney (3). In contrast, LAmB achieves higher concentrations in brain tissue (75). ABLC achieves higher lung concentrations (50,76).

Studies have examined the pharmacokinetics of amphotericin B (including LBFAmB) in a variety of special populations, including patients with renal dysfunction (78,79) (see section Dosing and Administration). Amphotericin is poorly removed by dialysis (80). Studies have also been conducted to examine the pharmacokinetic profile of AmBd (81–83), LAmB (55,84), and ABLC (85) in pediatrics. In general, significant differences between adult and pediatric patients justifying alterations in weight-based dosing ranges have not been observed.

ADVERSE EFFECTS

It is perhaps the adverse event profile that most limits the current use of amphotericin B.

Electrolyte Disturbances

A variety of electrolyte abnormalities have been associated with amphotericin B administration, most commonly hypokalemia and hypomagnesemia (86). Recent clinical studies (87–90) report hypokalemia in approximately 10% to 20% of patients receiving various amphotericin B formulations. Hyperkalemia is less frequently reported, and has been more frequently associated with rapid infusions (91).

Infusion-Related Reactions

Infusion-related reactions related to intravenous administration of amphotericin B occur frequently, ranging from 20% to 90% (depending largely upon population, preparation, administration, and the use of premedications) (92,93). Such effects usually occur during the infusion or within one to three hours following therapy. These include headache, fever, chills, and rigors. Gastrointestinal complaints (such as nausea, vomiting, and abdominal discomfort) may also occur during or directly following administration. Less common reactions during or immediately following infusion include bronchospasm, hypotension, thrombophlebitis, and cardiac arrhythmias. Hypertension has also been reported (94–96). Anaphylaxis associated with amphotericin B administration has rarely been reported (97).

Rapid infusion of AmBd (i.e., less than four to six hours) may increase the incidence of infusion-related reactions (98,99). In addition, the formulation of amphotericin B may also influence the frequency of infusion-related reactions (22,93). For example, infusion-related reactions on day 1 of empiric therapy without premedications were reported in 88.5% of febrile neutropenic patients receiving ABLC 5 mg/kg/day compared to 52% and 48% of those receiving LAmB 3 mg/kg/day or 5 mg/kg/day, respectively ($p < 0.001$) (93). Infusion-related reactions have also been reported more frequently among subjects receiving ABCD than those receiving AmBd (100,101). Select reactions have also been reported to occur more frequently with certain formulations. For example, a triad of hypoxia, back pain, and chest pain has been reported following administration of LAmB (102–104). A prospective analysis found a 20% mean overall frequency (range 0% to 100%) of acute infusion-related reactions among 84 patients at 64 centers (102). While these reactions rarely required discontinuation of therapy, slowing the infusion rate had no effect on the infusion-related reactions described. ABCD administration has been associated with hypoxia, dyspnea, and respiratory distress, which may necessitate cessation of therapy and the need for supportive care (105,106). Reactions in individual patients may also be formulation specific and not necessarily recur upon rechallenge with a different formulation.

Numerous strategies have been proposed to minimize the frequency and severity of amphotericin B–related infusion reactions, including the administration of premedications and the use of LAmB (see section Dosing and Administration).

Nephrotoxicity

Renal dysfunction secondary to amphotericin B administration is often the treatment-limiting adverse effect of amphotericin B. Proposed mechanisms include direct interaction with epithelial cell membranes (causing cellular disruption) and renal vasoconstriction (with resulting reductions in renal blood flow) (107,108). Manifestations may include renal tubular acidosis, casts in the urine, azotemia, oliguria, and magnesium and potassium wasting (107,108). The incidence of amphotericin B–induced nephrotoxicity varies widely between studies because of differences in definition, study population, underlying risk factors, duration of therapy, and use of premedications. However, such reactions (generally described as a doubling of the baseline creatinine value) have been reported up to 50% of patients receiving AmBd (109–113). Risk factors include underlying renal dysfunction, formulation, concomitant nephrotoxins, and dosing (daily and cumulative) (111,114–117). Significant economic and clinical consequences can result (112,118,119).

Numerous strategies have been employed in attempts to reduce the incidence and severity of amphotericin B–induced renal dysfunction. This includes careful patient selection and (whenever possible) minimizing concomitant nephrotoxins. Use of saline loading (108,120,121), aggressive fluid resuscitation (122), and continuous infusions (123,124) have also been investigated (see section Dosing and Administration). More common today in patients at increased risk or experiencing AmBd-related nephrotoxicity is the use of LBFAmB, which exhibit a reduced incidence of nephrotoxicity when compared to AmBd (93,101,125,126). For example, ABLC-associated nephrotoxicity (defined as a doubling of baseline serum creatinine) was observed in 13% of 3514 patients with suspected or proven IFI in a multicenter, open-label retrospective study (125). Studies comparing the incidence of nephrotoxicity between preparations has also been evaluated. For example, doubling of serum creatinine was significantly less frequent with LAmB 3 mg/kg/day than with AmBd 0.6 mg/kg/day when given as empiric therapy in persistently febrile neutropenic patients (18.7% vs. 33.7% respectively, $p < 0.001$) (109). LAmB (3 or 5 mg/kg/day) has also been compared to ABLC (5 mg/kg/day) in this patient population, with rates of doubling of serum creatinine from baseline observed in 29.4%, 25.9%, and 42%, respectively (93). Other reports comparing LAmB and ABLC for a variety of indications and ranges of doses for both agents (i.e., between 3 and 5 mg/kg/day) failed to detect significant differences between these preparations (126). ABCD 6 mg/kg/day demonstrated a reduced incidence of nephrotoxicity relative to AmBd 1 to 1.5 mg/kg/day in patients with invasive aspergillosis (12.5% vs. 38.4%, respectively) (101).

Other

Elevations in liver function tests have less frequently been associated with amphotericin B administration (127). Anemia (usually normochromic, normocytic) has been reported secondary to amphotericin B administration, and may be a consequence either of direct inhibition of erythropoietin (128) or secondary to renal toxicity.

OVERVIEW OF CLINICAL APPLICATIONS

This section is not intended to be comprehensive and readers should refer to other chapters for a comprehensive discussion of this topic.

Amphotericin B's status as the "gold standard" for the prevention and treatment of IFIs has been put into question by the recent introduction of new options, namely extended-spectrum triazoles and echinocandins (3,4). Despite these new alternatives, amphotericin B is continually cited by numerous consensus guidelines as an option for serious and treatment-refractory IFIs (5–11). With few exceptions, AmBd is frequently replaced by LBFAmB, largely based on the potential for increased safety (3,129). However, controlled studies examining LBFAmB as primary therapy for IFIs are limited. In addition, while ABCD may be associated with increased infusion-related reactions (100,101), clinically significant differences between other LBFAmB (i.e., LAmB and ABLC) may be less clear (129).

Candidiasis

Nystatin for the treatment of infections due to *Candida* spp. is restricted to the treatment of mucocutaneous forms. This may include oropharyngeal, cutaneous, mucocutaneous, and vulvovaginal infections.

The efficacy of AmBd caused by many *Candida* spp has been established in invasive candidiasis, including candidemia, osteomyelitis, disseminated candidiasis, endophthalmitis, and endocarditis (130–132). Extensive experience with AmBd has also been documented for invasive candidiasis in the neonatal population (133,134). A comparative trial of AmBd with fluconazole in nonneutropenic patients with candidemia has failed to demonstrate differences in efficacy between the two treatments (130). More recently, AmBd has been used as the comparative agent against both caspofungin (88) and voriconazole (135). In general, efficacy for AmBd is comparable to these new agents. However, newer agents are generally better tolerated than AmBd. In the case of voriconazole, an alternative exists for continued oral therapy once the patient is stable.

LBFAmB has also been used in the treatment of invasive candidiasis. The efficacy of ABLC has been reported in open-label studies (136,137). For example, cure (30%) or improvement (30%) was noted in the treatment of invasive candidiasis in ABLC-treated patients in a retrospective observational trial (137). Use of LAmB for the treatment of invasive candidiasis has also been reported in pediatric patients (138,139). Published experience with ABCD is somewhat limited. It has been studied in an open-label, phase-I (101) and retrospective analysis of open-labeled trials. Early trials comparing ABCD with azoles (fluconazole) demonstrated comparable efficacy but the ABCD was less well tolerated (131). More recently, LAmB was demonstrated to be equally effective but less well tolerated than micafungin for candidemia and invasive candidiasis in adults (87). ABLC and LAmB are FDA-approved as second-line therapy for use in proven candidiasis in patients intolerant or refractory to AmBd.

The potential role of AmBd as part of combination therapy (with fluconazole) was examined in a randomized study in nonneutropenic patients with candidemia (140). In this trial, 30-day success rates were not different between subjects receiving fluconazole plus placebo (57%) or fluconazole in combination with AmBd (69%, $p = 0.08$). The rate of clearance from the bloodstream trended toward improvement in patients receiving combination therapy.

The availability of equally efficacious and better-tolerated agents limit the role of AmBd for candidiasis. Current expert guidelines for the treatment of invasive candidiasis limit the role of amphotericin B in severe, refractory infections, CNS infections, and in pregnant patients (6). In addition to its continued role in severe disease (141), some authorities (142) recognize the potential for a continued role in the treatment of neonatal infections.

Aspergillosis

For many years, AmBd represented the standard of care for the treatment of invasive disease (143). Overall efficacy rates varied with preparation, population, site of infection, and confirmation (possible, probable, definite). Since higher doses of AmBd (1–1.4 mg/kg/day) and longer durations of therapy were required, LBFAmB have assumed a growing role. Published data included experience with LAmB (104,144–146), ABCD (101), and ABLC (136,147,148).

Based on trials demonstrating superiority of voriconazole over AmBd (5,149) and improved mortality (150), AmBd and LBFAmB (including ABLC and LAmB) are now used as alternative or salvage therapy (5). Other options for refractory infections (i.e., echinocandins) also exist.

Cryptococcal Infections

While mild-to-moderate forms of cryptococcosis outside the central nervous system (CNS) may be managed by an azole such as fluconazole or itraconazole, amphotericin B has become the standard of care for the initial therapy of severe, disseminated disease and those involving the CNS (7). The efficacy of AmBd for the treatment of cryptococcal meningitis has been established by randomized controlled trials in both HIV and non-HIV patient populations (151–156). Combination therapy of AmBd with flucytosine has improved clinical efficacy, time to sterilization of the CSF, and the reduction in relapse rates (152). In HIV-infected patients, AmBd (0.7 mg/kg/day) combined with flucytosine 100 mg/kg/day for two weeks followed by azole

maintenance therapy was superior to AmBd + placebo, but more prolonged courses of flucytosine were of no additional benefit (154).

Current expert treatment guidelines recommend that AmBd be combined with flucytosine as initial "induction therapy" for cryptococcal meningitis in both HIV-infected and non-HIV-infected persons (7). However, the optimal dose of AmBd for this indication is unknown. The potential role of AmBd dose escalation for induction therapy of cryptococcal meningitis was recently examined in HIV-positive patients (157). Initial doses of 0.7–1.0 mg/kg/day (plus 5FC) × 2 weeks were followed by fluconazole. In this study, increasing doses demonstrated more rapid fungicidal activity.

There is less published experience with LBFAmB for cryptococcal infections. A comparison of LAmB 4 mg/kg/day and AmBd 0.7 mg/kg/day as induction therapy in HIV-infected patients ($n = 28$) concluded that LAmB was more effective than AmBd in sterilizing the cerebrospinal fluid ($p < 0.05$) (158). However, overall clinical response was similar between the two treatments. The efficacy of ABLC has also been reported for cryptococcosis (159) and cryptococcal meningitis (160). In patients with HIV-associated cryptococcal meningitis ($n = 21$), successful treatment was reported in 86% of subjects receiving ABLC 5 mg/kg/day (160). However, rates of CSF sterilization at six weeks were only 58%. The current IDSA treatment guidelines recommend LAmB 4 mg/kg/day be used as the LBFAmB, although such treatment is not currently FDA-approved (7). In addition, while initial treatment of serious cryptococcal disease in solid organ transplant recipients has not been evaluated by prospective controlled clinical trials, LBFAmB (either LAmB or ABLC) may also be considered over AmBd in this population due to the frequent coadministration of nephrotoxic calcineurin inhibitors (161).

Zygomycoses

Limited options exist for the treatment of invasive zygomycoses. While amphotericin B maintains activity *in vitro* against *Zygomycetes*, treatment outcomes (especially in the immunocompromised host) remain poor (162,163). AmBd (164) or lipid-based formulations (164,165) of amphotericin B are frequently prescribed in this clinical setting, especially as initial therapy and frequently in combination with surgical intervention (163,164). The introduction of newer agents with activity against zygomycoses (i.e., posaconazole) may impact the role of amphotericin B in the future to use for initial management of severe infections (163).

Visceral Leishmaniasis

Published data have reported the clinical applications of AmBd (166), ABCD (167), ABLC (168), and LAmB (169) for visceral leishmaniasis. Of these treatment options, LAmB has received FDA approval. Investigations with LAmB have also reported efficacy of single-dose therapy for this infection (170,171).

Endemic Mycoses

Current expert treatment guidelines for histoplasmosis identify AmBd as initial management of severe infections (such as pulmonary and CNS infections, mediastinitis, and disseminated disease) (8). It is rarely used today, except in severe cases (172). In general, higher doses of AmBd (i.e., 0.7–1 mg/kg/day) have been employed as initial therapy, with reduced doses (i.e., 0.5–0.6 mg/kg/day) for patients unable to tolerate higher doses. Patients improved or with stable disease following initial AmBd therapy (usually two weeks) can often be transitioned to azole therapy. LBFAmB may also have a role, especially for patients intolerant to AmBd (8). ABLC was evaluated in 25 patients, with efficacy (cure + improvement) observed in 84% (165). In a randomized, double-blind multicenter study for disseminated histoplasmosis comparing AmBd (0.7 mg/kg/day) to LAmB (3 mg/kg/day) as initial (i.e., 2 week) induction therapy for moderate-to-severe disease in AIDS patients, clinical success rates were 64% and 88%, respectively ($p = 0.014$) (173). Fewer deaths and improved treatment tolerability were reported in patients receiving LAmB. However, no difference in time to defervescence, rate of blood culture conversion, or change in *H. capsulatum* antigen levels was observed.

Similar to its role in histoplasmosis and despite lack of published data from large controlled clinical trials, AmBd is considered as primary therapy for pulmonary, disseminated, and CNS blastomycosis based on published guidelines (9). AmBd is also the preferred therapy for

immunocompromised or pregnant patients (9,174). Cure rates for AmBd have been reported to range between 70% and 91% (9,174) with LBFAMB 3–5 mg/kg/day or AmBd 0.7–1 mg/kg/day and can be administered for one to two weeks or until improvement, followed by a transition to an oral agent (9). Low relapse rates have been reported when cumulative doses of AmBd greater than 1 g have been employed. Similar to histoplasmosis, higher doses of AmBd (0.7–1.0 mg/kg/day) should be considered for disseminated disease with blastomycosis, in which cumulative doses of 1.5 to 2.5 g or higher (at least 2 g) for CNS disease are used. Published experiences with LBFAMB are limited. Some open-label experience exists with ABLC, with cure or improvement in 9/14 (64%) cases (165). Despite this relative lack of published data, LBFAMB are generally recommended for CNS disease (9).

In general, the use of amphotericin B for the treatment of sporotrichosis is restricted to pregnant patients or those with osteoarticular, pulmonary, or disseminated infections (10). LBFAMB 3–5 mg/kg daily or AmBd 0.7–1.0 mg/kg daily can be used in such settings (10). Similar to other guidelines, LBFAMB has been preferred by some clinicians for CNS disease. In a similar circumstance, fluconazole and itraconazole have largely replaced the need for the use of amphotericin B in the treatment of coccidioidomycosis (11,175). AmBd 0.5 to 1.5 mg/kg IV daily or on alternating days may be considered in patients with rapidly progressing disease, hypoxia and/or respiratory failure, or pregnant women. Experience with LBFAMB for the treatment of coccidioidomycosis is largely limited to case reports (136,165).

Emerging Mycoses

Due to lack of drugs active against many of the emerging mycoses, limited positive clinical experience (primarily case reports or case series) and/or *in vitro* data has been reported for amphotericin B against *Exophiala oligosperma* (176), mucormycosis (177), and rare molds (178).

Neutropenic Fever

Numerous studies have examined the efficacy and safety of amphotericin B in the treatment of fever in neutropenic patients. Early published experience with AmBd helped establish a role of antifungal therapy in empiric regimens for patients persistently (>7 days) febrile despite broad-spectrum antibacterials (179). The addition of AmBd substantially reduced rates of infection and/or shock (2/18 or 11%) compared to broad-spectrum antibacterials alone (6/16 patients) or discontinuation of broad-spectrum antibacterials (9/16 patients). Later studies confirmed this by treatment success (defined by absence of fever and infection) in AmBd-treated patients when compared to no treatment (69% vs. 53%, respectively) (180). More recently, AMBd has been compared to voriconazole (181), fluconazole (182,183), and caspofungin (89) in this patient population.

LBFAMB has also been evaluated for persistent fever in neutropenics. LAmB (3 mg/kg/day) was compared to AmBd (0.6 mg/kg/day) among 660 patients in a large, double-blind, multicenter, randomized trial (109). Similar rates of success (based on a composite endpoint of defervescence, survival, treatment of baseline infection, and absence of breakthrough IFI or toxicity requiring treatment discontinuation) of 50% (172/343) for LAmB versus 49% (170/344) for AmBd were reported. However, LAmB-treated patients experienced fewer proven breakthrough fungal infections [3.2% (11/343) vs. 7.8% (27/344), $p = 0.009$]. In addition, a reduction in infusion-related reactions [including fever (17% vs. 44%) and chills or rigors (18% vs. 54%)] was observed in LAmB versus AmBd treatments. Other studies have compared LAmB to AmBd (109,184,185) and voriconazole (186). ABCD (4 mg/kg/day) therapy has also been compared to AmBd (0.8 mg/kg/day) in a prospective, randomized, double-blind study among neutropenic adults and children (100). Success rates were 50% (49/98) and 43.2% (41/95), respectively ($p = 0.31$), while documented or suspected breakthrough fungal infections were reported in 14.3% and 14.7%, respectively. Although ABLC has been compared to both LAmB (93,126) and AmB (187) in this patient population, such evaluations were designed primarily to evaluate the safety and tolerability rather than efficacy.

Given the expanded options of alternative therapies and the underlying risks for toxicities associated with amphotericin B, published guidelines for the empiric management of fever in neutropenic oncology patients restrict the role of amphotericin B (188). Examples include treatment of patients at high risk and/or with clinical evidence of nasal and/or sinus infection

(in whom CT or MRI findings suggest the potential for invasive fungal infections such as aspergillosis or mucormycosis) or those receiving prior azole therapy at risk of invasive mold infections. In these cases, either LAmB or ABLC should be considered.

Prophylaxis

Numerous investigations have involved use of polyenes orally (in the case of nystatin and AmBd), parenterally, and via aerosol (amphotericin B formulations) in attempts to reduce IFIs in selected high-risk patient populations in the prevention of fungal infections in high-risk patient populations. Detailed discussions regarding this indication can be found elsewhere (189).

Orally administered nystatin has been examined as an antifungal prophylaxis in a variety of populations, including low birth weight infants (190), oncology (191), and solid organ transplant recipients (189,192). While some of these investigations reported the effectiveness of nystatin (relative to placebo) in the reduction of mucocutaneous disease, its role in the prevention of IFIs is not well established (189). In select patient populations (such as solid organ transplant recipients), use of nystatin has largely been replaced by azoles (189,192). Similar investigations have used oral AmBd. In addition to its lack of superiority over other options (such as azoles), combined with a lack of a commercially available oral formulation and definitive studies demonstrating success, the use of orally administered AmBd as a strategy to prevent IFIs is also very limited.

In patients with hematologic malignancy, the application of AmBd as prophylaxis has been examined in a variety of studies, including comparisons between AmBd and LBFAmB (193), fluconazole (182), and voriconazole (181). LBFAmB has also been studied in this patient population (105,194–197). Studies of the LBFAmB generally reflect the use of alternate dosing strategies (i.e., ABLC 2.5 mg/kg three times weekly prophylaxis and LAmB 3 mg/kg three times weekly) (196). There is less experience with ABCD, since a study evaluating it as prophylaxis in neutropenic patients was discontinued prematurely because of severe infusion-related adverse events (105).

For patients undergoing hematologic stem cell transplant (HSCT), AmBd has been compared to placebo (198,199) and fluconazole (200,201). Reduced doses of AmBd (i.e., 0.1–0.2 mg/kg/day) are more commonly studied in these settings. Use of LBFAmB has also been studied in this patient population, including LAmB (194,202,203) and ABLC (196,203).

Current published guidelines for the prevention of invasive fungal infections in cancer patients reflect that the toxicities of amphotericin B, along with the expanding options for alternate strategies, limit the routine use of amphotericin B in this setting (188). However, amphotericin B (preferably either LAmB or ABLC) should be considered as an option for prophylaxis in patients at intermediate- or high-risk of IFIs (such as those with allogeneic stem cell transplant recipients, myelodysplastic syndrome, acute myelogenous leukemia, or graft-versus-host disease).

Amphotericin B has also been examined as an antifungal prophylaxis in select solid organ transplant recipients (204–206). For example, a randomized, placebo-controlled trial evaluated LAmB 1 mg/kg/day \times 7 in liver transplant recipients (compared to fluconazole or placebo) (206). Active treatments demonstrated superior infection- and colonization-free rates when compared with placebo (40.6%, 34.9%, and 2.3% for LAmB, fluconazole, and placebo, respectively; $p < 0.01$). Comparison of LAmB 50 mg/day to AmBd 15 mg/day has also been conducted in this population (207). IFIs occurred in 4/44 (9%) and 3/48 (6%) patients, respectively. A statistically significant survival benefit was attributable to LAmB in this study (79.6% vs. 59.5%; $p = 0.038$).

Prophylactic strategies for solid organ transplant recipients vary widely between transplant centers and patient populations. However, use of amphotericin B (in the form of LBFAmB) as a prophylaxis against IFI in this setting may be restricted primarily to patients at significant risk of invasive candidiasis (such as pancreas, small bowel, or liver transplant recipients) unable to receive azole prophylaxis (either due to increased risk of intolerance or azole resistance) or select patients at increased risk of aspergillosis (such as lung transplant recipients) (204).

Secondary prevention against recurrence of some fungal infections is needed in select patients with HIV (208). Such infections may include cryptococcosis, histoplasmosis, and

coccidioidomycosis. However, due to the availability of alternate agents (such as fluconazole and itraconazole), amphotericin B plays a limited role in such prevention.

Use of systemic administration of amphotericin B formulations may be problematic, mostly due to tolerability issues. As reviewed extensively elsewhere (209), aerosol administration of various formulations of amphotericin B as a prophylactic strategy has been studied in a variety of patient populations, including AmBd in neutropenic cancer patients, heme-onc, HSCT and lung transplantation (210–217). Animal models suggest that aerosolized lipid formulations of amphotericin B achieve higher tissue penetration (relative to AmBd) (218–222). Despite this, several issues regarding aerosol therapy persist, including (but not limited to) determination of the optimal agent, dose, frequency, duration, and nebulizer (223).

DOSING AND ADMINISTRATION

Nystatin

As previously described, treatment with nystatin is restricted to cutaneous and mucocutaneous forms of candidiasis. Doses of nystatin used for prophylaxis varied by population, with the usual range in adult patients of 300,000 to 7.5 million international units per day in divided doses (189–192).

Amphotericin B

The optimal dose and duration of amphotericin B is unknown. For AmBd, the recommended dosing for most treatment indications range from 0.6 to 1.0 mg/kg/day, administered as a single daily dose. Doses as high as 1.5 mg/kg/day may be used, but generally reserved for infections with severe, life-threatening invasive infections and less-responsive organisms (such as *Aspergillus* spp. or *Mucor*). Studies examining AmBd for prophylaxis have employed lower doses and/or alternate day (sometimes three times weekly) administration. FDA-approved doses for LBFAmB generally range from 3 to 5 mg/kg/day. The safety of higher doses has been examined for LAmB (104). While not FDA-approved, LAmB doses probably should not exceed 10 mg/kg/day.

Because of its concentration-dependent pharmacodynamic profile, dose-escalation has been proposed as a potential strategy to improve efficacy. However, limited drug solubility and reversible binding of AmB in tissues, however, may limit the benefit of dose-escalation (51–53) (see section pharmacokinetics). Existing studies involving LAmB which compared initial therapy at either 3 mg/kg/day and 10 mg/kg/day × 14 days followed by 3 mg/kg/day for suspected or documented invasive mold infections observed increased toxicity without improvements in efficacy at higher doses (117).

Dosage modification is unnecessary for patients with underlying renal dysfunction. For those experiencing AmB-induced nephrotoxicity, dose reductions or the use of twice the daily dose administered on alternate days has been described, but data to support this practice is lacking (224,225). Such strategies are now largely replaced by substitution of AmBd with a LBFAmB or (in some cases) alternate therapies (such as echinocandins or extended-spectrum azoles). Amphotericin B is poorly removed by hemodialysis (78,79). Pharmacokinetic data are lacking for patients with hepatic insufficiency, and currently no adjustments are recommended in this population.

Several issues surround the administration of amphotericin B, including the use of test doses, premedications, infusion rate, and various strategies to reduce nephrotoxicity and electrolyte abnormalities. Administration of a 1 mg test dose (without premeds) has been recommended to screen for anaphylaxis. This practice is of questionable clinical value, and will not rule out subsequent adverse events (including infusion-related reactions). If performed, administration of a test dose should not delay institution of therapy. An alternate solution is to deliver a 1 mg aliquot from first dose, observe and then complete the infusion.

For the prevention of select infusion-related reactions, administration of acetaminophen and/or diphenhydramine prior to the infusion may reduce the frequency and/or severity (92). Pretreatment with corticosteroids in this setting has been described (226) but is less desirable. Heparin has been recommended by some to treat phlebitis, although controlled trials are lacking to support this practice. When possible, use of a central line may assist in reducing phlebitis.

Meperidine has been reported to treat the rigors, but is less frequently employed as a prophylactic strategy (227). Ibuprofen may significantly decrease the reaction (226).

Measures to reduce the frequency and/or severity of nephrotoxicity include careful patient selection, minimizing concomitant nephrotoxins, "saline loading," alternate day therapy, continuous infusion, combination of AmBd with intralipids, and use of LBFAmB (122). Administration of normal saline prior and/or subsequent to the infusion is thought to reduce AmBd-related nephrotoxicity, but controlled clinical data to support is lacking (108,120,121,228). The optimal method of saline administration is unknown, but often described as 500 mL normal saline before and following infusion. Aggressive fluid resuscitation has also been explored (122). Caution should be used in patients with heart failure and renal dysfunction. It is also unknown if saline loading is necessary as a routine practice in patients receiving LBFAmB, since such practices are not usually described in clinical studies. In all cases, assuring eunatremia and euvolemia prior to AmB administration is desirable. The safety and tolerability of combining AmBd with intralipids has also been reported (229–233). However, efficacy, toxicity, and quality control data are generally lacking with such preparations, and therefore cannot be recommended (234). Most commonly, LBFAmB would be selected for patients requiring continued therapy with amphotericin B and at risk or experiencing nephrotoxicity.

The rate of infusion may also impact toxicity. Therefore, rapid infusions should be avoided (235). Despite pharmacodynamic studies that illustrate the concentration-dependent nature of AmBd's fungicidal activity (15,46), continuous infusion of the daily dose over 24 hours as a strategy to reduce nephrotoxicity has been investigated (123,124,236,237). For AmBd, continuous infusion has decreased infusion-related reactions, nephrotoxicity, and mortality relative to a four-hour infusion (123). Other open-labeled trials have also documented the reductions in nephrotoxicity (124). It is unknown, however, whether such data applies to LBFAmB, since these preparations were not studied. Models accounting for human serum albumin fail to show significant pharmacodynamic differences between methods of administration (15). In addition, such a method of administration cannot be recommended due to lack of efficacy data in patients with documented infections.

In addition to clinical monitoring for efficacy and adverse effects, patients receiving amphotericin B should receive close laboratory monitoring, including baseline blood counts, hepatic and renal function, and serum chemistries. In addition to serum creatinine, serum electrolytes (most notably potassium) should be monitored frequently in patients receiving AmB preparations. For patients experiencing hypokalemia, judicious monitoring and replacement of potassium should occur. Successful coadministration of agents to prevent potassium wasting have been described, including amiloride (238–240) and spironolactone (241). Currently, there is no clinical role for monitoring amphotericin B serum concentrations in attempts to improve either safety or efficacy (47).

DRUG INTERACTIONS

Drug interactions with amphotericin B are largely a consequence of its adverse effects (2). For example, declines in renal dysfunction as a result of amphotericin B administration may alter the elimination of agents which undergo significant renal clearance. An increased incidence of nephrotoxicity may be seen with concomitant use of other nephrotoxins. Electrolyte abnormalities secondary to amphotericin B administration may be enhanced by other agents known to produce such imbalances (such as corticosteroids). Amphotericin B–induced hypokalemia may enhance the activity of agents such as nondepolarizing skeletal muscle relaxants and cardiac glycosides.

As previously noted, data regarding the combination of amphotericin B with other antifungals is highly dependent upon test conditions. Perhaps the interaction best studied clinically is the favorable combinations of amphotericin B with flucytosine in the initial management of patients with cryptococcal meningitis (7). However, it should be noted that declines in renal function secondary to amphotericin B may necessitate flucytosine dosage reductions. While antagonistic effects were noted previously when combined with the azole class, no antagonism was noted in a clinical trial of patients with candidemia (140). Clinical data regarding the potential interaction between amphotericin B and echinocandins are lacking, although case reports and historically controlled studies suggest a potential role in treatment-refractory patients (36).

SUMMARY

Nystatin, while continuing to represent a treatment option for selected forms of cutaneous and mucocutaneous forms of candidiasis, currently plays a limited role in the prevention and treatment of IFIs due primarily to its lack of systemic absorption after oral administration and the lack of a commercially available parenteral formulation.

Amphotericin B continues to be the broadest-spectrum antifungal with cidal activity against many pathogens and a very low potential for treatment-emergent resistance. Its efficacy has been established for numerous IFIs in a variety of patient populations. Despite the recent introduction of new options for prevention and treatment of IFIs, amphotericin B is still prominent in numerous treatment guidelines for severe, life-threatening infections (4,242,243).

Requirements for parenteral administration, along with treatment-related adverse effects, restrict the widespread use of amphotericin B. Of particular note is the significant clinical and economic impact of AmB-induced nephrotoxicity (112). The introduction of LBFAmB has expanded the population that can safely receive the drug (77,244). However, increased efficacy with LBFAmB is less clear (245). Increases in LBFAmB drug acquisition cost (relative to AmBd) may be offset by reductions in costs associated with amphotericin B nephrotoxicity (112,118,119,246). Despite these advantages, AmBd may continue to play a role in select uses and patient populations, such as neonates and children, intrathecal use in patients with *Coccidioides immitis* meningitis, or in patients otherwise at low risk of amphotericin B nephrotoxicity (3,122,247) or in azole/echinocandin-resistant fungal strains.

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11 | Flucytosine

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INTRODUCTION

Originally developed as a treatment for leukemia, flucytosine (5-fluorocytosine; 5FC) is an antifungal agent with a restricted role in present-day therapy. Its use as a monotherapy is significantly limited, primarily due to concerns regarding both primary and secondary flucytosine resistance. In contrast, combination therapy of flucytosine with amphotericin B has been proven to improve treatment outcomes in patients with cryptococcal meningitis when used as the initial part of therapy (1–5). Its role in combination with azole antifungals for the treatment of disseminated cryptococcal infections is less clear. In addition to concerns regarding resistance, flucytosine use is also limited by toxicities (hematologic and gastrointestinal) and the lack of a parenteral preparation in the United States. Therapeutic drug monitoring and dose adjustment in patients with renal insufficiency should be employed in an attempt to optimize therapy and reduce the potential for serum concentration–related side effects.

MECHANISM OF ACTION

Flucytosine penetrates the fungal cell wall with the aid of cytosine permease (6) and is then deaminated to 5-fluorouracil (5-FU) by cytosine deaminase (7) (an enzyme absent in mammalian cells). Fluorouracil then incorporates into fungal RNA (in place of uracil), interrupting protein synthesis (8,9). The 5-FU is then converted to 5-fluorodeoxyuridylic acid monophosphate, a noncompetitive inhibitor of thymidylate synthetase, which interferes with DNA synthesis (10–12). Depending upon test conditions, activity may be either fungistatic or fungicidal (13).

Flucytosine possesses postantifungal effect (PAFE) *in vitro*, depending upon the organism, concentration, and test conditions (14,15). Against *Candida albicans*, PAFEs ranged from 0 to 4.2 hours after a 0.5-hour exposure and extended beyond 10 hours after the exposure was increased to 1 to 2 hours (15). When concentrations exceeded the minimum inhibitory concentration (MIC) by fourfold, the rate and extent of fungistatic activity were limited (15). Similar findings have been reported in other *Candida* spp. and *Cryptococcus neoformans*, with PAFEs ranging from 0.8 to 7.4 hours and 2.4 to 5.4 hours, respectively (16). Prolongation of PAFE was observed with the combination therapy of fluconazole with flucytosine against *C. albicans* (17–19). Prolongations of PAFE have also been reported with the combination of flucytosine with amphotericin B against *C. albicans* (20). Combination PAFEs in this study were 6.3 to 21.8 hours, which were increased relative to agents tested individually.

The effects of flucytosine on immunity have been investigated using both *in vitro* and animal models. In a guinea pig model, flucytosine had no effect on cellular immunity (21,22). Exposure of *C. albicans* to amphotericin B and flucytosine significantly enhanced neutrophil killing (23). Combination with immunoglobulin therapy (i.e., an IgG1 monoclonal antibody to *C. neoformans* capsular glucuronoxylomannan) has also been investigated *in vitro* and in a murine animal model of cryptococcal infection, with the combination more effective than either agent alone in reducing the numbers of *C. neoformans* colony-forming units (CFUs) (24). The clinical relevance of these findings, however, is unknown.

RESISTANCE

Flucytosine resistance may be due to several mechanisms, including alterations in cytosine permease, cytosine deaminase, and uracil phosphoribosyltransferase (6,11,12,25–28). The exact mechanism may depend upon the isolate. For example, use of cytosine as a nitrogen source has been proposed as a mechanism of resistance in *Aspergillus* spp. (12). In contrast, *C. albicans* isolates have demonstrated both decreased UMPP activity and decreased cytosine deaminase activity (26).

The genetic basis of susceptibility to flucytosine has been described in *C. albicans* (26,29). While isolates possessing heterozygous resistance traits (FCY1 and FCY2) exhibit minimal elevations in MICs, they may occur at a significant frequency among clinical strains. It has been proposed that single-step mutation promoted by drug exposure selects for homozygous isolates, resulting in significant elevations in MIC, which contribute to treatment failure (26). However, high-level resistance has also been reported in isolates with heterozygous resistance (29).

PHARMACODYNAMICS

Pharmacodynamic studies of flucytosine are lacking in humans. However, the pharmacodynamic parameters in the animal models have been investigated. For example, in a neutropenic murine model of invasive candidiasis, minimal concentration-dependent killing was observed (30). However, a dose-dependent suppression of growth was reported, and regrowth occurred after serum concentration fell below the MIC. Increasing dosing intervals resulted in increases in the dose necessary for fungistatic activity. Time above MIC best predicted outcome, with maximal efficacy observed when concentrations exceeded the MIC for 20% to 25% of the dosing interval. AUC/MIC was only slightly less predictive of outcome in this study. In contrast to these findings, AUC/MIC was the pharmacodynamic parameter best correlated with survival in a nonneutropenic mouse model of aspergillosis (31).

SPECTRUM OF ACTIVITY

Results of in vitro susceptibility testing of flucytosine vary with culture media, serum, pH, buffering agents, inoculum, temperature, and incubation time (32–39). As a result, significant interlaboratory variability has been reported (34). Standards have been approved by the Committee for Laboratory Standards Institute (CLSI) using macrodilution broth techniques for flucytosine and other antifungal agents (40). MICs of ≤ 4 $\mu\text{g}/\text{mL}$, 8.0 to 16 $\mu\text{g}/\text{mL}$, and ≥ 32 $\mu\text{g}/\text{mL}$ for *Candida* spp. are considered susceptible, intermediate, and resistant, respectively.

Most common clinical isolates of *Candida* spp. (with the exception of *C. krusei*) are considered susceptible to flucytosine in vitro (41,42). However, variability in results has been noted according to geographic location, serotype, and species (43). In one report, 3% of 5208 isolates of *C. albicans* were reported as resistant (44). Other reports of resistance range between 11.5% and 15.5% (37,45–47). Such ranges depend upon the serotypes of the isolates tested, since serotype B is much less susceptible to flucytosine (43). *Candida glabrata* is also highly susceptible in vitro to flucytosine (44,48,49), with 99% of 1267 isolates susceptible in one report (44). For 27 clinical isolates of *C. lusitanae*, the MIC₉₀ reported in one study was <0.125 $\mu\text{g}/\text{mL}$ (50), while others reported that 93% are susceptible at breakpoints <4 $\mu\text{g}/\text{mL}$ (44). In contrast, the MIC₅₀ and MIC₉₀ for *C. krusei* to flucytosine were reported to be 16 and 32 $\mu\text{g}/\text{mL}$, respectively (44,51). Therefore, only 5% of the isolates were considered susceptible according to CLSI breakpoints (44). Similar findings have been reported by other investigators (52,53).

The in vitro activity of flucytosine against *Cryptococcus* spp. varies significantly between reports. Primary resistance rates have reported to range from 1% to 24.5% of *C. neoformans* isolates (37,54–56). The influence of serotypes on susceptibility, however, is less clear. Lower susceptibilities in B/C serotypes in one report (55) were not observed by others (57). Higher MICs have been observed in non-*neoformans* strains of *Cryptococcus* (58).

In vitro susceptibilities of flucytosine have also been reported for pathogens causing chromomycoses. *Cladosporium* spp. and *Phialophora* spp. appear sensitive (59,60). For 31 isolates of *Saccharomyces cerevisiae*, an MIC₉₀ of 0.2 $\mu\text{g}/\text{mL}$ was reported (61). Dimorphic fungi (i.e., *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Histoplasma capsulatum*, and *Coccidioides immitis*) and most dermatophytes are also considered resistant (37). Flucytosine also lacks significant activity in vitro against fusariosis (62), zygomycosis (63–66), and aspergillosis (33).

In vitro evaluations of antifungal combinations are complex, and standards for methodology and interpretation of results are generally lacking (67–71). When tested in combination with amphotericin B against *Candida* spp., some form of positive interaction was reported in 35 of 40 (85%) isolates (72). Synergy with amphotericin B may be more common in flucytosine-sensitive organisms (73). However, antagonism of amphotericin B with flucytosine for *Candida* spp. is infrequent. Against cryptococcal isolates, conflicting data has been reported with in vitro combinations of flucytosine and amphotericin B. Synergistic, additive, neutral, or antagonistic effects

have all been reported with this combination (68,74,75). As was seen in *Candida* spp., pretreatment sensitivity to flucytosine may influence such results, since flucytosine-sensitive isolates more frequently demonstrate at least additive effects (73). In contrast, antagonism was more common in isolates resistant to flucytosine. There are extensive studies with flucytosine and fluconazole against *C. neoformans* (76–80). In combination with fluconazole against *Cryptococcus* spp., synergy was observed in 62% of the 50 clinical strains isolated, while no antagonism was observed (76). In the presence of fluconazole, flucytosine MICs for cryptococcal isolates were markedly reduced. However, flucytosine did not improve the in vitro activity of fluconazole against fluconazole-resistant isolates. Itraconazole in combination with flucytosine has also been studied against *C. neoformans* and synergistic (63%) and additive (31%) effects were reported (81). Finally, amphotericin B and flucytosine combinations against *Aspergillus* spp. have resulted in additive (82) or indifferent (83,84) activity.

PHARMACOKINETICS

Flucytosine is highly absorbed after oral administration (approximately 80–90%) (85) with peak serum concentrations (C_{max}) of 30–45 $\mu\text{g}/\text{mL}$ 1 to 2 hours after a single 150 mg/kg dose (85,86). A significant increase in serum concentrations of flucytosine was noted in one report when the drug was administered in a lipophilic vehicle (87). In contrast, reductions in serum concentrations after oral administration have been reported in a pediatric patient with Schwachmann syndrome (87) and in patients with advanced HIV infection (88). Significant systemic absorption of flucytosine has also resulted from patients receiving intraperitoneal administration (89–91).

The protein binding of flucytosine is low (approximately 4%) (92). As a result, it is widely distributed in body water, with a volume of distribution reported to range between 0.6 and 0.9 L/kg (92,93). Flucytosine penetrates into bone (94), vertebral disks (94), and synovial fluid (95,96). Based on a rabbit model, flucytosine also achieves high concentrations in both the vitreous and aqueous humor (97,98). Concentrations in spleen, heart, liver, kidney, and lung also have been reported to be comparable to simultaneous serum concentrations (92). Concentrations in the cerebrospinal fluid (CSF) are approximately 80% of simultaneous serum concentrations (99,100). Significant peritoneal fluid concentrations have been reported following oral administration (101). In patients with chronic respiratory disease, mean peak concentrations of $7.76 \pm 7 \mu\text{g}/\text{mL}$ in bronchial secretions were reported following a single 25 mg/kg intravenous dose (102). Serum and bronchoalveolar lavage (BAL) fluid concentrations ranged from <0.2 to $9.3 \mu\text{g}/\text{mL}$ and <0.4 to $1.5 \mu\text{g}/\text{mL}$, respectively, after oral administration of 4.5 to 6.0 g/day (103). Concentrations of flucytosine in the urine generally exceed that of serum by several-fold (85). It may also cross the placental barrier, with one report in amniotic fluid of $168 \mu\text{g}/\text{mL}$ 4 hours after a 2-g dose (104).

As much as 96% of the total flucytosine dose may be eliminated as unchanged drug (105). For the small metabolized fraction, several metabolites have been reported. Intestinal microflora may deaminate flucytosine to 5-fluorouracil (5-FU) (10,12,106–108). Deamination to 5-FU may be influenced either by chronic flucytosine exposure (106) or by use of broad-spectrum antibacterials (107). Other metabolites include 5-fluorodeoxyuridine monophosphate (5-FC-UMP) and fluororotic acid (12). *o*-Fluoro-*p*-alanine (FBAL), 5-hydroxy-5-fluorocytosine, 0-2P-glucuronide, 6-hydroxy-5-fluorocytosine (60HFC), and fluoride ion (F^-) have been reported in urine (105,109,110).

Flucytosine undergoes a high degree of renal elimination, with 60% to 95% of the dose eliminated by glomerular filtration (85,105,111). The serum half-life in patients with normal renal function ranges between 3 and 8 hours (85,112) and may be prolonged (i.e. 60–250 hours) in patients with significant renal disease (85,112). In patients undergoing continuous hemofiltration, serum half-lives ranging between 15.9 and 37.2 hours following an intravenous dose of 2.5 g have been reported (93). Removal may vary with ultrafiltration flow rate, serum concentration, and hemofilter type (93,113). Mean clearance of $77.0 \pm 15.6\%$ (SD) and $51.0 \pm 5.7\%$ (SD) of the ultrafiltrate flow rate were observed with polysulfone and polyacrylonitrile membranes, respectively (113). Therefore, continuous hemofiltration can remove an appreciable quantity of flucytosine. Hemodialysis also removes a significant quantity of flucytosine, depending on the

flow rate (114). A dialysate clearance ratio of approximately 70% has been reported (114,115). Peritoneal dialysis may also significantly enhance flucytosine elimination (90,116).

ADVERSE EVENTS

Hematologic, gastrointestinal, and hepatic toxicities are considered the most frequent and clinically relevant side effects of flucytosine (117). Conversion of flucytosine to 5-fluorouracil *in vivo* is thought to be responsible for most toxicities (106,118). The incidence of such reactions, however, is difficult to quantify because of the underlying infection, comorbidities, and concomitant therapies of patients receiving flucytosine.

The hematologic toxicity resulting from flucytosine may be treatment-limiting, especially in patients at increased risk. Leukopenia and thrombocytopenia are thought to occur more frequently in patients with serum flucytosine concentrations exceeding 100 $\mu\text{g}/\text{mL}$ (5,117,119–121). Rarely, bone marrow aplasia thought to be due to flucytosine has been reported (122,123). Hematologic effects are especially common in patients with advanced HIV infection receiving high doses (i.e. up to 150 mg/kg/day) (80,124).

Gastrointestinal complaints (most commonly nausea, vomiting, and diarrhea) may result from flucytosine therapy. However, the relationship between such events and serum concentrations is not clear (117). Hepatotoxicity (including hepatic necrosis) may result from flucytosine administration (125,126). In one report, approximately 5% of patients receiving flucytosine experienced elevations in hepatic transaminases and/or alkaline phosphatase (37).

Less-frequent side effects associated with flucytosine include photosensitivity reaction (127), anaphylaxis (128), and CNS abnormalities (such as headache, drowsiness, vertigo, confusion, and hallucinations). Flucytosine is generally not considered nephrotoxic (129). Early reports of nephrotoxicity were likely due to interference with selected laboratory methods used to determine serum creatinine (130) or to the concomitant administration of nephrotoxic agents.

CLINICAL APPLICATIONS

This section is not intended to be comprehensive and readers should refer to other chapters for a comprehensive discussion of this topic.

Cryptococcus

Current clinical trials with flucytosine for serious cryptococcal infections utilize combination therapy (131). Medical literature related to flucytosine monotherapy of cryptococcosis is limited. While the failure rate in 27 patients with invasive infection was 57%, it was not associated with drug resistance in most cases (132). Flucytosine monotherapy has also been reported for the treatment of pulmonary cryptococcosis (133,134).

Various animal models and human trials have established a role of flucytosine (as part of combination therapy with amphotericin B) in the treatment of serious cryptococcal infections. Flucytosine treatment of cryptococcal infections in humans is best studied in the treatment of central nervous system (CNS) infections. Amphotericin B (0.3 mg/kg/day) plus flucytosine (150 mg/kg/day) was compared to monotherapy with amphotericin B (0.4 mg/kg/day) for cryptococcal meningitis in patients without AIDS (1). Combination therapy for six weeks reduced the time to CSF sterilization, had similar efficacy and fewer failures/relapses and less nephrotoxicity than amphotericin B monotherapy administered for 10 weeks.

While retrospective evaluations of patients with AIDS and cryptococcal meningitis failed to detect a survival benefit of combination therapy over monotherapy (124), prospective studies in this population were subsequently performed in this patient population (2). Amphotericin B (0.7 mg/kg/day) with or without flucytosine (100 mg/kg/day) for two weeks was followed by either itraconazole or fluconazole therapy for eight weeks in a randomized trial (2). At two weeks, 51% and 60% of patients had sterile CSF cultures receiving monotherapy and combination therapy, respectively. While clinical outcomes were similar between treatment groups, addition of flucytosine was associated with improved rates of sterilization at week 2. Subsequent investigations comparing maintenance therapy with either fluconazole or itraconazole in this population detected an increased risk of relapse in patients not receiving initial therapy with flucytosine (relative risk = 5.88; 95% CI = 1.27–27.14; $p = 0.04$) (3). Further supporting data for the combination comes from a small, randomized, comparative study between initial

combination therapy (amphotericin B 0.7 mg/kg/day plus flucytosine 150 mg/kg/day) and oral fluconazole (400 mg/day) (4). In contrast to the 57% of the 14 patients who failed fluconazole therapy, none of the six patients receiving the combination regimen failed. Similar to previous studies, a shorter time to negative CSF cultures (15.6 ± 6.6 days) was observed in the combination therapy group. Similar observations were made when the combination was compared to itraconazole (135).

In contrast to studies evaluating the benefits of flucytosine in combination with amphotericin B, limited data are available evaluating the potential benefit of combining flucytosine with triazoles (e. g., fluconazole or itraconazole) in the treatment of cryptococcal meningitis in patients without AIDS (136,137). For patients with AIDS and acute cryptococcal meningitis, higher failure rates were reported in patients receiving fluconazole monotherapy when compared with the combination of fluconazole with flucytosine in a retrospective study ($n = 76$) (138). Finally, a significant increase in six-month survival was reported in patients receiving short-course combination therapy (two weeks) of flucytosine (150 mg/kg/day) plus fluconazole compared to fluconazole monotherapy in a randomized trial of 58 patients with AIDS-associated cryptococcal meningitis (139). Most recently, flucytosine improved fluconazole treatment outcomes in the investigations of larger doses of fluconazole therapy for cryptococcal meningitis in HIV-positive patients (140). While definitive data are lacking, itraconazole-containing combinations may also have benefit in treatment of cryptococcal infections in patients with AIDS (137).

Data regarding the use of flucytosine-containing regimens used to treat disseminated forms of other cryptococcal infections (including osteomyelitis, pneumonia, and prostatitis) are restricted to case reports and case series. As most of these reports involve treatment-refractory cases with prior and/or concomitant antifungal therapy, the contribution of flucytosine to the treatment outcome is difficult to assess.

Treatment guidelines for the management of pulmonary and CNS cryptococcal disease indicate flucytosine should be combined with amphotericin B for either two weeks of an "induction" regimen (prior to the conversion to fluconazole therapy) or is continued in combination with amphotericin B for 6 to 10 weeks (131). While fluconazole combined with flucytosine (100–150 mg/kg/day) for six weeks may be considered as an alternative option in HIV-infected patients, such regimens may frequently be associated with drug intolerance (especially those receiving higher flucytosine doses; i.e., 150 mg/kg/day) (131).

Candida

Flucytosine has been investigated in a variety of animal models and in some clinical studies for the treatment of various forms of candidal infections. In contrast to combination therapy, investigations of flucytosine monotherapy for candidal infections in humans are infrequent (owing primarily to concerns regarding the rapid development of resistance). Candiduria is perhaps the best-studied use for flucytosine monotherapy in humans (141,142). While initial clinical success was reported in 94% of 225 patients with genitourinary candidiasis caused by sensitive strains in vitro, 6% subsequently received supplemental therapy with systemic or bladder irrigations of amphotericin B due to failure or relapse (141). Other investigators have also reported similar experience in treating candidal urinary tract infections with flucytosine (142,143). In one of these reports, patients received 20–150 mg/kg/day flucytosine for the treatment of *Candida* ($n = 51$) or *Trichosporon beigelii* ($n = 6$) (143). Overall, 89.5% of the organisms was eradicated. Treatment guidelines by the IDSA identify monotherapy with flucytosine 25 mg/kg dosed four times daily as a treatment option for candiduria in patients requiring therapy, especially in cases involving non-*albicans* *Candida* (41).

With high concentrations achieved in peritoneal fluid following oral administration (101), flucytosine had potential as adjunctive therapy for treatment of fungal peritonitis. Case reports and case series described the use of flucytosine (both orally and intraperitoneally), often as part of combination therapy, for the treatment of patients with *Candida* peritonitis (89,90,144,145). Some investigators reported high rates of relapse after intraperitoneal flucytosine administration despite initial response (89,146).

Mucocutaneous forms of candidiasis have been successfully treated with flucytosine, administered either systemically or locally. Topical creams have been successfully used in

the treatment of vulvovaginal candidiasis (41,147–152). Treatment of esophageal candidiasis has also been investigated. Flucytosine 100 mg/kg daily was compared with fluconazole and placebo in the treatment of the first episode of esophageal candidiasis in 60 patients with AIDS in a double-blind crossover study (153). Endoscopic cure was reported in 9 (70%) and 9 (33%) patients in the fluconazole and flucytosine groups, respectively.

The potential role of flucytosine (in combination with other antifungals) for the treatment of various forms of invasive candidiasis has been identified (41,154,155). Combination with amphotericin B is generally considered in the more invasive candidal infections because of the potential synergism and protection against primary and secondary flucytosine resistance (156,157). Flucytosine may be used in combination with amphotericin B for the treatment of conditions such as endophthalmitis, candidal endocarditis, pericarditis, suppurative phlebitis, and meningitis (41,100,158). However, treatment outcomes in immunocompromised patients may remain poor despite flucytosine-containing combination therapy. For example, combination therapy of amphotericin B with flucytosine failed to demonstrate a benefit over amphotericin B monotherapy in a randomized study in persistently neutropenic patients with microbiologically and/or histologically documented systemic mycoses in patients with advanced disease (159). In contrast to these findings, in a retrospective study of neutropenic patients with *C. tropicalis* fungemia, success was reported in 5 of 9 patients receiving a combination of amphotericin plus flucytosine compared to 4 of 25 patients receiving amphotericin B monotherapy (160).

Combinations of flucytosine with azoles, such as fluconazole (161) and itraconazole (153), have been reported for select invasive forms of candidiasis. While the efficacy of flucytosine use in combination with fluconazole has been reported in HIV patients (80,139), such combinations are not considered optimal as initial therapy for CNS disease (131) and have been associated with significant toxicity in this patient population (80). In general, such combinations in HIV-infected patients are generally restricted to those with mild-to-moderate pulmonary infections (131). Published reports on flucytosine-containing combinations with echinocandins are sparse. One case report described the use of flucytosine in combination with caspofungin for the treatment of a prosthetic joint infection due to *C. glabrata* (162).

Aspergillus

Animal models of infection evaluating flucytosine in combination with azoles (163) and amphotericin B (163,164) generally report no effect, weakly additive, or indifferent effect. Prior to the availability of newer agents for the prevention and treatment of aspergillosis, secondary prevention utilizing amphotericin B plus flucytosine was reported in nine patients who received 13 subsequent courses of myelosuppressive chemotherapy for leukemia (165). Radiographic findings suggestive of invasive aspergillosis occurred during 2 of the 13 courses. Similar to other fungal pathogens, only observational and uncontrolled data are available to describe flucytosine's use in the treatment of disseminated aspergillosis, including meningitis, pulmonary infections, and endocarditis.

Availability of newer treatment options (such as voriconazole, posaconazole, and echinocandins) and the relative lack of data demonstrating flucytosine's effectiveness have significantly limited its usefulness in the treatment of invasive aspergillosis (166).

Chromomycoses

In addition to extended-spectrum azoles, flucytosine is considered a treatment option for certain chromomycoses (167–170). Susceptibility testing of isolates prior to therapy was recommended by one investigator due to the potential for drug-resistant relapse (171). However, availability of safer, alternative therapy options for these infections (such as itraconazole, voriconazole, and posaconazole) limits the clinical application of flucytosine for this indication.

Amebiasis

The use of flucytosine (in combination with other therapies, including pentamidine, fluconazole, and sulfadiazine) was reported in five AIDS patients with disseminated acanthamebiasis without CNS involvement (172). The contribution of flucytosine to the outcome, however, cannot be determined by this report.

DOSING AND ADMINISTRATION

While pharmacodynamic modeling has suggested doses as low as 25 mg/kg/day may be sufficient to treat patients with disseminated candidiasis (173), published recommended ranges for oral dosing of flucytosine in adult patients with normal renal function are generally between 100 and 150 mg/kg/day in four equally divided doses (41,131). In most situations, 25 mg/kg orally four times daily should be used (41,131). Higher amounts (i.e., up to 150 mg/kg/day in four equal doses) may be associated with higher incidences of dose-related toxicities in patients already at increased risk of adverse events, and should be considered (as part of combination therapy) in unique treatment issues of select HIV patients with cryptococcal disease (131) or invasive candidiasis (such as endocarditis) (41). Because 250 and 500 mg capsules are commercially available, individual oral doses are usually rounded to the nearest 250 mg increments in adult patients. For patients who are morbidly obese, ideal body weight may be best for dosing (based on limited information) (174).

In general, the oral pediatric weight-based dose of flucytosine is the same as the adult dose (175,176). However, extreme variability in half-life, volume of distribution, and clearance has been reported in pediatric patients (especially low birthweight neonates) (176). Therefore, divided doses of 100 mg/kg/day may be excessive in some children, and serum concentration monitoring should be employed in this patient population (177).

In patients with renal dysfunction, the dose of flucytosine should be modified based on estimates of creatinine clearance (37,85,86,111,117) (Table 1). Since flucytosine is most often administered in combination with amphotericin B (which may produce renal dysfunction), careful monitoring of renal function is required in order to optimize the dose. For patients undergoing either hemo- or peritoneal dialysis, supplemental doses of 25 to 50 mg/kg after dialysis have been recommended (114). Further adjustments may be indicated based on the results of serum concentration monitoring (113,115). Dosing guidelines have also been proposed in patients undergoing continuous hemofiltration based on filtration rate (93,178). Dosing adjustment has not been recommended in patients with hepatic insufficiency (179).

Alternate formulations and routes of administration for flucytosine have been described in the literature. Intravenous preparations are available in select countries outside the United States (175). The advantages of the intravenous formulation are unclear, and similar divided dosing to oral administration (i.e., 100 mg/kg/day) may be excessive (88). A liquid formulation for oral administration has been described (180,181). Intraperitoneal administration has been reported in patients with peritonitis undergoing peritoneal dialysis (90), and topical administration has been described in the treatment of candida vaginitis (147,148).

Because flucytosine may be converted in the gut to 5-fluorouracil (a teratogen as demonstrated in animal models), use is considered contraindicated in pregnancy unless benefit is considered to exceed risk. It is currently classified by the FDA as pregnancy category C. Despite such recommendations, isolated reports of flucytosine use during pregnancy have indicated normal pregnancy with delivery of normal infants (182–187).

Table 1 Dosage Adjustment of 5-Flucytosine in Patients with Renal Insufficiency

Creatinine clearance (mL/min)	Reference		
	37	133	117
>50	Full dose ^a q6 h	Full dose q6 h	37.5 mg/kg q6 h
41–50	Full dose q12–24 h	Full dose q6 h	37.5 mg/kg q6 h
31–40	Full dose q12–24 h	One-half dose q6 h or full dose q12 h	37.5 mg/kg q12 h
21–30	Full dose q12–24 h	One-half dose q6 h or full dose q12 h	37.5 mg/kg q12 h
11–20	Full dose q12–24 h	One-fourth dose or full dose q24 h	37.5 mg/kg q24 h
≤10	Full dose q24–48 h	One-fourth dose or full dose q24 h	37.5 mg/kg q36 h

^aFull dose = 100 mg/kg/day in divided doses (as indicated). The clinical trial with fluconazole for cryptococcal meningitis in HIV-infected patients used 150 mg/kg/day in divided doses. Dosing should be guided by target serum concentrations (as indicated in text).

Source: From Ref. 200.

The role of serum drug concentration for antifungals has been reviewed in detail elsewhere (188). Monitoring flucytosine serum drug concentrations may aid in individualizing therapy by avoiding excessive serum concentrations (associated with increased risks of hematologic toxicity) while obtaining "target concentrations" recommended for the effective of select invasive fungal infections (189). Bioassay (23,86), fluorometric (190), high-pressure liquid chromatography (HPLC) (191–193), enzymatic (194–196), and gas chromatography (197) methods for analysis have been reported. Clinical studies have established association of flucytosine toxicity and peak serum flucytosine concentrations ≥ 100 $\mu\text{g}/\text{mL}$ or more during two or more weeks of therapy (5,117,119–121,175). While some authors recommend determining serum levels two hours after and immediately before a dose (157,198), others have found minimal difference between such levels (117). Routine monitoring should include obtaining a sample for analysis two hours post-dose after three to five doses. For efficacy, target serum concentrations of 40 to 60 $\mu\text{g}/\text{mL}$ have been identified for invasive candidiasis (41), while a two-hour post-dose range of 30 to 80 $\mu\text{g}/\text{mL}$ has been recommended for the treatment of cryptococcosis (131). Monitoring may be repeated on a weekly basis or earlier if alterations in renal function indicate the potential for altered flucytosine clearance when timely drug concentration data are unavailable (120). Frequent blood counts should also be performed, and may be particularly useful in the absence of available serum concentration assays to predict the need for dose modification and reduce the potential for serious hematologic toxicities.

DRUG INTERACTIONS

Aluminum hydroxide/magnesium hydroxide, when administered concomitantly with flucytosine, may delay its absorption (92). However, effects of other agents altering gastric pH (such as other antacids, histamine 2 antagonists, or proton pump inhibitors) have not been reported. While the FDA-approved product information states that cytarabine may inactivate the antifungal activity of flucytosine (175), there are no published data to support this statement (175,199). Concomitant administration of flucytosine with agents possessing similar toxicities (most notably hematologic, hepatic, or gastrointestinal) should be done with caution.

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12 | Pharmacology of Azole Antifungal Agents

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INTRODUCTION

Azole antifungal drugs are the most widely employed antifungal agents in clinical practice. When first introduced in the late 1970s, these agents greatly changed the way fungal infections were managed by introducing choice for clinicians as well as oral therapy options for treating systemic fungal diseases. Today, their clinical utility spans every aspect of treating fungal infection from topical therapy for superficial diseases to intravenous preparations for the most severe, life-threatening invasive mycoses.

This class can be divided into two distinct groups based on the number of nitrogen atoms within the five-membered azole ring of their chemical structure (1). The imidazoles (butoconazole, clotrimazole, econazole, ketoconazole, miconazole, oxiconazole, sulconazole, terconazole, and tioconazole) are limited to largely topical use owing to their suboptimal pharmacokinetics and intolerable safety profiles. Comparatively, the triazoles (fluconazole, itraconazole, voriconazole, posaconazole) demonstrate a broader spectrum of activity and have become first-line treatment options for many serious infections.

The azoles cannot be thought of as a single class with similar pharmacologic profiles. Instead, each member must be specifically understood for its unique role in the management of fungal disease. This chapter focuses on the pharmacology of the azole class, primarily the triazole agents currently available for systemic administration. Where possible, similarities and differences are highlighted.

STRUCTURE AND MECHANISM OF ACTION

The key structural component of all members of the azole class is the five-membered azole ring (Fig 1). The imidazoles contain two nitrogen atoms in this ring, while the triazoles incorporate a third nitrogen into this structure. Many triazoles are derived from earlier members of the imidazole class such as itraconazole and posaconazole, which are structurally similar to the long lipophilic molecule, ketoconazole. This is in contrast to fluconazole that is less lipophilic and has a smaller molecular size that is more akin to the structures of clotrimazole and similarly appearing imidazoles. Although more lipophilic than fluconazole, voriconazole most closely resembles the structure of fluconazole (1).

All azole antifungal agents share a common, primary mechanism of action, inhibition of the cytochrome P450-dependent enzyme lanosterol 14 α -demethylase (2). This enzyme is necessary for the conversion of lanosterol to ergosterol, a vital component of the cellular membrane of fungi. Disruptions in the biosynthesis of ergosterol cause significant damage to the cell membrane by increasing its permeability, resulting in cell lysis and death.

SPECTRUM OF ACTIVITY

The agents within this class vary importantly in regards to spectrum of activity.

Fluconazole, the first member of this class, has activity limited to yeasts and some of the endemic fungi. It has excellent activity against most *Candida* species, but has less activity against *C. glabrata* and no activity against *C. krusei*. In a recent survey of over 200,000 yeast isolates collected from a seven-year period, fluconazole retained activity against most species of *Candida* (3). For many species, including *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida dubliniensis*, resistance remained less than 5% of all isolates tested. This was in contrast to nearly 80% resistance for *C. krusei* and more than 15% for *C. glabrata*. The activity of fluconazole against *Cryptococcus* spp. was reported at just above 70%. Overall, fluconazole activity for other yeasts including *Trichosporon* and *Saccharomyces* spp. was lower than that observed for candidal isolates (3).

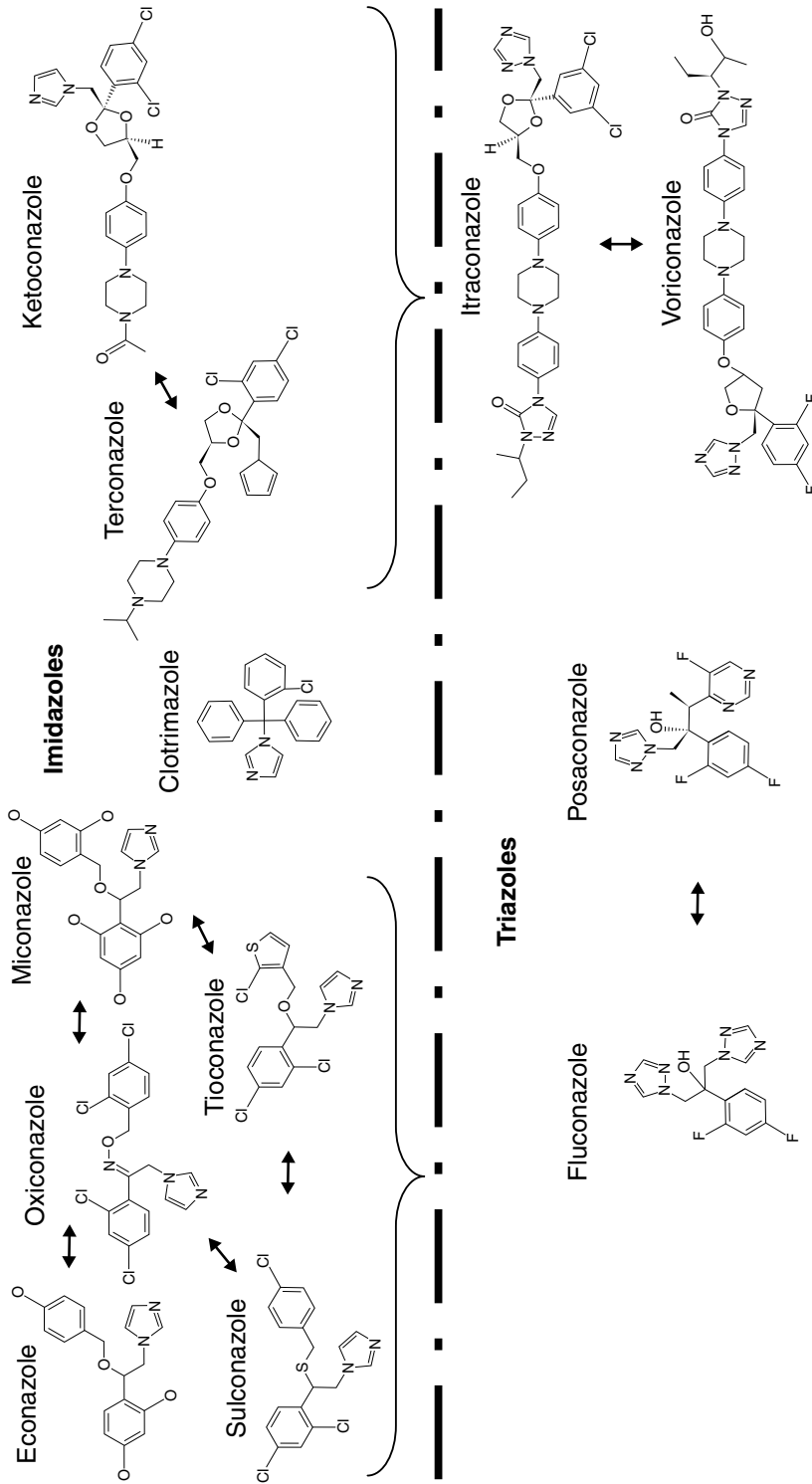


Figure 1 Similarities among the structures of the azoles.

Itraconazole offers a broader spectrum of activity than fluconazole, including some mould pathogens, such as *Aspergillus* species and dimorphic fungi. In a sample of nearly 20,000 clinical isolates, itraconazole susceptibilities compared favorably with other azole antifungal agents (4). The itraconazole MIC₅₀ was ≤ 1 $\mu\text{g}/\text{mL}$ for all species tested with the exception of *Fusarium* spp., *Scedosporium prolificans*, and many *Zygomycetes* (4).

The newer agent, voriconazole, has a broad spectrum of activity that includes the most frequently isolated yeasts and moulds causing opportunistic disease (5,6). Additionally, this agent has activity against common dermatophytes and the pathogens causing the endemic mycoses (7,8). In vitro data have shown that voriconazole is also effective against emerging fungal pathogens including *Scedosporium apiospermum*, *Trichosporon* spp., *Acremonium* spp., and *Fusarium* spp. (9–14).

Voriconazole, like fluconazole, has fungistatic activity against *Candida* spp.; however, it offers better activity against most isolates than its predecessor (3). Voriconazole also retains activity against some fluconazole-resistant strains of *Candida* that may represent a therapeutic niche for this agent when results of susceptibility testing are available (15). Fungicidal activity is seen against filamentous fungi including *Aspergillus* spp. In a survey of more than 400 clinical isolates, voriconazole was active against more than 95% of *Aspergillus* isolates, compared with only 90% being susceptible to itraconazole (16). The notable hole in the spectrum of voriconazole is the *Zygomycetes* (9,17,18).

Posaconazole provides a similar spectrum of activity to voriconazole with added activity against the *Zygomycetes* (9–14,18,19). With minimum inhibitory concentrations (MICs) ranging from 0.03 to 1 $\mu\text{g}/\text{mL}$ against filamentous fungi, posaconazole demonstrated similar potency to voriconazole and superior to that of itraconazole (19). Testing on more than 1400 *Aspergillus* isolates by Schering Plough yielded an MIC₉₀ of 0.5 $\mu\text{g}/\text{mL}$ for posaconazole, the most potent agent tested (4). Amphoterin B is the only antifungal agent with more activity against the *Zygomycetes* than posaconazole (18). MICs obtained in testing more than 250 isolates were 8- and 32-fold lower than those observed for itraconazole and voriconazole, respectively (4).

PHARMACOKINETICS

The kinetic properties of the azole antifungal agents differ between each member of the class and even among formulations for an individual agent. These distinguishing factors play an important role in drug selection, and can affect the ultimate success of the regimen if the agent is administered inappropriately. An understanding of the absorption, distribution, metabolism, and elimination of these drugs is the basis for many aspects of these therapies including use of certain formulations, optimal administration conditions, as well as drug–drug interactions.

Absorption

Currently, all systemic triazole antifungal agents are available as oral formulations and each achieves serum drug concentrations suitable for treating systemic disease. However, several factors are involved in optimal drug absorption.

Fluconazole is very hydrophilic allowing easy intravenous administration. It is almost completely absorbed with a reported oral bioavailability of 85% to 90% when compared with intravenous exposure, among the highest for the class (20). Unlike the other agents in the class, fluconazole absorption is unaffected by changes in gastric pH or contents.

In contrast, itraconazole has the most unpredictable serum drug concentrations following oral administration. The capsule formulation has a very limited bioavailability that is completely dependent upon an acidic gastric pH to attain even minimal serum drug concentrations (21). In order to optimize absorption, this formulation needs to be administered with a full meal and without any acid-suppressive therapies such as proton pump inhibitors or H₂ receptor antagonists (22–25).

In order to overcome some of the challenges with administering oral itraconazole, a cyclodextrin solution of itraconazole was developed. This formulation does result in improved drug exposure, with an increase in AUC of 30% compared with the capsule (26). This newer and now preferred formulation is free of effects from acid-suppressive therapy, but absorption can be impeded when concomitantly administered with food (27–29). Consequently, it is important

that this formulation be administered on an empty stomach and patients and caregivers alike need to be educated accordingly.

The cyclodextrin component of the itraconazole oral solution is employed as a solubilizing agent and this same excipient was used to develop an intravenous formulation of itraconazole initially marketed in the United States in the late 1990s. However, owing to limitations of administering cyclodextrin via this route and limited use following the availability of newer antifungal agents, this product has been removed from the U.S. market.

Similarly, voriconazole has limited solubility but readily goes into solution when formulated with cyclodextrin. This has allowed an intravenous preparation of voriconazole that is currently offered in addition to tablets and suspension both for oral administration. When administered orally, voriconazole demonstrates a greater than 90% bioavailability (30). This is enhanced by administration on an empty stomach (31). In a similar fashion to its azole predecessor, fluconazole, voriconazole does not otherwise rely on gastric pH for absorption as evidenced by a lack of any drug–drug interaction with acid-suppressive therapies.

Bioavailability of posaconazole is perhaps the most intriguing of the class. Currently, there is no available intravenous formulation of this agent and therefore, we rely on the oral route of administration. The formulation associated with highest serum drug exposure is the oral suspension that is currently the only marketed preparation (32). Interestingly, posaconazole demonstrates linear proportional increases in serum concentrations with rising drug doses that appears to maximize with a total daily dose of 800 mg (33,34). Exposure was improved, however, when the number of doses per day was increased; in other words, administering the total daily quantity of 800 mg in smaller increments optimizes absorption (33).

Gastric pH as well as the presence of food are important factors in posaconazole administration. Absorption is optimized when administered during a meal with a more than 2.5-fold increase in exposure (35). This is further enhanced with a high-fat meal resulting in a four times increase in drug concentrations compared with nonfat foods (32). Similar effects are seen with other sources of dietary fat including the nutritional supplement Boost Plus[®] (Nestle HealthCare Nutrition, Novartis Medical Nutrition, Fremont, MI) (36).

Initial studies indicated that gastric pH was not a factor in posaconazole administration since early trials with antacids did not alter posaconazole kinetics (35). However, more recent trials have concluded that posaconazole absorption is adversely affected by high gastric pH, especially administration with proton pump inhibitors (37). Conversely, use of an acidic beverage improved drug exposures (37). Therefore, posaconazole should ideally be given with foods having high fat content or an acidic beverage at minimum. Acid-suppressive therapies should be avoided if possible in patients requiring posaconazole treatment. Unfortunately, no data are available regarding administration of posaconazole with an acidic beverage in patients requiring chronic acid-suppressive therapy to determine if this strategy may effectively manage this interaction.

Distribution

Drug distribution is an important consideration when treating invasive fungal infections particularly when the infection occurs at a distant site that may not be amenable to drug penetration. In general, all of the triazole antifungal agents are well distributed throughout the body with volumes of distribution ranging from approximately 40 L (volume of total body water) for fluconazole to more than 1500 L for posaconazole (20,38). While initially appearing to vary largely, these values reflect the volume of distribution for total drug rather than free active drug. This highlights the importance of considering the degree of protein binding for each of these agents, which is smallest for fluconazole (10–12%) and highest for itraconazole and posaconazole (>98%) (20,38,39).

In addition to total body exposure of a drug, certain anatomical sites are of particular interest regarding drug penetration including cerebrospinal fluid (CSF), urine, and eye.

Azole agents are attractive options for treating diseases of the central nervous system (CNS). This is secondary to their oral routes of administration for patients who require long treatment courses as is the case with CNS disease. CSF penetration is the best for fluconazole (>60%) compared to other agents in the class (40,41). Voriconazole is also detected in the CSF at high concentrations approximately 50% of those observed in serum (42,43). CSF

concentrations of both itraconazole and posaconazole are minimal or not well described; however, this does not mean that these agents cannot effectively treat diseases of the CNS. For example, the success of posaconazole in treating cryptococcal meningitis as well as other fungal diseases of the CNS has been reported (44). Similarly, itraconazole has been shown to effectively treat patients with cryptococcal meningitis despite nearly undetectable CSF concentrations although this activity is still inferior to fluconazole (45).

Fungal infections of the urinary tract remain an area of controversy as positive cultures often represent colonization rather than true infection. There have been limited clinical trials to assess the efficacy of antifungal agents in treating fungal infections of the urine. Owing to their routes of elimination, fluconazole is the only azole antifungal with detectable concentrations in the urine that are adequate to treat lower urinary tract disease (30,46–48).

Additional data exist regarding penetration of the azole agents into other anatomic sites. Data are most complete for fluconazole, which has demonstrated concentrations in saliva, sputum, vitreous, blister fluid, skin, nails, prostate, liver, spleen, kidney, and vaginal tissues exceeding half of those detected in serum and in many cases at or above serum concentrations (20,49,50). Voriconazole, which is the azole most structurally similar to fluconazole, is detected in saliva, vitreous humor, brain, liver, kidney, heart, lung, epithelial lining fluid, and spleen (42,51–54). Both itraconazole and posaconazole also have decent vitreal penetration of greater than 10% (55,56). Itraconazole penetration into sputum is variable, but concentrations within lung tissue appear adequate to treat pulmonary disease (47,57,58). It also has excellent concentrations in the skin and nails thus supporting its use for treating infections due to dermatophytes as well as onychomycosis (59,60). Although posaconazole concentrations from lung and bone have not been published, clinical data and its large volume of distribution suggest that it adequately reaches these sites (61).

Metabolism and Elimination

All of the azole antifungal agents undergo some degree of hepatic metabolism (Table 1) (2,20,27,28,30–32,38,39,42,43,46,52,53,55,62–64). For fluconazole, this only contributes minimally to overall elimination with more than 80% excreted unchanged in the urine (46). Oxidative metabolism is the primary process involved in the metabolism for voriconazole and itraconazole while glucuronide conjugation is more prominent with posaconazole (30,39,65). Of note, only one antifungal metabolite has clinically meaningful activity, the itraconazole metabolite hydroxyitraconazole (66). For the inactive metabolites of the remaining azole agents, elimination occurs via a combination of urinary and fecal routes.

The cytochrome P450 enzyme system plays a significant role in the metabolism of voriconazole (2C19, 2C9, and 3A4) and to a lesser extent, itraconazole (3A4) (30,39,67–70) (Table 2). In the case of voriconazole, this process is saturable and as a result, the drug exhibits nonlinear pharmacokinetics (51). Therefore, clinicians should be cautious when considering dose escalation for this agent. Another variable that influences the kinetics of voriconazole is genetic polymorphisms that can lead to a complete lack of 2C19 expression in various patient populations. Just over 2% of Caucasians and 15.8% of Asians are poor metabolizers via 2C19 (71). The influence of this polymorphism on drug exposure has been demonstrated with voriconazole and may be an indication for serum concentration monitoring (72,73). Less frequently, genetic polymorphisms of CYP 2C9 also occur, although not in Southeast Asians and in only 1% of Caucasians (74). Limited experience in patients who are poor metabolizers via 2C9 did not result in altered voriconazole kinetics (75).

As previously mentioned, metabolism of posaconazole occurs via a noncytochrome P450 mediated pathway carried out by UDP-glucuronosyltransferase (UGT) enzymes (76). The two glucuronide metabolites are excreted in the urine with the majority of drug being eliminated unchanged in the stool (48).

Special Populations

Organ Dysfunction

The effect of organ dysfunction on drug elimination has been studied with each of the azole agents. Renal dysfunction is a concern for patients receiving fluconazole. In patients with renal

Table 1 Properties and Pharmacokinetics of the Azole Antifungals (2,20,27,28,30–32,38,39,42,43,46,52,53,55,62–64)

	Fluconazole	Itraconazole ^a	Voriconazole	Posaconazole
<i>Available formulations</i>	IV/PO	PO	IV/PO	PO
<i>Drug dosing ranges (adult)</i>	50–800 mg once daily	200–800 mg daily (doses greater than 400 mg/day should be divided)	IV Load: 6 mg/kg q12h × 2 doses Maintenance: 4 mg/kg q12h Oral Load: 400 mg q12h × 2 doses Maintenance: 200–300 mg q12h	800 mg daily in 2–4 divided daily doses
<i>Oral bioavailability (%)</i>	95	50	96	ND
<i>Food effect</i>	No effect	Decreases	Decreases	Increases—optimal with high-fat meal
<i>Distribution</i>				
<i>Total C_{max} (µg/mL)</i>	0.7	11	4.6	7.8
<i>AUC (mg·h/L)</i>	400	29.2	20.3	8.9
<i>Volume of distribution (L)</i>	40	796	322	1774
<i>Protein binding (%)</i>	10	99.8	58	99
<i>CSF penetration (%)</i>	>60	<10	60	NR
<i>Vitreous penetration (%)</i>	28–75	10	38	26
<i>Urine penetration (%)</i>	90	1–10	<2	<2
<i>Metabolism</i>	Minor hepatic	Hepatic	Hepatic	Hepatic
<i>Elimination</i>	Urine	Hepatic	Renal	Feces
<i>Half-life (h)</i>	31	24	6	25

^aData are for itraconazole oral solution.

dysfunction, it is advised to decrease the total daily dose of fluconazole by 50% (20). Studies have also been conducted in patients requiring renal replacement therapy with either hemodialysis or continuous hemofiltration. For a traditional hemodialysis session, 25% to 40% of fluconazole is removed depending on the duration of the session (77,78). Therefore, supplementation is suggested following each dialysis session (20). Continuous hemofiltration increased clearance of fluconazole ranging from 20 to 400 times baseline elimination in patients with acute renal failure suggesting that daily dosing should be continued in this population (79).

While one would expect that the other triazole agents are free from kinetic changes due to renal dysfunction; this is actually not the case. The solubilizing agent cyclodextrin that is

Table 2 Azole Antifungal Activity via Cytochrome P450 and P-Glycoprotein (67–70)

	CYP3A4	CYP2C9	CYP2C19	P-glycoprotein
<i>Substrates</i>				
Fluconazole				Yes
Itraconazole	Major			Yes
Posaconazole				Yes
Voriconazole	Minor	Minor	Major	
<i>Inhibitors</i>				
Fluconazole	Moderate (>200 mg)	Moderate	Minor	
Itraconazole	Strong	Weak		Yes
Posaconazole	Moderate			Yes
Voriconazole	Moderate	Moderate	Moderate	

used in the intravenous formulation of voriconazole can accumulate in patients with renal disease. While the exact effects of this are largely unknown, prescribing information cautions against use of this preparation in patients with decreased renal function (defined as creatinine clearance <50 mL/min) (30). While the same agent is employed in the oral solution formulation of itraconazole, it is not absorbed from the gastrointestinal tract and therefore similar concerns do not exist. Studies of itraconazole in patients with renal disease, including those requiring dialysis, did not suggest that any dose modifications are required (80). Posaconazole is also free from significant kinetic changes in patients with varying degrees of renal dysfunction (81).

Hepatic dysfunction is a concern with itraconazole, voriconazole, and posaconazole therapy as each of these agents relies heavily on the liver for metabolism. Unfortunately no firm guidelines exist for any of these agents in regards to specific dosing for patients with hepatic dysfunction. The most precise recommendation is available for voriconazole that suggests considering a dose modification in patients with mild to moderate cirrhosis (30). Data from the manufacturer indicate that in patients with moderate hepatic insufficiency defined as Child-Pugh class B, the mean C_{max} for voriconazole increased by 20% compared with subjects receiving traditional doses and that dose reductions should be considered in these patients accordingly (30,82). In patients with chronic liver disease, maximum serum concentrations of posaconazole were decreased while half-life and time to maximum serum concentration were prolonged; however, data were inconclusive to recommend dose modifications (83). Therefore, current recommendations for both itraconazole and posaconazole are to carefully monitor patients when administering them to patients with liver disease (38,39).

Pediatric Patients

The azole antifungal agents have all been used in pediatric patient populations. The kinetic properties for each drug are altered in this population leading to different dosing strategies depending on patient age.

Fluconazole clearance in pediatric patients is accelerated when compared with adults as evidenced by a shorter half-life (20 vs. 30 hours, respectively) (84). Some advocate addressing this by doubling the daily dose of fluconazole in children who are more than three months old (85). The FDA-approved prescribing information for fluconazole also offers guidance depending on the adult target dose (Table 3) (20,86–89).

Experience with the cyclodextrin solution of itraconazole in children has resulted in much lower concentrations than those seen in adults, particularly when a once daily dosing regimen is used (90). In order to obtain equivalent exposure to once daily doses of itraconazole in adults, a comparable total daily dose needs to be divided into a q12h regimen (87).

Voriconazole, which demonstrates nonlinear pharmacokinetics in adult patients, has linear elimination in children (86). As a result, children are able to eliminate more voriconazole per kg of total body weight than their adult counterparts, and higher daily doses may be needed in this population. Data from pharmacokinetic studies in children aged 2 to 12 years suggest that an intravenous dose of 4 mg/kg every 12 hours in children was equivalent to a 3 mg/kg dosed at the same interval in adults (30,86). Simulated data suggest it might take as much as 11 mg/kg twice daily in children to achieve AUC values equivalent to a 4 mg/kg dose in adults (86). Based on data in hospitalized children receiving voriconazole, the current expert

Table 3 Equivalent Pediatric Dosing (20,86–89)

	Pediatric patient age	Pediatric dose	Equivalent adult dose
Fluconazole	>3 mo	3 mg/kg	100 mg q24h
		6 mg/kg	200 mg q24h
		12 mg/kg (not to exceed 600 mg/day)	400 mg q24h
Itraconazole	> 5 yr	2.5 mg/kg q12h	200 mg q24h
Posaconazole	8–17 yr	800 mg/day	800 mg/day
Voriconazole	1–11 yr	4 mg/kg	3 mg/kg
		Recommended dose: 7 mg/kg q12h	

recommendation is to administer 7 mg/kg q12h or 200 mg orally q12h for children between the ages of 1 and 11 years (88). Any additional dose increases beyond this are based on results of serum concentration monitoring as well as clinical need. This strategy remains untested in clinical trials.

Data for use of posaconazole in pediatric patients are limited to sparse pharmacokinetic sampling in juvenile patients (8–17 years old), who were being treated for refractory fungal infections. All of these patients received total daily doses of 800 mg/day of posaconazole. Compared with adults on the same protocol, drug concentrations were similar (89). Therefore, a total daily dose of 800 mg is suggested for all patients.

ADVERSE EFFECTS

As a class, the azoles are generally well tolerated. Gastrointestinal symptoms are most frequently reported, including nausea, abdominal pain, vomiting, and diarrhea (20,30,38,39). The latter is most notable with itraconazole oral solution and is caused by the cyclodextrin vehicle, which enhances its solubility (91).

The other most common class-wide adverse effect is hepatic dysfunction. These reactions range from mild elevations in transaminases occurring in 1% to 20% of patients to severe hepatic reactions including hepatitis, cholestasis, and fulminant hepatic failure resulting in transplantation or death (92–99). In the case of voriconazole, this reaction appears to be concentration related (100,101).

Careful monitoring of liver function is recommended for all patients receiving systemic azole therapy, as this adverse effect does not appear to be associated with duration of antifungal therapy. Current recommendations are to monitor patients at baseline and routinely throughout the entire course of treatment for development of elevations in hepatic enzymes (20,30,38,39). Interestingly, hepatotoxicity to one azole agent does not predict reactions to other members of the class (102).

Another serious effect common to the whole azole class is prolongation of the QT interval and although rare, is associated with cases of torsade de pointes (103–109). Many of these cases are associated with drug–drug interaction between the azoles and other agents also associated with QTc prolongation or additive QTc prolongation with agents, such as the anthracycline antineoplastic agents. It is important to consider this effect when adding azoles to complicated medication regimens and monitor accordingly (20,30,38,39,110).

Adrenal insufficiency is also common to all of the azole antifungal agents (20,30,38,39). This is most often associated with high-drug doses and may go undetected or underreported especially in the critically ill (111–115).

Fluconazole should be avoided in pregnant women because of well-documented cases of fetal abnormalities (116,117). Although recent experiences suggest that short courses at low doses may be tolerated and no studies have been performed with the other triazoles in pregnant women, the class as a whole should be routinely avoided in this patient population (30,38,39,118).

In addition to the class effects already discussed, each of the triazole agents also carries a unique side effect profile that is discussed below.

Fluconazole

Fluconazole is associated with minimal side effects with safety data in millions of patients; however, there are two distinct reactions worth noting. Alopecia has been reported following long courses of high-dose fluconazole (119). This condition is reversible after discontinuation of the agent. Another, more serious condition associated with fluconazole is the development of Stevens–Johnson syndrome following administration (120).

Itraconazole

High doses of itraconazole (600 mg/day) have been associated with an aldosterone-like effect, with manifestations including hypertension, hypokalemia, and, less often, peripheral edema (115). Peripheral edema can also be seen with lower doses of itraconazole. Cases of heart failure have been described (121). The manufacturer recommends reconsideration of the use of the

drug in patients with a history of heart failure and to avoid use of the agent altogether for treatment of onychomycosis in patients with evidence of ventricular dysfunction (39,122).

Voriconazole

Voriconazole is associated with two distinct adverse reactions compared with the other triazoles: abnormal vision and rash. Abnormal vision (photophobia, color changes, or blurred vision) is reported in up to 20% of patients who receive this triazole (30). This transient effect is temporally associated with drug dosing, occurring within 30 minutes of oral administration. Symptoms usually last for approximately 30 to 60 minutes. Photopsia (flashes of light) and visual hallucinations have also been reported (101,123,124). Ocular toxicity has been linked to elevated serum concentrations of voriconazole (101,125,126).

Rash associated with voriconazole therapy is reported in approximately 10% of patients (30). This photosensitivity reaction is not prevented by administration of sunscreens. Therefore, avoidance of sun exposure is required to prevent this condition. Although the rash is usually mild and will abate with withdrawal of therapy, it has precipitated discontinuation of voriconazole, particularly in pediatric patients and caused more significant reactions in isolated patients (101,127–133).

Another rare complication of voriconazole therapy is pancreatitis and resultant hypoglycemia (134,135). This can be difficult to predict in patients, particularly in the complex scenarios that are often associated with invasive fungal disease, but withdrawal of this agent should be considered in patients who develop pancreatitis while on treatment.

Posaconazole

In general, posaconazole did not result in a significant increase in adverse events compared with other azole agents in randomized clinical trials (136,137). In general use, no specific adverse events have emerged beyond those seen for the class as a whole (138).

DRUG–DRUG INTERACTIONS

Drug–drug interactions involving the azole antifungal agents pose some of the most difficult challenges in administering these therapies to patients. Their occurrence is common with more than 70% of patients on azole treatment concurrently receiving another agent with the potential for interaction (122). The importance of understanding these complicated relationships cannot be understated. In addition to those involving altered azole absorption already discussed, there are numerous interactions involving drug metabolism. The complexity of drug–drug interactions arise from the similarities between the mechanism of action for the azole antifungals and the physiologic effects of the cytochrome P450 enzyme system and p-glycoprotein transport proteins. In addition, select azole antifungals are themselves substrates of p-glycoprotein and the cytochrome P450 enzymes resulting in multidirectional interactions that can be more difficult to predict.

Cytochrome P450–Mediated Interactions

CYP 3A4

All azole antifungals and some of their metabolites inhibit cytochrome P450 3A4 to a degree (67–69,139,140). These agents vary in their affinity for the 3A4 isoenzyme leading to different degrees of inhibition. Of the available agents, itraconazole and voriconazole are the most potent inhibitors of 3A4 followed by fluconazole (67,69,141). Head-to-head comparative studies have not yet been conducted with posaconazole; however, its structural similarity to itraconazole and relative equivalent requirement in dose modifications for immunosuppressive agents when compared with voriconazole imply that it is also a potent inhibitor of CYP3A4 (30,38,140).

Interestingly, the weakest of the inhibitors, fluconazole appears to exhibit dose-dependent inhibition of CYP3A4 that is only seen at doses exceeding 200 mg/day (142,143). There have been reports of interactions with fluconazole occurring at daily doses less than 200 mg/day; however, one must carefully consider renal function in these cases as systemic drug exposure may approximate that of a 200 mg daily dose in patients with compromised renal function (144,145).

Interactions involving CYP3A4 are particularly problematic. This isoenzyme is prevalent in the liver and gastrointestinal tract and accounts for the majority of cytochrome P450 enzymes present in humans (146). It is also involved in the metabolism of numerous medications. Therefore, inhibition can significantly affect the metabolism of these agents and lead to negative consequences such as added physiologic effect or toxicities of target medications.

Perhaps the most prominent of these are the immunosuppressants cyclosporine, tacrolimus, and sirolimus (147–149). Significant toxicities have occurred with each of these agents and concomitant triazole therapy (147). Empiric dose reductions are required when an azole agent is added to a regimen containing any of these drugs (20,30,38,39). Failure to recognize the impact of this interaction and appropriately increase immunosuppressant doses when terminating azole therapy has resulted in negative consequences including loss of the graft and death (150–152). In some cases, azole agents have been proposed as a method to decrease the required daily dose of immunosuppressant, a practice more commonly employed with calcium channel blockers (153–155). However, given the rising incidence of azole resistance and toxicities of these agents, azoles should not be used for the sole purpose of decreasing daily requirements of immunosuppressive agents.

Many clinicians do not realize that glucocorticoid metabolism is also completed in part by intestinal and hepatic cytochrome P450 3A4 leading to potential interactions with the triazole class (147,148). This is best described with itraconazole; however, it is a theoretical concern with each of the other triazoles as well (156–158). While there are no empiric dose modifications suggested, it should be considered in patients demonstrating symptoms of excess glucocorticoid therapy or rapid steroid withdrawal in the setting of azole initiation or discontinuation, respectively.

Several antineoplastic agents also rely on CYP3A4 for metabolism or activation. Inhibition of the clearance of vincristine by itraconazole has been noted to result in added neurotoxicity of vincristine and other vinca alkaloids (159,160). Prescribing information for itraconazole, posaconazole, and voriconazole cautions against use of these drugs in combination with any vinca alkaloid (30,38,39). Cyclophosphamide metabolism is also affected by azole antifungals (161). Inhibition of CYP3A4 leads to preferential production of the more toxic metabolite of cyclophosphamide. Interestingly, inhibition of 2C9 as is seen with fluconazole and voriconazole may be protective against this effect. Busulfan kinetics is known to be affected by itraconazole therapy, an effect that has been attributed to its elimination via cytochrome P450 (162). As a result, conventional wisdom is to try and avoid this agent with any of the azoles, if possible.

Several cardiac medications including members of the dihydropyridine calcium channel blockers and the statin class of lipid lowering agents are also metabolized via cytochrome P450 3A4 (147,148,163–165). Management of these interactions ranges from close monitoring for signs and symptoms of excess drug concentrations (calcium channel blockers) or use of an agent from the class less prone to interactions such as pravastatin for the HMG CoA reductase inhibitors (164).

Several additional classes of medications are also known substrates of CYP450 3A4 leading to potential interactions with the azole antifungals. These include benzodiazepines, macrolide antimicrobials, protease inhibitors, and the antiarrhythmic agent quinidine (147,148). Given the complexity of drug interactions involving the azole antifungal agents, it is always prudent to consult current prescribing information and other drug information resources to determine the most appropriate course of action for managing the azole as well as the other medication therapy. Several internet-based sources maintain current information regarding drug–drug interactions and are listed in Table 4.

In addition to drug–drug interactions occurring as a result of triazole inhibition via 3A4, three members of this class (voriconazole, fluconazole, and itraconazole) are also substrates

Table 4 Internet Resources for Drug–Drug Interactions Involving Azole Antifungals

Information provided	Website
Cytochrome P450 interactions	www.medicine.iupui.edu/flockhart/table.htm
P-glycoprotein interactions	www.mhc.com/pgp/

of this isoenzyme (30,39,67). As a result, inducers of 3A4, including phenytoin, carbamazepine, phenobarbital, and the rifamycins (rifampin and rifabutin) can result in decreased concentrations of the azole antifungal agents (30,166–172). Great caution should be exercised if azoles are to be administered with any of these agents.

CYP 2C9

Voriconazole and fluconazole are both moderate inhibitors of CYP 2C9 (149,173). Fortunately, fewer drugs are metabolized via this route than 3A4; however, there are some notable interactions worthy of discussion. Most significant on this list are the sulfonylurea hypoglycemic agents and the active enantiomer of warfarin (S-warfarin) (147,148,174–176). The magnitude of these interactions can be significant. For example, when voriconazole was added to a stable warfarin regimen, the average increase in prothrombin time was 17 seconds in one trial (174). Careful monitoring is essential when either voriconazole or fluconazole are added to a regimen containing any of these agents.

CYP 2C19

Fluconazole and voriconazole are also the only azole agents known to inhibit CYP 2C19 (149,173). The most clinically significant interaction that has been identified via this mechanism occurs with voriconazole and omeprazole. Voriconazole can lead to elevated omeprazole concentrations if daily doses of the proton pump inhibitor exceed 40 mg (177). As a result, prescribing information for voriconazole recommends halving the daily omeprazole dose for patients receiving more than 40 mg daily when voriconazole is initiated (30).

UGT-Mediated Interactions

The majority of drug–drug interactions involving posaconazole stem from its inhibition of cytochrome P450 3A4 (149). Given the unique elimination route of this agent compared with other triazoles, posaconazole itself is relatively free of drug–drug interactions unrelated to absorption. However, some agents are able to induce the UGT enzymes responsible for posaconazole metabolism and decrease concentrations of the drug. These include the rifamycins and phenytoin, which should be used cautiously with posaconazole (38,178,179).

P-Glycoprotein–Mediated Interactions

P-glycoprotein is an ATP-dependent transport protein that is found throughout the human body and is involved in the transport of many drugs. There are significant similarities in the list of agents that are substrates and inhibitors between cytochrome P450 3A4 and p-glycoprotein (Table 4) (70). As a result, the azole antifungals can also inhibit p-glycoprotein and many, with the exception of voriconazole, are also substrates (149). Often it is difficult to differentiate the role of p-glycoprotein in azole-induced drug–drug interactions from that of CYP 3A4.

The best described pure interaction involving an azole agent and p-glycoprotein is that of itraconazole and digoxin (180,181). Itraconazole is known to increase digoxin concentrations, which is surprising given that digoxin is not a substrate for any of the cytochrome P450 enzymes (182–184). The mechanism of this interaction is p-glycoprotein inhibition by itraconazole. This is further supported by the lack of an interaction between digoxin and voriconazole, which is not a p-glycoprotein inhibitor (185).

THERAPEUTIC DRUG MONITORING

Assays to determine azole drug concentrations are available for each member of the class (186). The role of drug concentration monitoring in antifungal therapy continues to evolve; however, available data for each of the agents has established a framework for where this practice may be most useful (Table 5).

Fluconazole

Of all the azole antifungals, fluconazole achieves relatively consistent drug exposures when administered via the oral route. In addition, available susceptibility breakpoints are based in part on efficacy data from clinical trials (187). Together, these two factors have largely obviated the need for routine therapeutic drug monitoring of this agent.

Table 5 Recommendations for Therapeutic Drug Monitoring of Triazole Antifungal Agents

	Indication	Minimal time to first measurement after initiation of therapy (days)	Target trough concentration for efficacy ($\mu\text{g/mL}$)	Target trough concentration for safety ($\mu\text{g/mL}$)
Itraconazole	Routine during first week of therapy, lacking response, gastrointestinal dysfunction, drug–drug interactions	4–7	Prophylaxis: > 0.5 Treatment: > 1–2	NA
Voriconazole	Lacking response, gastrointestinal dysfunction, drug–drug interactions, children, intravenous to oral switch, severe hepatopathy, unexplained neurologic symptoms	4–7	Prophylaxis: > 0.5 Treatment: > 1–2	<6
Posaconazole	Lacking response, gastrointestinal dysfunction, therapy with proton pump inhibitors, drug–drug interactions	4–7	Prophylaxis: > 0.5 Treatment: 0.5–1.5	NA

Source: Adapted from Ref. 186.

Itraconazole

Serum drug concentration monitoring for itraconazole has become an established practice and is recommended in treatment guidelines for many fungal infections when itraconazole is used (188–190). The erratic absorption associated with each of its oral formulations underlies this practice (26,47). Initially, these concentrations were obtained solely to confirm oral absorption of the drug. However, data from studies linking success of therapy with drug concentration have been conducted and support thresholds for efficacy with many indications (187,191–196) (Table 5).

When obtaining serum drug concentrations with itraconazole, the effect of the active metabolite, hydroxyitraconazole, also needs to be considered (66). If monitoring is performed via bioassay, this activity is accounted for in the result. High performance liquid chromatography (HPLC), however, measures itraconazole and hydroxyitraconazole separately, sometimes leaving the impression that HPLC results can be 2- to 10-fold lower than those obtained by bioassay (197). The true activity of the drug can be determined, however, by adding the results for hydroxyitraconazole to those for itraconazole.

Voriconazole

Voriconazole has several qualities that make it an attractive candidate for therapeutic drug monitoring. These include its oral bioavailability that is dependent on optimal gastric conditions (31), significant inter-patient variability in pharmacokinetics (51), and that it is subject to cytochrome P450-mediated metabolism and drug–drug interactions that can decrease its systemic exposure (30,39,67). Several toxicities of voriconazole have also been linked to serum drug concentrations (123,125,126,134,198).

Voriconazole concentration monitoring data are available beginning with the earliest clinical trials for this agent (101). Clinical failures of voriconazole in patients with invasive fungal disease are often linked to low or undetectable serum drug concentrations (101,199,200). Low drug exposures can also be associated with breakthrough fungal infections in patients receiving prophylactic voriconazole (201,202).

Posaconazole

Posaconazole is also an attractive candidate for routine drug concentration monitoring as it is only available orally and is relied upon to prevent or treat severe, life-threatening infections. Other reasons to promote monitoring include variable drug exposure as a result of changes in gastric pH, fat content of food, and frequent use in patients with altered gastrointestinal integrity (32,37,137). Although limited, data are available that support a positive linear relationship between success and drug exposure in patients with aspergillosis (203). A similar experience has been noted for antifungal prophylaxis (137).

SUMMARY

The triazole antifungal agents are the most widely used antifungal drugs today. Their development has revolutionized treatment of many invasive fungal diseases. Part of their attractiveness is a perception that this drug class is relatively easy to administer and is less toxic than systemic alternatives. However, each member of this drug class has unique properties that need to be understood and considered when these therapies are being administered. Based on available data regarding the kinetics, drug–drug interactions, toxicities, and appropriate monitoring of these agents, clinicians can now further optimize therapy in complex patient populations.

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13 Echinocandins for Prevention and Treatment of Invasive Fungal Infections

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INTRODUCTION

Echinocandins are the most recent addition to the antifungal armamentarium, with a unique mechanism of action. There are currently three echinocandin antifungal agents in clinical use: caspofungin, micafungin, and anidulafungin. These agents block fungal cell wall synthesis through inhibition of β -(1,3)-D-glucan synthase, resulting in fungicidal effects against *Candida* spp. and fungistatic effects against *Aspergillus* spp. Echinocandins have also been shown to have some activity, alone or in combination with other agents against a variety of other fungal pathogens. Several clinical trials have evaluated performance of echinocandins in the setting of oropharyngeal/esophageal candidiasis, and invasive candidiasis. Others have reported experience with echinocandins as salvage therapy for invasive aspergillosis, and case reports describe efficacy of these agents in the treatment of a variety of other fungal infections. In general, echinocandins have become preferred agents in the hospital setting for invasive candidiasis because of broad activity against non-*albicans Candida* as well as *Candida albicans* infections while having an excellent safety profile and relative lack of drug interactions. Since they are available only in intravenous form, their use is limited in patients where oral antifungal therapy is preferable.

CHEMICAL STRUCTURE AND FORMULATIONS

Structurally, echinocandins are all large lipoprotein molecules containing an amphiphilic cyclic hexapeptide. All three echinocandins have a unique N-linked acyl lipid side chain, which imparts different physicochemical properties to each agent (1,2). Caspofungin was derived from *Glarea lozoyensis*, while micafungin and anidulafungin are fermentation byproducts of the fungi *Coleophoma empetri* F-11899 and *Aspergillus nidulans*, respectively (2–5). Owing to their lipophilicity and large size, echinocandins have low bioavailability and are therefore only available for parenteral (intravenous) delivery. With its fatty acid side chain, caspofungin is soluble in water. Similarly, micafungin is soluble in water due to its complex aromatic side chain. In contrast, anidulafungin is insoluble in water due to its alcoxytriphenyl side chain. Both caspofungin and anidulafungin are somewhat soluble in ethanol, but micafungin is not. Therefore, reconstitution of vials containing echinocandin must be done carefully, according to the manufacturers specific instructions (Table 1).

MECHANISM OF ACTION

Echinocandins exert their antifungal effects by inhibiting the synthesis of β -(1,3)-D-glucan, an integral component of the fungal cell wall (6). β -(1,3)-D-Glucan is a major component of the cell wall of many fungi, along with other cell wall components including β -(1,6)-D-glucan, chitin, galactomannan, and various glycoproteins. The effects of echinocandins result in decreased β -(1,3)-D-glucan production, loss of cell integrity, and eventually cell lysis (6).

SPECTRUM OF ACTIVITY

Echinocandins have antifungal activity against the most common yeasts and molds, although these agents generally lack activity as single agents against *Cryptococcus neoformans* and

Table 1 Dosing, Chemical, and Pharmacological Properties of Echinocandin Formulations (2–5)

	Caspofungin	Micafungin	Anidulafungin
Brand name	Cancidas	Mycamine	Eraxis
Relative molecular weight	1213.42	1292.26	1140.3
Origin	<i>Glarea lozoyensis</i>	<i>Coleophoma empetri</i>	<i>Aspergillus nidulans</i>
FDA-approved dose			
Adults			
Candidemia/invasive candidiasis	70 mg IV loading dose followed by 50 mg IV daily	100 mg IV daily	200 mg IV loading dose followed by 100 mg IV daily
Esophageal candidiasis	50 mg IV daily	150 mg IV daily	100 mg IV loading dose followed by 50 mg IV daily
Children			
3 mo to 17 yr	70 mg/m ² loading dose followed by 50 mg/m ² daily, up to 70 mg daily maximum		
Other FDA-approved indications	Invasive aspergillosis (intolerant/refractory to other therapies), and empirical therapy in febrile neutropenics: 70 mg loading dose followed by 50 mg IV daily	Prophylaxis in HSCT recipients: 50 mg IV daily	
Reconstitution	Any of the following: <ul style="list-style-type: none"> • 0.9% Sodium chloride Injection • Sterile water for injection • Bacteriostatic water for injection with methylparaben and propylparaben • Bacteriostatic water for injection with 0.9% benzyl alcohol 	<ul style="list-style-type: none"> • 0.9% Sodium chloride injection, USP Alternative: <ul style="list-style-type: none"> • 5% Dextrose injection 	Use only the companion diluent supplied by the manufacturer [20% (w/w) dehydrated alcohol in water for injection]
Dilution	0.9%, 0.45%, or 0.225% Sodium chloride injection or lactated ringers injection	0.9% Sodium chloride injection or 5% dextrose injection	0.9% Sodium chloride injection or 5% dextrose injection
Duration of infusion/infusion rate	1 hr	1 hr	Rate not to exceed 1.1 mg/min

Zygomycetes. Because of their mechanism of action, beta-glucan content of the specific fungus may affect activity of echinocandins against individual fungal species (7). Echinocandins have fungicidal activity against *Candida* spp. and fungistatic activity against many invasive molds (8–14) (Tables 2 and 3). The CLSI recently recommended that *Candida* spp. with minimum inhibitory concentrations (MICs) $\leq 2 \mu\text{g}/\text{mL}$ are considered susceptible to echinocandins (15). The guidelines also recommend performing the MIC determination for echinocandins after 24 hours of incubation (16). In a recent in vitro comparison of more than 5000 *Candida* isolates collected from patients with invasive *Candida* infections at 91 medical centers from 2001 to 2006, the majority of *Candida* isolates demonstrated MICs $\leq 2 \mu\text{g}/\text{mL}$ to all three echinocandins with no change in susceptibility observed over the six-year observation period (Table 2) (14). Six of the isolates tested had MIC $> 4 \mu\text{g}/\text{mL}$: *C. guilliermondii* (three isolates with caspofungin

Table 2 MIC₉₀ of 5346 Invasive *Candida* spp. from Six Years of Global Surveillance (14)

Organism (number of isolates tested)	MIC ₉₀ µg/mL		
	Caspofungin	Micafungin	Anidulafungin
<i>C. albicans</i> (2857)	0.06	0.03	0.06
<i>C. parapsilosis</i> (771)	1	2	2
<i>C. glabrata</i> (747)	0.06	0.015	0.12
<i>C. tropicalis</i> (625)	0.06	0.06	0.06
<i>C. krusei</i> (136)	0.25	0.12	0.06
<i>C. guilliermondii</i> (61)	1	1	2
<i>C. lusitanae</i> (58)	0.5	0.25	0.5
<i>C. kefir</i> (37)	0.015	0.06	0.12
<i>C. famata</i> (24)	1	1	2
<i>Candida</i> spp. (30)	0.25	0.5	1
Total (5346)	0.25	1	2

MIC \geq 8 µg/mL), *C. glabrata* (one isolate with caspofungin MIC \geq 8 µg/mL), *C. tropicalis* (one isolate with caspofungin MIC \geq 8 µg/mL), and *C. rugosa* (one isolate with anidulafungin MIC \geq 8 µg/mL).

In many studies, the MIC₉₀ for *C. parapsilosis* for all the echinocandins has been 2 µg/mL, which is at the breakpoint of susceptibility (17,18). The clinical relevance of this finding among patients with invasive *Candida* spp. infections is not known. A naturally occurring proline-to-alanine substitution in the region of *FKS1p* may explain the higher MICs observed with *C. parapsilosis* and echinocandins (19).

The in vitro activity of echinocandins against dimorphic fungi, such as *Hisptospasma capsulatum*, *Blastomyces dermatitidis*, and *C. immitis*, has been variable and it is not clear if this translates to clinical efficacy. Caspofungin MICs of 0.5 to 8 µg/mL for *B. dermatitidis* and 0.5 to 4 µg/mL for *H. capsulatum* have been reported (20). Anidulafungin MICs of 2 to 8 µg/mL have been reported for *B. dermatitidis* and 2 to 4 µg/mL for *H. capsulatum*, respectively (20).

Activity of echinocandins against *Trichosporon* spp. has not been investigated extensively, but may be insufficient; cases of breakthrough infections with this species have been reported (20–23). Activity of echinocandins against mold species has been described in several in vitro studies (10,24,25). Minimum effective concentration (MEC) is the lowest concentration of drug that produces growth of small, rounded, compact hyphal forms of the organism and has shown to have better reproducibility than MIC testing for echinocandins against molds (26). MECs have not been correlated with clinical outcome in studies to date, and breakpoints for susceptibility or resistance have not been approved by CLSI for in vitro testing of mold species. MECs for most echinocandins against *Aspergillus* spp. are generally \leq 1 µg/mL. In one study, *A. versicolor* was found to have slightly higher MICs to caspofungin (MIC₉₀ = 0.12 µg/mL, range: 0.015–4 µg/mL) and approximately 90% of strains were inhibited at an MEC of \leq 1 µg/mL (10). In other studies, anidulafungin and micafungin have been found to have similar in vitro activity against *Aspergillus* spp. with MIC₉₀s of $<$ 0.03 µg/mL (24,25). Other molds including *Scedosporium apiospermum*, *S. prolificans*, *Exophiala jeanselmei*, and *Fonsecaea pedrosoi* may be inhibited by echinocandins, but extensive incubation periods have sometimes been used to demonstrate this activity (27). Appreciable in vitro activity of echinocandins against *C. neoformans*, *Fusarium* spp., *Rhizopus* spp., or *Mucor* spp. as single agents has not been demonstrated (28).

Table 3 Comparative MEC Values for *Aspergillus* spp. (40)

Organism (number of isolates tested)	Median (range) MEC µg/mL		
	Caspofungin	Micafungin	Anidulafungin
<i>A. fumigatus</i> (11)	1 (0.5–1)	0.06 (0.06–0.12)	0.03 (0.015–0.03)
<i>A. terreus</i> (8)	0.5 (0.5–1)	0.12 (0.06–0.12)	0.03 (0.015–0.03)
<i>A. flavus</i> (8)	1 (0.5–1)	0.12 (0.03–0.12)	0.03 (0.03–0.06)

MECHANISM OF RESISTANCE

Recent reports have documented isolates with higher MICs to echinocandins, and mechanisms by which these isolates may be echinocandin resistant. Generally, *Candida* isolates with higher MICs in patients failing caspofungin, micafungin, or anidulafungin therapy have had mutations in the *FKS1* and *FKS2* genes (29–35). A recent investigation also highlighted apparent differences in susceptibility between echinocandins among *C. parapsilosis* in an outbreak situation in a burn unit (36). However, these differences in susceptibility were not due to differences in *FKS1* gene mutations. In another study, isolates from other patients failing caspofungin with MICs of 0.5 to 1 $\mu\text{g}/\text{mL}$ were found to have upregulation of cell wall integrity pathway genes and increases in the minimum fungicidal activity without changes in *FKS* genes (37). There are other reports of clinical failures due to isolates with reduced susceptibility to echinocandins, suggesting that other unknown mechanisms may also contribute to echinocandin resistance (38).

A paradoxical effect of fungal organisms regrowth at high echinocandin concentrations has been reported in several *in vitro* investigations, and further complicates the study of echinocandin resistance (39). In the presence of high concentrations of echinocandin, it has been suggested that resistance mechanisms among *Candida* and *Aspergillus* spp. are derepressed. The majority of reports have occurred with caspofungin, but one recent paper also observed these effects with micafungin and anidulafungin against certain isolates (39–44). These concentrations are much higher than those typically achieved with human doses, and notably these *in vitro* findings have not been consistently replicated in animal models (45). This effect was also not apparent in clinical studies of invasive candidiasis (42,46–48). Based on these data, additional studies are needed to further elucidate the clinical and microbiologic significance of the paradoxical effect reported with echinocandins.

PHARMACOKINETICS

The pharmacokinetics of echinocandins has been described in both animal models and humans. All present echinocandins have limited oral bioavailability. Thus, they are only available as a therapeutic solution for parenteral administration.

Caspofungin

Caspofungin is extensively bound to albumin in serum (~97%), with rapid distribution into tissues after intravenous dosing in humans (3,49,50). It has a relatively low volume of distribution (9.67 L after a single 70 mg dose in healthy adults) (49). Caspofungin exhibits triphasic elimination from plasma, with a short α -phase following infusion ($t_{1/2}$ 1–2 hours), and then a β -phase 6 hours to 48 hours post-dose ($t_{1/2}$ 9–11 hours) that accounts for most of the plasma clearance. The third or γ -phase has a half-life of 40 to 50 hours (49). Administration of a 70-mg loading dose followed by 50 mg intravenously typically results in serum concentrations exceeding 1 $\mu\text{g}/\text{mL}$.

Caspofungin is metabolized to inactive metabolites by hydrolysis and N-acetylation (51). It is also spontaneously degraded to a ring-opened peptide compound called L-747969 (3). Inactive caspofungin metabolites are then excreted in urine (41%) and feces (35%) (51,52). However, a very small amount of active, unchanged drug is excreted in bile (<1%) or urine (1.4%). It is important to note that urine caspofungin concentrations are minimal, and since it lacks active metabolites its clinical utility in treating urinary infections with *Candida* spp. might be compromised. However, it has been effective in isolated cases of candiduria (53).

Results of four different pharmacokinetic studies using various doses of caspofungin on a single- and multiple-dose basis were published in one paper (49). In one study, pharmacokinetics of caspofungin was compared for healthy men receiving 50 mg IV daily or a loading dose of 70 mg IV followed by 50 mg daily IV for 14 days total. Serum concentrations at the end of a one-hour infusion and at 24 hours were higher for the loading dose group on day one ($C_{1\text{h}}$ 7.64 $\mu\text{g}/\text{mL}$ and 12.09 $\mu\text{g}/\text{mL}$, $C_{24\text{h}}$ 0.76 and 1.41 $\mu\text{g}/\text{mL}$), and this finding indicated that a loading dose is necessary to achieve an inhibitory target concentration over 24 hours of 1 $\mu\text{g}/\text{mL}$ which is predicted to be effective against most *Candida* species. After 14 days of dosing, the differences between those who had received a loading dose and the nonloading dose group were less pronounced, with $C_{1\text{h}}$ approximately 9 $\mu\text{g}/\text{mL}$ and $C_{24\text{h}}$ approximately 1.7 $\mu\text{g}/\text{mL}$. Caspofungin doses of 70 mg daily for 14 or 21 days resulted in higher serum concentrations

at the end of infusion (approximately 15 $\mu\text{g}/\text{mL}$) and higher concentrations 24 hours after the dose (approximately 2.5 $\mu\text{g}/\text{mL}$) (49). Day 21 to Day 14 ratios for $\text{AUC}_{0-24\text{h}}$, $C_{1\text{h}}$, and $C_{24\text{h}}$ indicated that there was also accumulation of serum concentrations between 14 and 21 days of dosing. This suggests that steady-state was not reached by day 14, but subjects were approaching steady-state by the third week of dosing (49).

Micafungin

Like other echinocandins, micafungin is extensively bound to proteins in serum, primarily albumin and to a lesser extent alpha acid glycoprotein. In animals, micafungin has also been shown to bind high-density lipoprotein (HDL) and gamma globulin (54). Following doses of 50 to 150 mg/day, micafungin exhibits linear pharmacokinetics with dose-proportional increases in serum concentrations. Single doses of 100 or 150 mg result in trough concentrations of 2 and 2.5 $\mu\text{g}/\text{mL}$, respectively. Steady-state concentrations typically are achieved after four days of intravenous dosing. Micafungin undergoes ring opening and liver metabolism to three metabolites: M1, M2, and M5. M1 undergoes arylsulfatase transformation to a catechol form, which is then transformed by catechol-*o*-methyltransferase to a methoxy form (M2). M5 is a minor metabolite, and is the result of CYP450 hydroxylation of micafungin's side chain. Like other echinocandins, <1% of unchanged drug is excreted in urine. Fecal excretion is the primary route of elimination, with 71% of the dose eliminated as parent drug and metabolites 28 days after dosing.

A population pharmacokinetic analysis based on a range of doses from 12.5 to 200 mg micafungin daily in adult hematopoietic stem cell transplant recipients (HSCT) suggested that body weight significantly impacted micafungin AUC (55). The investigators estimated that patients weighing ≥ 66.3 kg or < 66.3 kg would have $\text{AUC}_{0-24\text{h}}$ of 81 mg \cdot h/L or 121 mg \cdot h/L, respectively. Doses of 150 mg daily would be necessary in those weighing ≥ 66.3 kg to achieve exposures similar to that observed after 100 mg doses among lower-weight adults. The clinical significance of these weight-based differences in pharmacokinetics among adults has not been established.

Anidulafungin

Anidulafungin is highly protein bound (>99%) and has a larger apparent volume of distribution (30–50 L) and somewhat longer half-life (26–40 hours) than the other echinocandins (Table 4). This echinocandin is also unique in that it is not metabolized in the liver, but rather undergoes chemical degradation to inactive open-ring products. Less than 10% of the drug is excreted unchanged in feces (56).

Doses of 100 mg once daily, following a loading dose of 200 mg intravenously result in trough concentrations of 2.5 $\mu\text{g}/\text{mL}$ on day 1. Population pharmacokinetics have been

Table 4 Comparative Pharmacokinetics of Echinocandins in Adults (64,71,132–135)

	Caspofungin	Micafungin	Anidulafungin(69)
Steady state C_{max} (range) ($\mu\text{g}/\text{mL}$)	12 (11–13)	10 (SD \pm 4.4)	9 (% CV 16)
Steady state AUC_{0-24} ($\mu\text{g} \cdot \text{h}/\text{mL}$)	101 (range 88–115)	97 (SD \pm 29)	112 (% CV 24.9)
β for half-life (h)	11 (SD 1.1)	11–17	36(69)– 52 (%CV 12)
Clearance (mL/min)	10–13	11	0.94–0.99 L/h (SD 0.1)
V_d (L/kg)	0.15	0.2	0.5
Half-life in hepatic impairment (h \pm SD)	Prolonged	14 (\pm 0.8)	Mild: 34 (\pm 2.5) Moderate: 42 (\pm 8.6) Severe: 35 (\pm 7)
Half-life in severe renal impairment (h \pm SD)	NA	14 (\pm 1.5)	33–39 (\pm 5–7)
Protein binding	96	99.8	>99%
Urine concentration (% of plasma)	1.4%	<1%	<1%
CSF concentration	Low	Low	Low

determined for anidulafungin on the basis of combined data from four Phase II/III clinical trials receiving 50 to 100 mg of anidulafungin daily (57,58).

Overall, pharmacokinetic parameters in infected patients were similar to that previously reported among healthy volunteers. Drug clearance was faster among males and those with increased body weight, but only explained a small proportion (20%) of intersubject variability observed with anidulafungin. In addition, both males and females with invasive candidiasis appeared to have approximately 30% faster clearance than subjects with oropharyngeal/esophageal candidiasis or invasive aspergillosis. Body weight also explained a small amount of variability in volume of distribution observed among study subjects, but overall body weight is not currently recommended as a consideration when dosing anidulafungin in adults.

Tissue Concentrations

In animal models, echinocandins had the highest ratio of tissue-to-plasma concentrations in the liver, lungs, and kidneys. After intraperitoneal administration in murine models (50), caspofungin had the highest ratio of tissue-to-plasma concentrations in the liver (16), followed by kidneys (2.9), large intestine (2), small intestine (1.3), lungs (1.1), and spleen (1). In this model, caspofungin had little penetration into heart (0.3), thigh (0.2), and brain (0.1) tissue.

In rats, micafungin had the highest concentrations in lung, kidney, and liver with tissue:plasma ratios of 3.6, 3.2, and 7.8, respectively, after 1 mg/kg of nonradiolabeled drug (59). Micafungin was shown to achieve concentrations in the retina-choroid similar to plasma in rabbits, but the drug could not be detected in vitreous humor (60). In a rabbit model of *C. albicans* hematogenous meningoencephalitis, micafungin was found in highest concentrations in the meninges and choroid, but had low and variable concentrations in the CSF (61). This suggested that high doses may be necessary to achieve therapeutic concentrations in the CNS (61). CSF concentrations of micafungin were measured clinically in a patient receiving treatment for CNS aspergillosis. CSF:plasma concentration ratio was 0.2% to 0.05%, despite a dose of 300 mg daily. The patient responded clinically, however, suggesting that tissue concentrations rather than CSF may be an important factor in treating these infections.

Administration of a single intravenous dose of 5 mg/kg of radiolabeled anidulafungin in rats resulted in the highest tissue-to-plasma concentrations in liver (12.4), lung (10.4), kidney (10.7), and spleen (9.2) (62). Anidulafungin had little to no measurable concentrations in cerebrospinal fluid, brain tissue, or the eye.

PHARMACOKINETICS IN SPECIAL POPULATIONS

The pharmacokinetics of echinocandins have also been reported among special populations including elderly patients (63,64), women (65), adults with renal or hepatic insufficiency, intensive care unit (ICU) patients, and children (66).

Elderly

Pharmacokinetics of caspofungin and micafungin has been studied in elderly subjects, and were similar to nonelderly adults (63,64). With caspofungin dosing, six elderly men and six elderly women (67–77 years of age) with creatinine clearances ≥ 60 mL/min had slightly higher

Table 5 Comparative Pharmacokinetics of Echinocandins in Older Children (Single Dose Administration)

	Echinocandin		
	Caspofungin	Micafungin	Anidulafungin
Age range	2–11 yr	3–17 yr	2–17 yr
Dose	50 mg/m ²	2 mg/kg	0.75 mg/kg
C _{max} (μg/mL)	13	15	4
AUC _{0–24 h} (μg · h/mL)	96	83	48
t _{1/2 β} (h)	8	13	21
Plasma clearance (L/h/kg)	0.016	0.02	0.018
Vd _{ss} (L/kg)	N/A	0.31	0.45

Source: Adapted from Ref. 80.

serum concentrations of caspofungin at one-hour and 24-hours after infusion compared to young men (aged 24–44 years) (63). Mean $AUC_{0-24\text{ h}}$ was higher among elderly men (28% greater) and women (18% greater), but these differences were not statistically significant. With micafungin, pharmacokinetics among 10 elderly (66–78 years of age) volunteers was similar to 10 young men (20–24 years of age) receiving single intravenous doses of micafungin 50 mg (64). Pharmacokinetics of anidulafungin has not been specifically reported in elderly patients.

Women

Serum concentrations among women receiving echinocandins may be relatively higher than that among men, but no dosage adjustments are currently recommended on the basis of gender (3–5). Mean $AUC_{0-24\text{ h}}$ at day 14 was increased a mean of 22% among healthy women when compared to healthy men receiving caspofungin (70 mg IV loading dose, followed by 50 mg IV daily for a total of 14 days) (65). AUC was increased by 23% in women compared to men after 14 days of 150 mg micafungin daily, but this difference in serum concentrations was attributed to lower body weight among women (5). In a population pharmacokinetic model based on serum concentrations obtained from participants in Phase II/III studies with anidulafungin, gender impacted clearance of anidulafungin but in concert with body weight and a diagnosis of invasive candidiasis, explained less than 20% of intersubject pharmacokinetic variability (4).

Renal Insufficiency

Dosage adjustment of echinocandins is not recommended for those with renal insufficiency or those requiring hemodialysis (3). Plasma concentrations of caspofungin among patients with mild- to end-stage renal disease receiving 50 mg daily for treatment of invasive aspergillosis or candidiasis were similar to that among patients with no renal impairment (3). In a single dose study of 70 mg, caspofungin serum concentrations were moderately higher (AUC 30–49% increased) among those with moderate, advanced, or end-stage renal insufficiency. However, dosage adjustments are not recommended for those with renal insufficiency receiving caspofungin. Hemodialysis does not remove caspofungin, so dose supplementation is not necessary in patients requiring dialysis. There are clinical data but no published pharmacokinetic data from patients requiring peritoneal dialysis; however, continuous ambulatory peritoneal is not likely to affect plasma concentrations of caspofungin. Renal impairment ($\text{CrCl} < 30\text{ mL/min}$) also did not affect the pharmacokinetics of micafungin after single 100 mg doses (67). Continuous hemodiafiltration does not increase the elimination of micafungin (68). Anidulafungin pharmacokinetics among 26 subjects with renal impairment or end-stage renal disease were similar to eight subjects with normal renal function following single intravenous doses of 50 mg anidulafungin (69). Pharmacokinetics of anidulafungin was not affected in those undergoing hemodialysis, whether anidulafungin was given before or after a dialysis session (69).

Hepatic Insufficiency

Hepatic insufficiency appears to reduce elimination of caspofungin and micafungin, while plasma concentrations of anidulafungin are decreased in those with severe hepatic impairment receiving anidulafungin (3,70). Dose reduction should be considered when administering caspofungin to patients with moderate hepatic insufficiency (3). In a single dose study of 70 mg, those with Child Pugh scores of 5 to 6 experienced an increase in AUC of 55% compared to historical healthy controls, while those with Child Pugh scores of 7 to 9 experienced a mean AUC increase of 76% (52). These differences were not as pronounced with daily caspofungin doses of 50 mg, where subjects with mild hepatic insufficiency experienced increases in AUC of approximately 19% to 25% compared to healthy controls. In this study, subjects with moderate hepatic impairment received 35 mg daily, following the 70 mg loading dose. Patients with severe liver dysfunction (Child Pugh score > 9) were excluded from clinical trials with caspofungin, so there is limited clinical experience with the drug in this severely ill patient population.

Single 100 mg doses of micafungin have been administered to subjects with Child Pugh scores of 7 to 9, who experienced 22% lower values for C_{max} and AUC compared to age-, gender-, and weight-matched individuals with no hepatic impairment (67). Pharmacokinetics of micafungin in those with severe hepatic impairment was described among nine liver transplant recipients. A graft recipient with small-for-size graft had substantially higher AUC

and elimination half-life after micafungin doses of 100 mg, and the authors suggest that lower doses of 50 mg daily may be appropriate for such patients (71).

Pharmacokinetics of anidulafungin among subjects with mild-to-moderate hepatic dysfunction was similar to healthy controls receiving single 50 mg doses of anidulafungin. Peak serum concentration and AUC were 36% and 33% lower, respectively, in those with severe hepatic impairment (Child Pugh score 10–15) compared to healthy controls. Clearance was increased by 57% in those with severe hepatic impairment, with 78% increase in volume of distribution that could be potentially attributed to ascites and edema. Since concentrations remained above the MIC for most relevant organisms, the authors suggest that these differences in pharmacokinetic parameters among subjects with severe hepatic failure are probably not clinically relevant.

Critically Ill Patients in the Intensive Care Unit

In a recent study of 40 Surgical Intensive Care Unit patients, caspofungin doses of 50 mg daily were associated with higher concentrations 24-hours post-dose than that previously reported among healthy volunteers (mean C_{24} 2.16 $\mu\text{g/mL}$ vs. 1.41 $\mu\text{g/mL}$, respectively) (72). Serum concentrations were further reduced with increasing body weight (>75 kg), but were increased among those with albumin concentrations >23.6 g/L. Of the subjects studied, 75% were within the target concentration range of 1–3 $\mu\text{g/mL}$ for the duration of treatment. Of those with subtherapeutic concentrations, 10 of 17 weighed more than 75 kg, suggesting that weight may be an important covariate in caspofungin serum concentrations among critically ill adults.

Pharmacokinetics of micafungin was determined in nine Japanese ICU patients receiving doses of 150 mg to 300 mg daily (68). In this study, clearance of micafungin appeared to be greater than that previously reported among healthy volunteers (1.4 L/hr vs. 0.7 L/hr, respectively). The investigators suggest that this increased clearance observed in the ICU setting may be attributed to decreased albumin and total protein concentrations in serum of these critically ill patients, which may lead to increases in free drug available for clearance.

Pharmacokinetics of anidulafungin has not been specifically studied to date in ICU patients.

Older Children

Children have been the focus of several recent investigations of echinocandin pharmacokinetics. In general, clearance of echinocandins is increased in children relative to adults. In a small study of caspofungin prophylaxis in children and adolescents with fever and neutropenia, $\text{AUC}_{0-24\text{ h}}$ among children receiving 1 mg/kg daily doses was 46% lower than that of adults receiving 50 mg daily (66). The β -phase elimination half-life was similarly reduced by approximately 32% to 43% in these children compared to adults. These effects appeared to be more pronounced among younger, smaller subjects than older, larger subjects, so increases in dosage may be especially important for younger children. On the basis of these data, the authors suggest that a dose of 50 mg/m² in children and adolescents 2 to 17 years of age may more closely approximate pharmacokinetic parameters achieved in adults. Micafungin clearance was also increased in children relative to adults (73). Children 2 to 12 years of age receiving micafungin doses of 0.5 to 4 mg/kg/day or 13 to 17 years of age receiving 0.5 to 1.5 mg/kg/day for neutropenic fever experienced linear pharmacokinetics, with 1.5 to 2 times faster clearance among children/adolescents less than eight years of age and 1.3 to 1.5 times faster than that reported for adult patients. Additional studies have described pharmacokinetics of micafungin in Japanese children at dosages of 1–6 mg/kg (73,74). Anidulafungin pharmacokinetics was found to be similar among older children (2–17 years of age) receiving 0.75 mg/kg/day or 1.5 mg/kg/day in the setting of neutropenia. Concentrations achieved at these dosages approximated that of adults receiving 50 or 100 mg of anidulafungin daily (75). Body weight, but not age, was associated with variability in clearance and volume of distribution in this patient population.

Neonates

Among the echinocandins, micafungin has been studied most extensively in preterm infants. Clearance of micafungin among neonates appears to be substantially greater than that in older children and adults (61,76,77). Doses of 9 mg/kg in neonates have been estimated to yield

exposures similar to 150 mg daily in adults and 2 mg/kg in older children (61). Doses of 15 mg/kg/day in preterm (median 27 weeks of age, 775 grams at birth) yielded concentrations similar to 5 mg/kg/day in adults (76). A recent small multicenter study reported pharmacokinetics following caspofungin doses of 25 mg/m² among 18 infants less than three months of age with documented or suspected invasive candidiasis (78). Pharmacokinetics in neonates receiving this dose was similar to adults receiving 50 mg caspofungin daily for invasive candidiasis.

PHARMACODYNAMICS

The pharmacodynamics of echinocandins has been extensively evaluated in in vitro studies and animal models of infection. As mentioned previously, a paradoxical effect has been reported in vitro with some isolates, but this phenomenon has not been validated in the clinical setting. Post-antifungal effects have been reported for echinocandins against *Candida* infections, but not against *Aspergillus* (79,80). In animal models of invasive candidiasis, $C_{max}:MIC$ and the $AUC_{0-24}:MIC$ ratios were most predictive of echinocandin activity (81–84). In these studies, $C_{max}:MIC$ ratio >10 or $AUC_{0-24}:MIC$ >250 has been suggestive of efficacy. Likewise, in animal models of invasive pulmonary aspergillosis, the C_{max}/MIC ratio for caspofungin or $C_{max}:MEC$ ratio for micafungin was best correlated with efficacy (81,85). More recently, clinical experience has been published, and supports the hypotheses generated in laboratory investigations that the echinocandins exhibit concentration-dependent killing. A study in Japanese patients with candidiasis or aspergillosis has suggested that a $C_{max} > 5 \mu\text{g/mL}$ after doses of 50 mg or more of micafungin was associated with efficacy (86). A later study suggested that higher concentrations ($C_{min} > 5 \mu\text{g/mL}$) were necessary for optimal outcome in managing invasive aspergillosis (87). For anidulafungin, based upon data from Phase II and Phase III studies, a steady-state AUC of more than 35 mg · hr/L, a steady-state serum concentration of more than 1.5 mg/L, and trough concentration >1 mg/L have been suggestive of efficacy in oropharyngeal/esophageal candidiasis (88). These concentrations are readily achievable following daily doses of 50 mg.

SAFETY

Overall, echinocandins have been well tolerated with few serious side effects. In a summary evaluation of several caspofungin clinical trials, the most commonly reported drug-related adverse events were fever (12–26%) and infusion-related phlebitis (12–18%) (89). The most common laboratory abnormalities reported were increased liver transaminases, alkaline phosphatase, and decreased hemoglobin and hematocrit concentrations, although these rates were similar to those among comparators. There have been isolated reports of histamine-related symptoms with caspofungin; however, these reports are rare (90,91). In addition, there have been rare reports of hepatotoxicity in patients receiving caspofungin and cyclosporine concomitantly, but the contribution of caspofungin was uncertain (92). However, the benefit of the caspofungin therapy should be weighed against the risks carefully in those receiving cyclosporine, and liver function tests should be monitored (3).

Since micafungin and anidulafungin were FDA-approved later than caspofungin, safety experience with these agents is somewhat less, but is increasing. Micafungin has been approved for use in Japan for several years, and has a large body of evidence in Japanese literature. In a dose escalation study of 74 patients receiving doses of micafungin up to 200 mg/day, who were undergoing peripheral blood or stem cell transplantation, the most common adverse events were rash, headache, arthralgia, and hypophosphatemia (93). In clinical trials with micafungin, the most common adverse events were nausea (2.8%), vomiting (2.4%), increased AST (2.7%), increased ALT (2.6%), and increased alkaline phosphatase (2.0%) (5).

In clinical trials of patients receiving anidulafungin for the treatment of candidemia, the most frequent adverse events were diarrhea (3.1%), hypokalemia (3.1%), and increased ALT (2.3%), which were not different than that seen with the comparator (94). There have been several reports of infusion-related toxicities including hypotension and flushing with anidulafungin (4,95). However, the overall incidence of infusion-related reactions can be minimized by infusing anidulafungin at a rate not to exceed 1.1 mg/min (4).

DOSING AND ADMINISTRATION

Dosing of the echinocandins differs among the agents and according to indication for use (3–5). FDA-approved recommended doses for each agent are summarized in Table 1. In addition to these doses, the prescribing information for some countries recommend doses of caspofungin of 70 mg IV daily throughout the course of therapy for adult patients weighing more than 80 kg (96). A recent study in adults with candidemia/invasive candidiasis also investigated higher doses of caspofungin (150 mg daily), and found no differences in safety compared to standard dosages (47). Dosages of caspofungin may need to be increased to 70 mg daily (or 70 mg/m² in older children) in the setting of invasive aspergillosis when patients have not responded to 50 mg (or 50 mg/m²) daily, or in those receiving inducers of drug clearance such as rifampin, nevirapine, efavirenz, carbamazepine, dexamethasone, or phenytoin (see drug interactions below). Dose reductions of caspofungin to 35 mg daily are recommended for adults with moderate hepatic insufficiency (Child Pugh score 7–9). Based on pharmacokinetic data, caspofungin doses of 25 mg/m² for children less than three months of age can be considered, but are not FDA approved for this use.

Among HSCT recipients, doses as high as 8 mg/kg/day (maximum 896 mg) of micafungin have been tolerated (93). However, micafungin is typically administered in doses of 50 to 150 mg daily for adults based on clinical indication (Table 1). There are no FDA-approved doses for children, but on the basis of pharmacokinetic data, doses of 4 to 8 mg/kg/day can be considered for children 2 to 8 years of age. Weight-based dosing of 1 to 3 mg/kg/day is probably sufficient for children 9 to 17 years of age to achieve concentrations similar to that among adults. In clinical studies, dosages of 2.1 (mean, \pm 1.25) mg/kg/day and 2 mg/kg/day have been administered for treatment of IA and candidiasis, respectively (97,98). In one study, dose escalation was permitted and as much as 325 mg daily (8.6 mg/kg/day) was administered to a child less than 16 years of age (97). Dose optimization is still being determined for neonates, but up to 15 mg/kg/day has been given safely for up to five days in neonates >48 hours of life and infants less than three months of age (76). Micafungin dosages do not need to be adjusted for drug interactions (see Drug Interactions below).

Anidulafungin is typically given as a 100 or 200 mg loading dose, followed by 50 or 100 mg daily for esophagitis or candidemia/invasive candidiasis, respectively (4). In clinical trials, a dose as high as 400 mg was administered on one occasion without adverse event in one patient, and in healthy subjects doses of 130 mg daily following a 260 mg loading dose were well tolerated (4). Among children, a dose of 1.5 mg/kg/day of anidulafungin can be considered on the basis of pharmacokinetics studies, but anidulafungin is not FDA approved for this use.

DRUG INTERACTIONS

Drug interactions with all three echinocandins are fairly limited. Caspofungin is not an inhibitor, inducer, or substrate for any of the cytochrome P450 isoenzymes, nor a substrate for P-glycoprotein. However, when caspofungin is coadministered with cyclosporine, a 35% increase in the AUC of caspofungin, without a concomitant increase in the AUC of cyclosporine has been observed (3). In addition, the AUC and C_{max} of tacrolimus is reduced by 20% and 16% respectively when coadministered with caspofungin (3). However, in patients receiving caspofungin concomitantly with cyclosporine and/or tacrolimus in the clinical setting, the need for dose adjustments of immunosuppressants is not common (3,99). Coadministration of caspofungin with rifampin also results in a 30% decrease in the caspofungin trough concentrations and an increase in caspofungin dose to 70 mg/day is recommended (3). The mechanism of the drug–drug interactions is unclear; however, this may be due to an overexpression of organic anion transporter proteins (OATP) responsible for hepatic uptake (100).

Although micafungin is a substrate for CYP3A4, this is a minor pathway of metabolism and inhibitors and inducers of this isoenzyme system do not affect the pharmacokinetics of either drug. However, sirolimus coadministration with micafungin resulted in a 21% increase in the AUC of sirolimus and nifedipine AUC was increased by 18% with an increase of 42% of the C_{max} with coadministration of micafungin (5). It is thus recommended to monitor for nifedipine and sirolimus toxicity if coadministration is necessary.

Anidulafungin has been evaluated in several drug interaction studies to evaluate the effects of CYP450 isoenzymes inducers, inhibitors, and substrates on the metabolism of

anidulafungin. Anidulafungin is not an inducer, inhibitor, or substrate for any of the common isoenzymes, and clinically relevant drug–drug interactions were not present between anidulafungin and cyclosporine, tacrolimus, liposomal amphotericin B, voriconazole, or rifampin (4).

CLINICAL EFFICACY

Clinical efficacy of all three echinocandins has been well established in clinical trials for oropharyngeal/esophageal candidiasis and treatment of candidemia/invasive candidiasis (17,48,94). All of these clinical trials, however, excluded subjects with meningitis, endocarditis, and osteomyelitis. Sufficient penetration of echinocandins into these spaces at concentrations required for clinical efficacy has been questioned. Some success has been reported with echinocandins for these indications (alone or in combination), but clinical failures have also been observed (101–105). None of the echinocandins has received FDA approval for such deeply invasive *Candida* spp. infections. In addition to efficacy in management of invasive candidiasis, the individual agents have demonstrated activity as salvage therapy for invasive aspergillosis (caspofungin), as empirical therapy in those with neutropenic fever (caspofungin), and prophylaxis in HSCT recipients (micafungin). Additional experience is evolving with micafungin for invasive aspergillosis (97,106), and for all candins as combination agents in the management of invasive fungal infections.

CASPOFUNGIN

Oropharyngeal/Esophageal Candidiasis

Efficacy of caspofungin has been demonstrated in the management of esophageal candidiasis in several clinical trials that involved primarily HIV-infected patients. In all of these trials, *C. albicans* was the most common pathogen isolated. Favorable responses in subjects with esophageal candidiasis at the end of IV therapy (14 days) with 50 or 70 mg of caspofungin daily were 85% and 96%, respectively. For the 70 mg caspofungin group, this response rate was significantly greater at two weeks after therapy than the comparison group receiving intravenous amphotericin B 0.5 mg/kg daily [89% vs. 63%, mean difference 26% (95% CI = 4–50%)] (107). In a dose ranging study, favorable responses 3 to 4 days after 7 to 14 days of 35, 50, or 70 mg of caspofungin were observed in more than 70% of patients with oropharyngeal/esophageal candidiasis. Favorable responses were observed in 63% of subjects randomized to 0.5 mg/kg/day of amphotericin B, but these differences were not statistically significant (108). In both these studies, fewer subjects receiving caspofungin experienced drug-related adverse events than those receiving amphotericin B. Caspofungin (50 mg daily) was also compared to fluconazole (200 mg daily) in HIV patients with esophageal candidiasis (109). The overall clinical response was 90% for the caspofungin-treated patients and 89% for the fluconazole-treated patients, with a median time to symptom resolution of 4 to 5 days in both groups. However, relapse rates four weeks after treatment were numerically higher in caspofungin-treated patients than in those who had received fluconazole, but this was not statistically significant (28% vs. 17%, respectively; difference = 12%; 95% CI = –5% to 29%, $p = 0.19$).

In a pooled analysis of data from four phase II/III clinical trials including 115 evaluable patients who received caspofungin for esophagitis, relapse was experienced by 17% of patients (110). In a multivariate analysis, only severe symptoms and extensive esophageal disease by endoscopic assessment at baseline were predictive of relapse at 14 days after the end of therapy. Duration of antifungal therapy (range 7–21 days) with caspofungin was not predictive of relapse.

Candidemia/Invasive Candidiasis

Caspofungin's efficacy in the management of candidemia/invasive candidiasis has been demonstrated in a large randomized, double-blind clinical trial of 239 patients (17). Caspofungin (50 mg daily following a 70 mg loading dose) or AmBd (0.6–0.7 mg/kg) were administered for at least 10 days, followed by oral fluconazole (400 mg daily), for a total of 14 days after the last positive culture for *Candida* spp. In the intent to treat population, the overall response rate was 73.4% versus 61.7% (difference = 12.7%, 95.6% CI = –0.7% to 26%, $p = 0.09$) for caspofungin- versus amphotericin B-treated patients, respectively. The difference in the treatment groups increased to 15.4% (95.6% CI = 1.1–29.7, $p = 0.03$) among clinically evaluable patients.

Caspofungin (50 mg daily, following a 70 mg loading dose) was also compared to two different doses of micafungin (100 and 150 mg) for candidemia/invasive candidiasis in a recent double-blind, controlled clinical trial of 595 subjects (48). Treatment success at the end of IV therapy was experienced by 72.3% of subjects receiving caspofungin, which was similar to the success rates among those receiving micafungin 100 or 150 mg (76.4% and 71.4%, respectively) (see Micafungin efficacy). Relapse, mortality, and treatment-related adverse rates were similar between the treatment groups. Treatment-related adverse events were experienced by approximately 23% of patients in the study, and most commonly included liver transaminase elevations, gastrointestinal disturbances, hypokalemia, and rash.

Another recent study investigated the safety and efficacy of high-dose (150 mg daily) caspofungin compared to standard caspofungin dosages (50 mg daily after a 70 mg loading dose) in adults with candidemia/invasive candidiasis (47). This study was designed primarily to assess safety, but efficacy was a secondary objective. At the end of caspofungin therapy, favorable overall response was experienced by 71.6% and 77.9% of patients, who received standard- and high-dose caspofungin, respectively. This difference was not statistically significant (6.3%; 95% CI = -5.9% to 18.4%). Numerical trends for fewer relapses in the high-dose arm were apparent at two and eight weeks after treatment discontinuation, but the numbers of cases were small and these differences were not statistically significant. Rates of significant or serious drug-related adverse events (2–3%) were similar between treatment groups, and overall adverse event rates were similar to rates from other published studies with caspofungin at approximately 20%.

Aspergillosis

Caspofungin has been investigated as a single agent and as part of combination antifungal therapy for invasive aspergillosis. In a multicenter, open label, noncomparative clinical trial, caspofungin (50 mg daily following a 70 mg loading dose) was administered to 48 patients with proven or probable aspergillosis (111). Most patients (75%) had pulmonary infections refractory (90%) or intolerant (10.4%) to initial therapy. Among those with pulmonary disease, response was experienced by 53%. Among those with extrapulmonary disease, there was only an 18% response, which probably reflects the challenge of treating disseminated aspergillosis.

Caspofungin has also been studied in combination with other antifungal agents in the management of invasive aspergillosis (112,113). In a small, single-center open label study, patients with pulmonary aspergillosis who were failing therapy with amphotericin B received either voriconazole ($n = 31$) or voriconazole in combination with caspofungin ($n = 16$) (112). Combination therapy was associated with improved survival relative to a historical cohort of patients who received voriconazole alone (HR = 0.28, 95% CI = 0.28 to 0.92, $p = 0.01$). In another study, the combination of caspofungin and voriconazole was used as initial therapy for invasive aspergillosis in a prospective, multicenter cohort of solid-organ transplant recipients ($n = 40$) and compared to a historical cohort of patients ($n = 47$), who had received a lipid formulation of amphotericin B (LFAB) as primary therapy. Pulmonary infection was most common among both cases and controls (92.5% and 87.2%, respectively). Treatment success was 70% among those receiving the combination of caspofungin plus voriconazole compared to 51% among those who received LFAB, but this difference was not statistically significant ($p = 0.08$). Other smaller studies have been performed and suggest that echinocandin combinations may be promising, but conclusive evidence of synergistic effects in combination with other agents for invasive aspergillosis remains to be shown (114–116).

Empiric Treatment of IFI in Febrile Neutropenia

Caspofungin has also demonstrated efficacy as empirical antifungal therapy in patients with neutropenia and persistent fever despite ≥ 96 hours of broad spectrum antibacterials. In a double-blind randomized trial, 1123 subjects received either caspofungin (70 mg loading dose, followed by 50 mg/day) or liposomal amphotericin B (L-AmB, 3 mg/kg daily). Overall clinical response based on a composite endpoint (successful treatment of any baseline fungal infection, absence of breakthrough fungal infection, survival for at least 7 days after the end of therapy, no discontinuation of drug due to treatment-related adverse event or lack of efficacy, and resolution of fever $< 38^{\circ}\text{C}$ for ≥ 48 hours) was similar between treatment groups: 33.9% and 33.7% of the caspofungin- and liposomal amphotericin B-treated patients, respectively (difference 0.2%,

95.2% CI = -5.6% to 6%). Caspofungin was associated with a higher rate of treatment success among those with baseline fungal infections (51.9% vs. 25.9%, difference 25.9%, 95.2% CI = 0.9–51%). In addition, the proportion of patients surviving to seven days was greater among those receiving caspofungin, compared to L-AmB ($p = 0.04$). Nephrotoxicity, infusion-related adverse events, and treatment discontinuation due to adverse events were significantly less common among patients receiving caspofungin. Thus, as empirical therapy in patients with fever and neutropenia, caspofungin was as effective and better tolerated than liposomal amphotericin B.

Efficacy in Pediatric Patients

Efficacy of caspofungin in children with invasive fungal infections has been demonstrated in an open-label, noncomparative study (117). Forty-nine children three months to 17 years of age received caspofungin 70 mg/m² as a loading dose, followed by 50 mg/m² daily as primary or salvage therapy for invasive candidiasis ($n = 37$), esophageal candidiasis ($n = 1$), or invasive aspergillosis ($n = 10$). Among those with invasive candidiasis, 92% had candidemia and 82% received caspofungin as primary therapy. All 10 patients with invasive aspergillosis were refractory to prior antifungal therapies, and 80% had pulmonary involvement. Overall, success at the end of caspofungin therapy was experienced by 81% of patients with invasive candidiasis and 50% of those with invasive aspergillosis. Treatment was also successful in one child with esophageal candidiasis. Doses were increased up to 70 mg/m² in four patients with candidiasis and one with aspergillosis. Of these, three patients with candidiasis responded favorably, while one patient each with candidiasis and aspergillosis had unfavorable responses and discontinued caspofungin therapy after one and three days, respectively. Caspofungin was generally well tolerated, with 27% of patients having at least one clinical adverse event and 35% having at least one laboratory adverse event that was possibly related to caspofungin. These rates are similar to those reported in other similar studies in adults.

Caspofungin has also been compared to L-AmB in a double-blind randomized trial as empirical antifungal therapy in children with persistent fever and neutropenia in children 2 to 17 years of age (118). Eighty-two children were randomized in a 2:1 fashion to receive caspofungin 70 mg/m² loading dose, followed by 50 mg/m² daily or L-AmB 3 mg/kg/day. Efficacy was assessed using the same composite endpoint used in the adult studies mentioned previously (119). Twenty-seven percent of patients in both treatment groups were considered high-risk (allogeneic HSCT recipients or had relapsed acute leukemia). Prior antifungal prophylaxis had been used in 51% of the 81 patients evaluable for efficacy. Success rates were 46% for caspofungin- and 32% for L-AmB-treated patients (3). Among high-risk patients, success was higher among those receiving caspofungin (9 of 15 patients, 60%) compared to L-AmB (0 of 7 patients, 0%). Among low-risk patients, success rates were similar between treatment groups: 17 of 41 children (41.5%) receiving caspofungin and 8 of 18 (44.4%) children receiving L-AmB had favorable responses. Overall rates of clinical and laboratory treatment-related adverse events were similar between groups. Final results of this study have not yet been published in a peer-review journal, so full details of the data are not yet available. However, this information suggests that caspofungin is safe and effective as empirical therapy in febrile neutropenic children.

Based on these studies, the FDA has approved caspofungin for use in children three months and older as treatment for candidemia and intraabdominal abscesses, peritonitis, and pleural space infections caused by *Candida* spp., esophageal candidiasis, invasive aspergillosis that is refractory to other therapies, and as empirical therapy for presumed fungal infections in febrile neutropenic patients.

MICAFUNGIN

Oropharyngeal/Esophageal Candidiasis

Micafungin has demonstrated efficacy in the treatment of oropharyngeal/esophageal candidiasis in several clinical trials. In a multicenter, randomized, double-blind study, micafungin (50, 100, or 150 mg) was compared to fluconazole (200 mg/day) in the treatment of esophageal candidiasis with or without oropharyngeal candidiasis in 245 HIV-infected adults (120). A dose-response was demonstrated with micafungin, based on end-of-treatment endoscopic cure rates

of 68.8%, 77.4%, and 89.8% for micafungin dosages of 50, 100, and 150 mg daily, respectively. Fluconazole response rates were 86.7% overall. Micafungin response rates were comparable to fluconazole at micafungin doses of 100 or 150 mg daily, but inferior at micafungin 50 mg daily doses. As in trials with caspofungin, relapse occurred more frequently with the echinocandin compared to the azole in the posttreatment phase period (9 vs. 0 patients).

A multicenter, randomized, double-blind study compared micafungin 150 mg to fluconazole 200 mg per day for 14 days in the treatment of esophageal candidiasis (121). Ninety-four percent of study subjects were HIV-infected, although antiretroviral therapy was only used in 10% of the study subjects. Treatment success, as determined by endoscopy at the end of antifungal therapy, was similar between the two treatment groups: 87.7% for micafungin- and 88.0% for fluconazole-treated patients. Relapse rates at week 2, week 4, and through week 4 were also similar between groups. Relapse through the week 4 visit was 15.2% for micafungin versus 11.3% for fluconazole ($p = 0.257$). No differences in overall adverse event rates were observed between treatment groups (27.7% micafungin vs. 21.3% fluconazole, $p = \text{NS}$).

Another multinational, double-blind randomized trial compared micafungin to caspofungin for oropharyngeal/esophageal candidiasis, but these results have not yet been published in a peer-reviewed journal (122). In this study, 454 subjects were randomized to receive micafungin 150 mg daily, micafungin 300 mg every other day, or caspofungin 50 mg daily. Approximately 90% of subjects had both oropharyngeal and esophageal candidiasis. There were no differences found between treatment groups in endoscopic cure rate, clinical response rate, or overall therapeutic response at the end of antifungal therapy. Incidence of relapse was numerically lower in the two micafungin treatment groups than in the caspofungin treatment group at the two-week follow-up visit and through four weeks of follow up. In particular, relapse rates were approximately 7% lower with micafungin 300 mg QOD than caspofungin 50 mg daily.

Together, these studies demonstrate that micafungin is safe and effective for management of oropharyngeal/esophageal candidiasis.

Candidemia/Invasive Candidiasis

Experience with micafungin for management of candidemia and invasive candidiasis has been published from both open-label noncomparative studies (123) and as well as randomized clinical trials (48,124). One hundred twenty-six adults and children with newly diagnosed ($n = 72$) or refractory candidemia ($n = 54$) received micafungin in a multicenter, open-label, noncomparative study (123). Patients with infections due to *C. albicans* were initially administered 50 mg daily, while those with infections caused by other species of *Candida* initially received 100 mg daily (or 1–2 mg/kg/day if <40 kg). Incremental dose escalation in 50 mg increments was permitted up to 200 mg daily after at least five days of dosing for those with stable or progressive disease. Combination antifungal therapy was allowed in those with refractory *Candida* spp. infections. Approximately 80% of the patients received ≤ 100 mg of micafungin daily. Of the patients receiving micafungin as initial therapy for candidemia, 87.5% experienced a complete or partial response at end of therapy. Among patients who had failed prior antifungal therapy, 76% responded to micafungin alone while 79.3% responded to micafungin in combination with another agent. These data provided preliminary evidence of the efficacy of micafungin as both primary and salvage therapy for candidemia.

Micafungin has been compared to other antifungals in two multicenter, randomized, double-blind controlled trials in patients with invasive candidiasis or candidemia (48,124). Micafungin 100 mg daily was compared to L-AmB 3 mg/kg/day in 531 patients ≥ 16 years of age (124). A large proportion of the patients were in the ICU (51%), and/or receiving mechanical ventilation (35%). Most infections were caused by non-*C. albicans* species and approximately 16% had sites of infection outside the bloodstream. Overall clinical and mycological response at the end of IV therapy in the per-protocol population was 89.6% for micafungin versus 89.5% for L-AmB. In the modified intention-to-treat analysis, overall success was lower but still similar between treatment groups [74.1% micafungin, 69.6% L-AmB, difference when stratified by neutropenic status: 4.9% (95% CI = -3% to 12.8%)]. No differences in treatment efficacy between the two groups were observed on the basis of infecting pathogen. There were more infusion-related reactions (28.8% vs. 17%) and increases in serum creatinine from baseline to above upper level of normal (29.9% vs. 10.3%) in the L-AmB group compared to the micafungin group.

Micafungin doses of 100 mg/day and 150 mg/day were compared to caspofungin (50 mg daily following a 70 mg loading dose) in a phase III, double-blind, multicenter study in 595 patients with invasive candidiasis or candidemia (48). Approximately 85% of the patients were candidemic, and 55% of the infections were due to non-*C. albicans* spp. Overall, success at the end of IV therapy was experienced in 73.9%, 70.3%, and 71.4% of patients treated with micafungin 100 mg, micafungin 150 mg, and caspofungin 50 mg, respectively ($p = \text{NS}$). Of the study participants, 29.6% died but there were no statistical differences in survival between the treatment groups. Rates of treatment-related adverse events were similar between treatment groups (range 22–23.8%), and rates of discontinuation due to treatment-related adverse events were quite low overall (3%). Based on the data in these studies, 100 mg has become the standard daily dose for micafungin in the treatment of adults with invasive candidiasis or candidemia (5).

Prophylaxis in Patients Undergoing Hematopoietic Stem Cell Transplant

The role of micafungin as antifungal prophylaxis has been established through a randomized, double-blind trial in 882 patients undergoing autologous (46%) or allogeneic (54%) HSCT (125). Micafungin 50 mg IV daily or fluconazole 400 mg IV daily was started within 48 hours of initiating pretransplant conditioning and continued up to a maximum of 42 days and discontinued when the patient's ANC was >500 cells/mm³. Treatment success (absence of systemic IFI) was higher among those receiving micafungin compared to fluconazole (80% vs. 73.5%, difference 6.5%, 95% CI = 0.9% to 12%). Accordingly, breakthrough infections were more common with fluconazole therapy. These breakthrough infections included two candidemia and seven aspergillosis (four proven and three probable) cases in patients who received fluconazole. Four cases of candidemia and one probable aspergillosis case were reported among those who received micafungin. Overall mortality was similar between treatment groups (4.2% micafungin vs. 5.7% fluconazole, $p = \text{NS}$). Discontinuations due to treatment-related adverse events were low in both treatment groups (4.2% micafungin vs. 7.2% fluconazole, $p = \text{NS}$). In summary, this study demonstrated that micafungin was superior in efficacy and as safe as fluconazole as antifungal prophylaxis in HSCT recipients.

Aspergillosis

No randomized comparative trials have been performed with micafungin as primary treatment for invasive aspergillosis, but experience from several open-label studies for this indication has been published (97,106,126).

In a small open label, multicenter Japanese study, experience with micafungin doses of 25 to 150 mg/day with 46 pulmonary aspergillosis patients was described (126). Among 42 patients evaluable for efficacy at the end of antifungal therapy, 57% of patients experienced complete or partial improvement. Response rates were similar for the range of aspergillosis diagnoses included in this study: 60% (6/10) in those with invasive pulmonary aspergillosis, 55% (12/22) in those with pulmonary aspergilloma, and 67% (6/9) in those with chronic necrotizing pulmonary aspergillosis.

Micafungin was also investigated as a single agent or in combination antifungal therapy in two recent studies (97,106). The first study describes 225 evaluable adults and children who received micafungin for proven or probable invasive aspergillosis refractory or intolerant to initial antifungal therapy (97). Eighty-five percent of these patients added micafungin to a failing antifungal regimen. Complete/partial responses were experienced by 35.6% (8% complete, 27.6% partial) of patients at the end of antifungal therapy, while 53.5% of patients experienced progression of infection. This study showed no advantage of combination antifungal therapy compared to micafungin alone as either primary (29.4% vs. 50%) or salvage (34.5% vs. 40.9%) therapy; however, the overall number of patients included in these groups was small so it was underpowered to detect such differences. In a second, multicenter, open-label study, 98 adult and pediatric HSCT recipients with invasive aspergillosis received micafungin as a single agent (8%) or in combination with other antifungals (92%) as primary (15%) or salvage (85%) therapy (106). Most of the patients (83%) had invasive pulmonary aspergillosis. Amphotericin B or its lipid formulations were most commonly used in conjunction with micafungin. Treatment success was experienced by 26% of patients, who had either complete (5%) or partial (20%)

responses. Success rates were similar among those receiving micafungin as primary therapy (22%) and as salvage therapy (24%). Success was 24% among those receiving micafungin in combination with other antifungals, and 38% among the eight patients receiving micafungin alone. Thus, micafungin demonstrated some success in treating invasive aspergillosis in this very challenging patient population. Together, these data demonstrate that micafungin may be an effective treatment option for patients with pulmonary aspergillosis and further blinded, comparative clinical trials are needed.

Efficacy in Pediatric Patients

Several studies investigating micafungin efficacy have included children as well as adults (97,98,106,123,125). Not all of these have reported outcomes separately for children. A multicenter, noncomparative study of micafungin for management of candidemia included 20 patients less than 16 years of age (123). Micafungin was given as a single agent for primary therapy ($n = 6$) or as salvage therapy ($n = 6$), or in combination with other antifungals as salvage therapy ($n = 8$). Eleven neonates were included, and all but one of these received micafungin as salvage therapy. Treatment success was experienced by 15 of 20 children (75%, 95% CI = 51% to 91%). This was similar to the rate among adults (84.9%, 95% CI = 77% to 91%). Eight of the eleven neonates (72%) had a complete response while three failed the therapy. In a larger substudy of a double-blind, randomized multicenter trial, micafungin (2 mg/kg/day) was compared to L-AmB (3 mg/kg/day) as primary therapy for candidemia/invasive candidiasis (98). Ninety-eight children under 16 years of age with confirmed candidiasis were included. 57 patients were less than two years of age, and 19 of these were premature infants. Approximately 18% of the children were neutropenic. Overall clinical and microbiologic success at the end of therapy was 73% versus 76% (difference -2.4%, 95% CI = -20.1% to 15.3%) for the children who had received micafungin versus L-AmB, respectively. Seven patients in each treatment group had persistently positive cultures at the end of antifungal therapy. Mortality and overall adverse event rates were also similar between treatment groups. During the posttreatment follow-up, three patients who had received micafungin and no patients who had received L-AmB experienced recurrent infection. Two cases involved recurrences of candidemia, and one additional recurrence involved *Candida* meningitis in a four-week-old infant who initially presented with disseminated candidiasis. Higher dosages of micafungin are subsequently being investigated in neonates to achieve greater CNS penetration, but efficacy of these dosages needs to be established in a clinical trial.

Children were included in two open-label studies of micafungin as primary or salvage therapy for invasive aspergillosis (97,106). The first study included 58 children, and 27 of these were less than 10 years of age (97). Premature neonates were excluded from the study, and the youngest child was three months of age. Micafungin was initially administered as 1.5 mg/kg/day for children weighing ≤ 40 kg, but dose escalation was allowed after seven days of dosing in 1.5 mg/kg/day increments. The mean dose administered to patients less than 16 years of age was 2.1 ± 1.25 mg/kg/day and mean maximum daily dose was 2.8 ± 1.7 mg/kg/day. Twelve children received 4 mg/kg/day or more, with daily doses of more than 200 mg in five children and as high as 325 mg (8.6 mg/kg) in one child. Overall treatment success (complete and partial response) in children was 45%, with a similar rate among children less than 10 years of age. These rates are comparable to the success rate of the overall study (36%). Treatment was discontinued due to adverse events in 24% of children, but this rate was similar to that in the overall study (26%). In the second study, HSCT recipients received micafungin (1.5 mg/kg/day) alone or as part of combination antifungal therapy for primary or salvage treatment of invasive aspergillosis. Twenty-seven children less than or equal to 16 years of age were included in the study. Treatment success was experienced by 19% (5/27) of these children, while success was experienced by 28% (20/71) among adult study participants. No additional information is provided about the outcomes among children in the study, but these rates of success are not surprising given the challenge of managing invasive aspergillosis in HSCT recipients.

Finally, 84 children (6 months to 16 years of age) were included in a large randomized, double-blind clinical trial of antifungal prophylaxis in HSCT recipients (125). Patients were randomized to micafungin (1 mg/kg/day in those < 50 kg, $n = 39$) or fluconazole (8 mg/kg/day

in those <50 kg, $n = 45$). Treatment success was experienced by 61% of children overall, which is somewhat lower than the success rates among adults 16 to 64 years of age (78%) and >64 years of age (86%). Of the children receiving micafungin, 69.2% had treatment success while only 53% of those receiving fluconazole were successfully prophylaxed (difference 15.9%). Statistical comparisons were not presented for children, probably reflecting the small sample size. However, the authors state that there were no differences between children and adults in frequency of treatment-related adverse events. In summary, micafungin has demonstrated that it is at least as effective as fluconazole as antifungal prophylaxis in children undergoing HSCT.

ANIDULAFUNGIN

Oropharyngeal/Esophageal Candidiasis

Efficacy of anidulafungin in managing oropharyngeal/esophageal candidiasis has been investigated in one small open-label study and one large clinical trial (95,127). In a noncomparative, open-label Phase II study, anidulafungin (50 mg daily following a 100 mg loading dose) was administered for up to 21 days to 19 adults with azole-refractory oropharyngeal/esophageal candidiasis (127). Eighty-nine percent of patients were HIV-infected, with a median CD4+ cell count of 9 cells/mm³. Study subjects had a median of 5.5 prior to episodes of mucosal candidiasis. Endoscopic success was achieved in 92% of study subjects with esophageal candidiasis at the end of therapy, with 75% experiencing cure and 17% experiencing improvement of lesions. Among those with oropharyngeal candidiasis, the success rate was similar (94%), with 61% cured and 33% improved at the end of therapy. Clinical success was not universally sustained through the posttreatment period 10 to 14 days after the end of therapy. Success was 47% at this visit, with 8/18 (44%) of patients with oropharyngeal candidiasis and 6/12 (50%) of patients with esophageal candidiasis experiencing success. Four patients were successfully re-treated with anidulafungin. Use of antiretroviral therapy was not described in this study, but 76% of patients had CD4 cell count ≤ 50 cells/mm³, which could have impacted ability to sustain a clinical response after antifungal therapy was discontinued.

Anidulafungin (50 mg daily, following a 100 mg loading dose) was compared to fluconazole (100 mg daily, following a 200 mg loading dose) in a randomized, double-blind clinical trial in 601 predominantly HIV-infected adults with esophageal candidiasis (95). Endoscopic resolution of lesions after 14 to 21 days of treatment was graded as either cured (complete resolution of lesions) or improved. In addition, clinical response, defined as an absence or improvement in symptoms compared to baseline was determined. At the end of treatment, success based on endoscopic assessment and clinical response was similar between anidulafungin and fluconazole. Endoscopic cure or improvement was present in 97.2% and 98.8% of anidulafungin- and fluconazole-treated patients, respectively. Clinical response was 98.8% with anidulafungin, versus 99.6% for fluconazole. However, at the two-week follow up, only 64.4% of the anidulafungin-treated patients had a sustained endoscopic success compared to 89.5% of the fluconazole-treated patients ($p < 0.001$). The lack of sustained response in the anidulafungin compared to the fluconazole-treated patients was complicated by higher use of antiretroviral therapy among patients receiving fluconazole. It has also been suggested that salivary enzymes or pharmaceutical replacement enzymes could digest anidulafungin through ring digestion, and this could contribute to the lack of a sustained response when treating esophageal candidiasis with anidulafungin (128).

Candidemia

Anidulafungin has been investigated in at least two prospective studies of invasive candidiasis (94,129). In a Phase II open-label, noncomparative study, 123 patients were randomized to 50, 75, or 100 mg of anidulafungin daily (129). Overall clinical success rate at the end of treatment was 84%, 90%, and 89% with 50, 75, and 100 mg daily, respectively. In a phase III, randomized, double-blind study of candidemia and invasive candidiasis, anidulafungin (100 mg daily, after a 200 mg loading dose) was compared to fluconazole (400 mg daily, after a 800 mg loading dose) (94). Treatment was continued for at least 14 days from the last positive blood culture. Patients included in this study were predominantly nonneutropenic (97%) with APACHE II (Acute Physiology and Chronic Health) scores less than 20 (80%). The majority of infections were

due to *C. albicans* (62%), and involved candidemia (89%). At the end of intravenous therapy, there was a 75.6% overall clinical response with anidulafungin compared to 60.2% response with fluconazole (difference = 15.4%, 95% CI = 3.9% to 27%, $p = 0.01$). This improvement in efficacy with anidulafungin was maintained at two weeks after the end of therapy. There was no apparent association between fluconazole MICs of the organisms and eradication rates observed in this study, but few isolates ($n = 5$) had fluconazole MICs $\geq 16 \mu\text{g/mL}$. Microbiologic and overall treatment success among patients with *C. parapsilosis* infections were numerically higher with fluconazole, but these differences were not statistically significant. Overall mortality among study subjects was lower among patients receiving anidulafungin (33%) compared to fluconazole (23%), but these differences were not statistically significant in a survival analysis. Fewer patients receiving anidulafungin had adverse events leading to discontinuation of study treatment (11.5% anidulafungin group vs. 21.6% fluconazole group, $p = 0.02$), but overall rates of treatment-related adverse events were similar between groups (24.4% anidulafungin vs. 26.4% fluconazole). In summary, anidulafungin had superior efficacy and was similarly safe as fluconazole in treating adults with candidemia/invasive candidiasis. Additional studies are needed to confirm these findings that echinocandins may be more efficacious than azole for *Candida* spp. infections.

SUMMARY OF EFFICACY AND RECOMMENDATIONS

Echinocandins have been employed in numerous clinical trials demonstrating efficacy in esophageal/oropharyngeal candidiasis. These agents are currently recommended by the Infectious Diseases Society of America (IDSA) as alternatives to itraconazole, posaconazole, or voriconazole for fluconazole-refractory esophagitis (130). Sustained response has been difficult to achieve in studies of mucosal candidiasis with echinocandins, and azoles appear to have higher rates of sustained responses after therapy than echinocandins. However, use of concomitant antiretroviral therapy may impact success in this setting, and the safety of echinocandins makes them an attractive treatment option for patients with azole-refractory disease.

In candidemia/invasive candidiasis, caspofungin and micafungin have demonstrated similar efficacy as polyenes. Anidulafungin is the only echinocandin to be compared to an azole for treatment of candidemia/invasive candidiasis in a randomized, double-blind clinical trial. This study suggested superiority of the fungicidal echinocandin compared to the fungistatic azole, but additional clinical data are needed to confirm this finding. When two echinocandins were directly compared for candidemia/invasive candidiasis, no differences were found in efficacy or safety between caspofungin and micafungin. Thus, these data suggest that echinocandins are generally equivalent to one another in the management of candidemia/invasive candidiasis. These agents are now recommended by the IDSA as first-line agents for neutropenic adults with invasive candidiasis/candidemia and nonneutropenic adults and children with moderately severe to severe candidemia with a history of recent azole exposure. The IDSA suggests echinocandins should be reserved as alternatives to fluconazole or amphotericin B in neonates, since additional experience with this new class of agents is needed in this setting (130).

Echinocandins are also a first-line option as empirical therapy for suspected invasive candidiasis in neutropenic patients and nonneutropenic patients (130). In addition, caspofungin is an option as prophylaxis among patients receiving induction chemotherapy for the duration of neutropenia, and micafungin is an option as prophylaxis in neutropenic HSCT recipients (130).

Experience with echinocandins in treatment of invasive aspergillosis is evolving, and is generally limited to smaller open-label, noncomparative studies. Caspofungin has shown promise as salvage therapy, and micafungin has generally been employed as part of combination antifungal therapy as salvage for invasive aspergillosis (97,106,111,112). Both caspofungin and micafungin are recommended as alternatives to voriconazole in the management of invasive aspergillosis by IDSA (131).

Caspofungin is the only echinocandin with an FDA indication for use in children. However, micafungin has been investigated in several pharmacokinetics and safety studies in older children as well as neonates. Most of the clinical studies performed with micafungin included children as well as adults, so there is a relatively large body of experience with this echinocandin in children with invasive fungal infections or who require antifungal prophylaxis.

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14 Novel Administrations of Antifungals

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INTRODUCTION

Despite the availability of numerous antifungal agents with potent activity *in vitro* against a variety of fungal pathogens, treatment outcomes for invasive fungal infections (IFIs) (especially in immunocompromised hosts) remain poor. Numerous strategies have been employed in an attempt to optimize therapy, including use of prophylaxis in high-risk patient populations, early diagnosis, pharmacodynamic-based dosing of antifungals, and the introduction of newer therapies. In an effort to optimize antifungal concentration at the infection site while minimizing the consequences of systemic administration (including adverse reactions and drug interactions), antifungals tested and approved for systemic therapy (i.e., oral and parenteral administration) have been administered in a variety of novel ways (1). While most reports involve administration of amphotericin B, other antifungals (such as flucytosine, nystatin, miconazole and voriconazole) have also been administered in novel methods, including (but not limited to) aerosols, irrigations, and local injections.

The novel administration of antifungals has been reviewed previously, and included descriptions of the administered preparations when available (1). As expected, much of the published data are restricted to uncontrolled case reports and case series involving prior and/or concomitant systemic therapy, making it difficult to determine the exact contribution of the novel method of antifungal drug delivery in treatment outcome. Adequate descriptions of the agent's preparation and stability are lacking in most reports. In many cases, the focus is on efficacy, and safety information is inconsistently reported. Along with this lack of adequate data, the recent expansion of available antifungals (such as echinocandins and extended-spectrum triazoles) with favorable pharmacokinetic and safety profiles is likely to further limit the role of such novel administrations.

It is the intent of this chapter to focus on the potential role of novel antifungal administration in the current treatment of a variety of IFIs. All of the descriptions below utilize FDA-approved drugs for unapproved indications and routes of administration. Inclusion of information in this chapter should not be considered an endorsement of such use, and clinicians should refer to the original publications and current treatment guidelines for details and verification regarding doses and formulations.

AEROSOLS

Since 1956 when the use of nystatin aerosol was reported to contribute to elimination of *Aspergillus fumigatus* from bronchial secretions (2), numerous case reports and clinical trials have been published evaluating the use of various formulations of amphotericin B aerosols in the prevention and adjunctive therapy of IFIs in select populations (3).

Although early reports described the use of nystatin (4–6), more recent investigations in both animals and humans describe various formulations of amphotericin B (3). The focus of recent human investigations has been in the prevention of invasive aspergillosis in high-risk patients (3). Since disease occurs after inhalation and deposition of the fungal propagule into the lungs, aerosolized formulations of amphotericin B may provide high antifungal concentrations at the site of initial infection while minimizing its potential for systemic side effects (3,7).

Aerosolized amphotericin B deoxycholate (aAmBd) has been studied for the prophylaxis of fungal infections in several patient populations, including neutropenic patients receiving chemotherapy (8–10), bone marrow transplant recipients (11,12), and solid organ transplant (SOT) recipients, most notably heart or lung transplant recipients (13–16). Doses vary between trials, ranging from 5 to 20 mg once daily to thrice daily with and without additional systemic

prophylaxis. The trials also utilize a variety of nebulizers and durations of drug exposure. More recently, aerosolized amphotericin B lipid complex (aABLC) (16–18) and aerosolized liposomal amphotericin B (aLAmB) (19) have been investigated for their potential roles in the prevention of IFIs in both solid organ recipients, hematopoietic stem cell transplants, and high-risk hematology–oncology patients. In one such study, a randomized, placebo-controlled trial was conducted in 271 patients with prolonged neutropenia (19). Patients were randomized to receive either aLAmB or placebo twice weekly. A total of 18/132 in the placebo group and 6/139 patients in the aLAmB developed invasive pulmonary aspergillosis (odds ratio, 0.26; 95% CI = 0.09–0.72; $P = 0.005$) and significantly favored the use of aLAmB in preventing pulmonary aspergillosis.

The potential role of aerosolized formulations of amphotericin B as adjuncts to systemic therapy for the treatment of IFIs has been studied in the animal model (20). However, reports in humans are generally restricted to case reports or case series in patients receiving concomitant systemic therapy (6,21–27), which make definitive statements regarding efficacy impossible. Doses of aAmBd in these reports range from 2.5 mg thrice daily to 50 mg once daily.

Adverse events associated with the administration of aerosolized amphotericin B formulations include nausea, bad taste, cough, dizziness, chest tightness, mild bronchospasm, and sputum production (8–15,19,27). The incidence of such reactions, however, varies between preparations and patient populations. When compared to aAmBd, aABLC has demonstrated improved tolerability in lung transplant recipients (16).

The current role of aerosolized amphotericin B formulations in the prevention and treatment of IFIs (most notably invasive pulmonary aspergillosis) in high-risk patients is still evolving. Numerous questions still need to be addressed, including optimal preparation, dose, delivery system, need for concomitant systemic antifungals, and timing and duration of therapy (28). Recent guidelines for the management of tracheobronchial aspergillosis (29) and for the prevention of infections in patients with solid organ transplantation (30) also recognize the need for further study. In contrast to recent prevention and treatment guidelines for hematology–oncology patients published by the National Comprehensive Cancer Center (31) where the role of aerosolized amphotericin B was not addressed, published guidelines by the German Society for Hematology and Oncology recommend aLAmB 12.5 mg twice weekly for fungal prevention in patients with prolonged neutropenia (32).

IRRIGATIONS

A variety of antifungal-containing irrigating solutions have been described in the literature. Descriptions of such irrigations are generally restricted to case reports or case series. Of most current interest is the use of nasal, intraperitoneal, and bladder irrigations utilizing amphotericin B deoxycholate.

Nasal Irrigations

Initial interest in the intranasal administration of antifungal-containing solutions was for the prevention of IFIs in high-risk patients. The vast majority of reports involve amphotericin B (33–36). Use of intranasal amphotericin B-containing solutions (administered via nasal spray) was first described in 1984 as a potential method to prevent pulmonary aspergillosis infections in neutropenic patients (33). Subsequent reports of nasal use describe a variety of doses, formulations, and durations of therapy (34–36).

While isolated reports have described the nasal use of alternative agents such as fluconazole (37) and liposomal amphotericin B (38), adjunctive use of nasal irrigations containing amphotericin B has been reported for the treatment of various forms of fungal sinusitis. Again, lack of adequately controlled clinical trials makes it difficult to determine its efficacy in these settings. However, surgery and (in select cases) systemic antifungal therapy are likely to be mainstays of therapy for most forms of fungal sinusitis.

Fungal colonization has been implicated as a precipitating factor in select patients with chronic rhinosinusitis. However, the role of amphotericin B-containing nasal solutions in the treatment of patients with chronic rhinosinusitis (with or without polyps) continues to be poorly defined (39–45). Some investigators have proposed that nasal polyps represent an allergic reaction to fungal colonization of the nares (43). Conflicting information exists regarding the effect of amphotericin B on inflammatory markers (44,46,47). A recent randomized, double-blind,

placebo-controlled trial in patients with chronic sinusitis without nasal polyps demonstrated improved symptoms and endoscopic findings in patients receiving intranasal amphotericin B (39). However, overall treatment outcomes did not improve.

In general, clinical trials evaluating the potential role of antifungal-containing nasal irrigants and sprays for the prevention of IFIs are significantly limited by the use of historical controls and/or the coadministration of systemic prophylaxis. However, the administrations were generally well-tolerated, with mild rhinorrhagia the most frequent adverse event reported. Currently, antifungal-containing nasal irrigations are not recommended for the prevention of invasive fungal infections in either SOT recipients (30) or in patients with malignancy at increased risk of IFIs (31,32). In addition, existing studies to date do not justify their routine use in patients with chronic rhinosinusitis (40,45).

Urinary Tract Irrigations

Prior to the availability of fluconazole, options for the treatment of fungal urinary tract infections (most commonly due to *Candida* spp.) were limited, with rare case reports of the use of miconazole (48), nystatin (49), and methylene blue (50). The overwhelming majority of clinical experience with antifungal urinary bladder irrigations is with amphotericin B deoxycholate. Amphotericin B bladder irrigations were first reported in two 59-year-old male patients with complaints of frequent urination, dysuria, and nocturia (51). Since that report, attempts have been made to determine the optimal dose, method of administration, duration of therapy, and comparative efficacy with alternate therapies.

Early experience with amphotericin B bladder irrigations was reported from an open-labeled, noncomparative study in 40 patients with noninvasive candiduria receiving amphotericin B 50 mg/L of sterile water (administered through a three-way catheter or an indwelling urethral catheter or suprapubic tube at a rate of 40 mL/hr until urine became clear of *Candida*) (52). *Candida* was eliminated from the urine in 92.5% of cases. Eight of the 14 patients with follow-up cultures were also negative while two patients experienced recurrence. Subsequently, a report of similar doses in 65 nursing home residents with candiduria determined a response rate of 72% after two days of therapy (53).

The use of a single amphotericin B bladder irrigation (30 mg in 100 mL of sterile water infused through a three-way catheter clamped for two hours) as a diagnostic strategy to distinguish between upper and lower urinary tract infections has been reported (54). Forty-four out of 62 (71%) single bladder irrigations caused clearance of *Candida* from the urine, with 12 of these experiencing recurrence one to three weeks after treatment. Persistence of positive cultures occurred in 18, of which 10 lacked evidence of upper tract infection or invasive candidiasis. Therefore, the use of irrigation as a diagnostic tool is questionable or (at best) has limited applicability.

Continuous versus intermittent administrations of amphotericin B bladder irrigation were compared in a randomized, prospective study (55). Ten men were randomized to either continuous (50 mg/L sterile water per day through a three-way catheter for 48 hours) or intermittent (10 mg/100 mL through a catheter which was clamped for 30 minutes and released three times) infusion. Eight out of 10 patients in the continuous infusion group had clearance of fungus from the urine at 72 hours versus only 3 out of 10 patients in the intermittent treatment group ($p = 0.035$). Reinfection at day 7 was seen in two patients and one patient, respectively. These findings do suggest the method of administration may be important.

Amphotericin B bladder irrigations have been compared to oral fluconazole therapy (56–59). In the first of these trials in patients with noninvasive candiduria, no difference in efficacy could be detected in patients receiving either fluconazole 200 mg daily for seven days, continuous amphotericin B bladder irrigations 50 mg/L for one day, or continuous amphotericin B bladder irrigations at 50 mg/L for seven days (56). A randomized, placebo-controlled trial comparing oral fluconazole (200 mg \times 1, then 100 mg/day \times 4), a single 15 mg dose of amphotericin B IV, and three concentrations of amphotericin B bladder washes (5 μ g/mL, 100 μ g/mL, or 200 μ g/mL thrice daily for three days) for treatment of fungal urinary tract infections in 180 adults failed to demonstrate differences between the three active treatment strategy groups (57). However, when oral fluconazole (200 mg on day 1 followed by 100 mg daily for four days) was compared with amphotericin B bladder irrigations (25 mg in 500 mL of D5 W continuous

infusion for five days) in elderly patients, with microbiologic response rates of 73% (33/45) and 96% (49/51), respectively ($p < 0.05$) (58). Finally, an observational trial in 530 patients with funguria reported resolution in 75.5% of untreated patients, 45.5% of patients treated with fluconazole alone, and 54.4% of patients treated with amphotericin B bladder irrigations alone (59).

The optimal concentration of amphotericin B to use as a bladder irrigation remains controversial. Recommendations generally range between 5 and 50 mg/L (53,60,61). Amphotericin B bladder irrigation solutions must be protected from light and heat, and are usually added to sterile water for irrigation, since amphotericin B will precipitate in normal saline (48,52,56,62,63).

Adverse effects associated with the use of amphotericin B bladder washes appear to be infrequent, but may include hematuria, cramping, bladder discomfort, dysuria, and burning during irrigation (61).

In the majority of patients with uncomplicated candiduria, no treatment is needed (64). Given the availability of alternative oral treatment options for fungal infections, the role of amphotericin B bladder irrigations for the treatment of uncomplicated candiduria is limited (64). Prior to use of amphotericin B irrigations, it is important to define desired goals (such as diagnosis of upper tract disease, relief of symptoms associated with symptomatic cystitis, or eradication of yeast in the urine in a patient undergoing urinary catheterization). Use of amphotericin B bladder irrigations does not treat upper urinary tract disease, while lower tract disease remains difficult to define. Use of amphotericin bladder irrigations is not needed in most patients with candiduria.

In addition to bladder irrigations, the use of antifungals as irrigants for nephrostomy tubes has also been reported. The majority of the published experience with amphotericin B in this role is in pediatric patients (65–69). Less experience is published in adult patients (70,71). In addition to amphotericin B, fluconazole has also been described in literature as an irrigation for nephrostomy tubes to treat upper urinary tract *Candida albicans* infections (72–75). Concentrations of fluconazole in these reports ranged from 10 to 1000 mg/L administered once to six times daily. As with many of the previous reports, most patients received concomitant systemic antifungal therapy, making the efficacy of the irrigant difficult to determine.

Orthopedic Irrigations

Use of fluconazole- (76) and amphotericin B- (77) containing irrigants has been described as adjuncts to surgical intervention and systemic antifungal therapy in the treatment of select fungal bone and joint infections. One description included local therapy with amphotericin B during surgical debridement for the treatment of mucormycosis (77). However, in select settings (such as mediastinitis due to *Candida*), use of amphotericin B-containing lavage solutions should be discouraged due to the potential for chemical mediastinitis to occur (64).

Endobronchial Instillations

Reports on endobronchial instillations of antifungals are generally restricted to treatment of pulmonary aspergillosis (78–83). Less frequently, these involve administration of ketoconazole (83) or fluconazole (81). Most of this endobronchial experience is from case reports involving the administration of amphotericin B in the treatment of aspergillomas (78–81). In patients who received amphotericin B deoxycholate, doses ranged from 5 to 50 mg administered between every other day and four times daily (78,79,81). Complete remission occurred in 2/7 patients (78,79). Four out of five patients not achieving complete remission demonstrated improvement (78,80,81). Adverse effects were cough and fever (81).

Given the availability of newer treatment options for the treatment of pulmonary aspergillosis (such as echinocandins and extended-spectrum triazoles) and the lack of sufficient efficacy and safety data to support its use, endobronchial instillations of antifungal agents should not be routinely used for the treatment of pulmonary aspergillosis (29).

Peritoneal Lavage

Direct instillation of antifungals into the peritoneal fluid in patient undergoing peritoneal dialysis with fungal peritonitis was first described in the early 1970s (84). As with most other alternative dosage forms, it was first described with amphotericin B deoxycholate (84–87) and most often due to infections caused by *Candida* spp. Concentrations in dialysate varied from

1 to 4 $\mu\text{g}/\text{mL}$ (85,87). Use with concomitant IV therapy with amphotericin B was common in these reports. Adverse effects have included mild/moderate abdominal discomfort (86,88) and hypokalemia (87).

Administration of alternative antifungal agents via peritoneal lavage has also been reported for flucytosine and fluconazole. Use of peritoneal lavage containing flucytosine 50 mg/L at a rate of 1.2 L/hr for five days was successful in the treatment of fungal peritonitis (89). Peritoneal lavage with flucytosine has also been reported in pediatric patients (88).

Use of intraperitoneal antifungals is generally considered as adjunctive to catheter management and administration of systemic antifungals (90). Continuous amphotericin B 1.5 mg/L dialysate or fluconazole 200 mg intraperitoneally in one exchange per day every 24 to 48 hours has been identified as an option (depending upon the pathogen). While their role may be limited in the treatment of peritonitis caused by *Candida* spp. (64), amphotericin B-containing lavage solutions (in addition to systemic administration and catheter removal) may play more of a role in the treatment of peritonitis caused by *Aspergillus* spp. (29).

PERCUTANEOUS DELIVERY

Percutaneous administration of antifungals has been reported in a variety of dosage forms, including injections of antifungal medications, infusions, pastes, and gelatins (1). Reports in the literature are generally restricted to use in patients with pulmonary aspergilloma, often accompanied by hemoptysis (80,91–103).

English language reports of delivery of antifungals via percutaneous catheter generally involve amphotericin B deoxycholate (93,94,98–103). Most reports have utilized amphotericin B 50 mg in 20 mL of D5 W (97–99,102,103). Total doses generally have ranged between 500 mg (102,103) and 3 g via catheter (100). Reported toxicities of the instillations include coughing (99,101–103), fever (98,102,103), headaches (103), and vomiting (103). Reports of fluconazole via percutaneous catheter instillation are rare (94). Percutaneous injections are less frequently reported than through a catheter, but also most frequently involve the administration of amphotericin B deoxycholate (96,103).

The percutaneous administration of pastes and gelatins containing either amphotericin B or nystatin has been described in the treatment of patients with aspergilloma (91,95). Final concentrations of amphotericin B and nystatin in one of these reports were 5 mg/mL and 45,000 units per mL, respectively (95). Patients were injected 5 mL (25 mg of amphotericin B and 225,000 units of nystatin) at one time, usually every five to seven days (95). In other reports, 10 mL of amphotericin B per dose was administered every one to three weeks (91). One published report to date described percutaneous injection of amphotericin B in a gelatin solution (92).

Based on the availability of alternative treatment options and the lack of adequate efficacy, safety, and stability data, percutaneous delivery of antifungals is not routinely recommended for the treatment of *Aspergillus* infections (29).

INTRATHECAL/INTRACISTERNAL ADMINISTRATION

Low penetration of intravenously administered amphotericin B into cerebrospinal fluid (104–106), combined with poor treatment outcomes for amphotericin B monotherapy for central nervous system (CNS) fungal infections, stimulated reports regarding the potential role of direct drug administration to the CNS. While intrathecal administration was first investigated for amphotericin B as adjunctive treatment for cryptococcal meningitis, it was soon investigated for other systemic fungal infections involving spread to the CNS (105,107–109).

Case reports and case series have been published which describe the intrathecal administration of amphotericin B for the treatment of cryptococcal (110–115), coccidioidal (116–121), and candidal (122) meningitis. In fact, with the incurable nature of coccidioidal meningitis, this condition is the most likely infection being treated with intrathecal amphotericin B deoxycholate. Less frequently, *Histoplasma* meningitis and CNS blastomycosis have been treated with intrathecal administration of amphotericin B (123,124). Doses generally ranged between 0.25 and 0.5 mg (maximum 1 mg) two to four times per week. As with other reports regarding novel administrations of antifungal therapy, patients were generally treated with concomitant systemic antifungal therapy, usually amphotericin B (112–115,119–121,123–125). Intrathecal

treatment as outpatients for several months was continued in some patients to prevent relapse (114,118,120,126).

Numerous and frequent adverse effects secondary to intrathecal administration of amphotericin B have been reported, including (but not limited to) paraplegia (114,123,124,127), pain in the back and legs (114), nausea and vomiting (114), loss of bowel and bladder control (123,127), and headache (114,115).

Other methods of administering antifungals directly into the CNS have been investigated in attempts to address the serious adverse effects associated with intrathecal administration via lumbar injection of amphotericin B. Cisternal administration of amphotericin B has been reported for the treatment of coccidioidal meningitis (126) and cryptococcal infections (128,129). Severe adverse effects have also been reported with cisternal administration, including a report of subarachnoid hemorrhage, brain stem decompensation, and subsequent death (130). Intrathecal administration of amphotericin B using ventricular reservoirs [such as the Ommaya reservoir (131–137) and Rickham reservoir (138,139)] has been used for the treatment of cryptococcal meningitis, coccidioidal meningitis, mucormycotic brain abscess, and other unidentified invasive mold infection of the CNS. Amphotericin B doses ranged from 0.05 mg (137) to 1 mg (132), most commonly one to three times per week (131,135,136). Less frequently, the Ommaya reservoir administration of amphotericin B was used for the treatment of mucormycotic brain abscesses (135,137) and *Aspergillus* (133). Chemically induced arachnoiditis and bacterial colonization of the reservoir have been reported to be complications of such administration (134).

Relative to administration of amphotericin B, direct administration of other antifungals into the CNS is infrequently reported. Miconazole has been administered intrathecally in the treatment of various CNS infections, including coccidioidal meningitis, cryptococcal meningitis, histoplasmosis meningitis, and *C. albicans* infections (140–145). Doses in adults ranged from 1 to 30 mg (140–145), with lower doses of 3 to 5 mg in children (144,145). Adverse effects of intrathecal miconazole may include arachnoiditis (105,142), cisternal hemorrhage (105,127,142), ventricle hemorrhage (140), transient numbness (140), and bacterial infections when administered via Ommaya reservoir (144).

Because of the complications associated with intrathecal or intracisternal administration of amphotericin B, current treatment guidelines for the management of various IFIs involving the CNS do not recommend the routine use of such administration (29,64,146–149). However, in desperate situations (such as select cases of coccidioidal meningitis), intrathecal amphotericin B is still considered by some experts.

INTRA-ARTICULAR INJECTIONS

Direct intra-articular injection of amphotericin B was first described in the literature in the late 1960s (150). Subsequent reports for the management of fungal synovitis and arthritis utilized doses ranging between 0.05 and 20 mg (most commonly 2–5 mg) (150–158). However, such administration is rarely employed today in the treatment of IFIs, since many of the systemic treatment options achieve high concentrations in joint fluid, and intra-articular administrations of amphotericin B may cause significant pain and irritation.

OPHTHALMIC ADMINISTRATION

Multiple novel methods of antifungal drug administration as adjuncts to systemic therapy are used in an attempt to overcome poor ocular penetration of amphotericin B (159). Among these are topical (i.e., eye drops or ointments), subconjunctival, intracameral injections, intravitreal injections, intraconal administration, and subtenonian drops.

Topical administration of antifungals has been described for multiple agents. Amphotericin B eye drops have been used in the treatment of a variety of fungal pathogens such as *Aspergillus*, *Candida*, *Curvularia lunata*, *Phialophora*, *Gibberella*, *Alternaria*, *Scopularisopsis brevicaulis*, *Rhinosporidiosis*, *Macrophoma*, and *Fusarium* (160–165). Solutions containing 0.5 to 1.5 mg/mL of amphotericin B were administered every 30 minutes to 1 hour (160–165). Miconazole 10 mg/mL solution has also been administered (in combination with subconjunctival miconazole) for the treatment of keratomycosis caused by *Candida* (166). Successful use of topical posaconazole (using the suspension containing 10 mg/0.1 mL) administered every hour (in conjunction with oral posaconazole) was reported for keratitis caused by *Fusarium solani*

(167). Flucytosine 1% eye drops given every hour was used in conjunction with oral flucytosine therapy for the treatment of corneal ulcers in two patients caused by *Candida* (168). Case reports also describe the use of both amphotericin B and miconazole administered as a topical ointment for corneal ulcers and endophthalmitis caused by *Aspergillus* and *Fusarium* (169,170). Miconazole 2% ointment has been utilized in the treatment of a patient with *Aspergillus conicus* endophthalmitis following a cataract extraction (170). Topical voriconazole (in combination with systemic administration) has also been reported (171,172).

Among the agents best studied for the topical treatment of fungal keratitis has been natamycin (173–175). In one study, natamycin 5% eyedrops was compared with itraconazole 1% eyedrops in 100 patients with fungal keratitis due to a variety of pathogens, including *Fusarium*, *Aspergillus*, and *Curvularia* (173). Overall favorable response rates were seen in 72% and 60% of natamycin and itraconazole-treated patients, respectively. In the subset of patients with fungal keratitis due to *Fusarium* spp., 19/24 (79%) and 8/18 (44%) demonstrated a favorable response, respectively ($p < 0.02$). Natamycin has become a treatment option primarily for the treatment of keratitis due to filamentous fungi (176). Natamycin 5% ophthalmic suspension is approved for use in the United States for the treatment of fungal blepharitis, conjunctivitis, and keratitis.

Subconjunctival administration of antifungals has been reported in combination with other local therapies (159,166,177,178). Both amphotericin B (159,177,178) and miconazole (166,179) have been used. Subconjunctival miconazole (in combination with amphotericin B IV) was used in the treatment of keratomycosis (166), and in one patient for the treatment of blastomycosis (179). Use of amphotericin B in this manner is likely to be limited due to poor aqueous penetration when given by subconjunctival injection (159).

Various case reports describe intracameral injection of amphotericin B in the treatment of keratomycosis caused by *Aspergillus* and other molds like *Colletotrichum* and in endophthalmitis caused by molds such as *Paecilomyces* (177,178,180–182). Six of seven patients treated with intracameral injections achieved resolution or significant improvement of this infection (181). The only reported adverse effect in these reports is uveitis.

Anterior chamber injections have been described for amphotericin B in the treatment of endophthalmitis in doses ranging from 5 to 50 μg for the treatment of endophthalmitis caused by *Paecilomyces* (183,184), *Coccidioides* (185), *Cylindrocarpum* (183), and *Acremonium* (183). Intravitreal injections of amphotericin B [concurrently with surgery (i.e., vitrectomy), systemic and other local antifungal therapies] have also been utilized in the treatment of ophthalmic fungal infections in an attempt to compensate for its poor penetration into vitreal fluid. Endophthalmitis due to *Fusarium* (183,186), *Acremonium* (183,186–188), *Aspergillus* (183), and *Candida* (183,188–194) has been treated with intravitreal doses of amphotericin ranging from 5 to 10 μg , and many reports have used multiple intravitreal injections per infection. Fibrinous iritis has been reported with intravitreal injections of amphotericin B at doses of 10 μg , but not after dosage reduction to 5 μg (191). Retinal or pigment epithelial toxicity secondary to such use has also been described (183).

Intravitreal and intracameral administration of other antifungals is less common. However, recent reports describe the use of voriconazole in this setting (195–197).

Intraocular amphotericin B (in combination with vitrectomy) is considered an option in the treatment of endophthalmitis and topical therapy for keratitis due to *Aspergillus* (29). For severe corneal infections, topical antifungals from several classes (i.e., polyenes and azoles) may be used in an alternating fashion (198). However, controlled trials to support the efficacy of most topical therapy in the treatment of fungal keratitis are limited (199). The role of intravitreal amphotericin B for the treatment of endophthalmitis due to *Candida* spp. is uncertain, but may be considered (along with vitrectomy) in cases of severe vision loss (64).

ANTIBIOTIC LOCK ADMINISTRATION

The instillation and retention of high concentrations of antibiotics within the lumen of a catheter with the intent to sterilize in situ have been described in the medical literature in situations where catheter-related infections occur and that catheter removal (generally considered essential to treatment success) is impractical. While most frequently reported in the treatment of catheter-related bacteremias, antibiotic lock therapy for fungemia utilizing amphotericin B has also

been reported (200–203). The first case report of antibiotic lock use in fungemia was for the treatment of *Malassezia furfur*, for which 2 mL of amphotericin B 2.5 mg/mL solution in normal saline was utilized (201). A second report examined the use of amphotericin B deoxycholate 2.5 mg/mL solution daily instilled into catheters to remain for 8 to 12 hours in two patients with *Candida* catheter-related infections (in addition to systemic therapy) (200). However, relapse was reported in both patients.

Animal models have suggested a potential role for the use of lipid-based formulations of amphotericin B (specifically liposomal amphotericin B) (204) and amphotericin B lipid complex (205) in catheter-related infections, owing to their increased activity (relative to AmBd) against organisms producing biofilms. Reports of the use of lipid-based formulations of amphotericin B as an antibiotic lock solution are infrequent. In one such report, liposomal amphotericin B was successful in fungal eradication in 4/4 cases despite continued catheter use (206).

BONE CEMENT

Limited published information is available to describe the use of antifungals incorporated into bone cement for the treatment of fungal osteomyelitis and/or prosthetic joint infections (207,208). Successful use of an amphotericin B-containing bone cement was reported in a patient developing a knee infection due to *Candida glabrata* (207). In addition to systemic therapy and local irrigations containing amphotericin B, bone cement saturated amphotericin B was inserted into the knee.

While controlled clinical data are lacking, the incorporation of amphotericin B appears to be a safe adjunct for the treatment of osteomyelitis caused by *Candida* spp. (64).

SUMMARY

Novel administration of antifungals most frequently involves the delivery of amphotericin B in a variety of manners other than intravenous administration. Adequately controlled clinical studies to support the use of novel methods of antifungal drug administration for the adjunctive treatment of IFIs (generally in combination with systemic antifungal therapy) are lacking for most indications.

Data are emerging for the use of aerosolized formulations of amphotericin B in the prevention of invasive aspergillosis in high-risk patients (most notably hematology–oncology patients with prolonged neutropenia and in lung transplant recipients). Use of antifungal-containing irrigating solutions (usually amphotericin B) most frequently involves their use as bladder irrigations (for the treatment of funguria), peritoneal lavage fluid (for management of fungal peritonitis), and nasal solutions (for the treatment of fungal sinusitis). Among these indications, amphotericin B bladder irrigations and nasal solutions are perhaps the best-studied. However, the role of such therapies (in the setting of adequate systemic antifungal therapy) is often questionable. In contrast, despite lack of controlled clinical studies, novel administrations of amphotericin B (such as in the use for the treatment of candidal osteomyelitis, or endophthalmitis due to *Candida* or *Aspergillus* spp.) have been identified by treatment guidelines as adjunctive therapy for these infections.

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15 | Dermatophytosis

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DERMATOPHYTOSIS

Cutaneous fungal infections are a worldwide health concern. While the majority of dermatophyte infections are benign when affecting otherwise healthy individuals, infections in elderly or immunocompromised patients may be accompanied by significant morbidity or mortality. Some forms of dermatomycosis are increasing in incidence, whereas others remain rare (1). Although newer oral antifungal agents for the treatment of invasive and superficial infections have significantly improved the efficacy of treatment for many fungal infections, side effects, drug interactions, and resistant organisms have created a challenge to find safer and more effective treatments. This chapter blends current clinical knowledge of superficial and cutaneous fungal infections with recent advances in management.

Introduction

Dermatophytes are fungi causing localized infection of the stratum corneum, hair, and nails that may infect up to 20% of the population at any given time (2). Three genera of dermatophytes account for the majority of infections: *Epidermophyton*, *Trichophyton*, and *Microsporum*. In the United States, *Trichophyton* species account for the majority of dermatophytoses (3,4). Clinical infections are commonly named based on the region of the body affected. For instance, “tinea pedis” refers to a dermatophyte infection of the foot. Likewise, tinea manuum, tinea corporis, tinea cruris, tinea faciei, tinea capitis, tinea barbae, and tinea unguium refer to dermatophyte infections of the hand, body, genitalia, face, scalp, beard, and nails, respectively.

History

Charif and Elewski highlight the history and epidemiology of dermatophytoses in Europe and the United States (5). *Trichophyton rubrum* originated in West Africa, Southeast Asia, Northern Australia, and Indonesia mostly in the form of tinea corporis. Tinea pedis was not reported in Europe until 1908 (Great Britain). Until then, tinea pedis was considered a rare, sporadic, nonrecurring phenomenon, likely due to the limited use of footwear. Increased population mobility through migration, recreational travel, as well as World War I allowed *T. rubrum* to translocate to the Americas, with the first case in the United States being reported shortly after World War I (5). Thereafter, the incidence of tinea pedis and onychomycosis increased as a result of future wars, frequent use of health clubs, occlusive footwear, and greater ease of travel. Although the incidence of tinea capitis initially remained relatively low due to rigorous screening of immigrants, tinea pedis flourished unchecked (5).

Epidemiology

Trichophyton rubrum is the most prevalent pathogen and most common etiologic agent in the United States for most dermatophytic infections except tinea capitis and fingernail onychomycosis. A recent epidemiological study in the United States from 1999 to 2002 reported increasing incidence of *T. rubrum* in onychomycosis, tinea corporis, tinea cruris, tinea manuum, and tinea pedis (6). *Trichophyton tonsurans* and *Candida albicans*, on the other hand, were the predominant species for tinea capitis and fingernail onychomycosis, respectively. The primary etiologic agents for the various dermatophytic infections are listed in Table 1.

Table 1 Etiologic Organisms in Cutaneous Mycotic Infections

Condition	Dermatophytic fungi	Nondermatophytic fungi	Yeasts	Molds	Miscellaneous
Tinea pedis	<i>Trichophyton rubrum</i> <i>Epidermophyton floccosum</i> <i>Trichophyton mentagrophytes</i>	<i>Scytalidium hyalinum</i>	<i>Candida albicans</i>		<i>Corynebacterium minutissimum</i>
Tinea corporis	<i>T. rubrum</i> <i>Microsporum canis</i> <i>T. richophyton tonsurans</i> <i>T. richophyton verrucosum</i>				
Tinea cruris	<i>T. rubrum</i> <i>E. floccosum</i> <i>T. mentagrophytes var interdigitale</i>				
Tinea faciei	<i>T. rubrum</i> <i>T. mentagrophytes</i>				
Tinea capitis	<i>T. tonsurans</i> <i>T. mentagrophytes</i> <i>M. icrosporum audouinii</i> <i>M. canis</i> <i>M. icrosporum gypseum</i> <i>T. richophyton equinum</i> <i>T. verrucosum</i> <i>T. violaceum</i> <i>T. richophyton schoenleinii</i> <i>T. tonsurans</i> <i>T. mentagrophytes</i> <i>M. icrosporum audouinii</i> <i>M. canis</i> <i>M. icrosporum gypseum</i> <i>T. richophyton equinum</i> <i>T. verrucosum</i> <i>T. violaceum</i> <i>T. richophyton schoenleinii</i>				
Tinea unguium					
DSO	<i>T. rubrum</i> <i>T. mentagrophytes</i> <i>T. tonsurans</i> <i>E. floccosum</i>				
WSO	<i>T. mentagrophytes</i>			<i>Aspergillus terreus</i> <i>Acremonium roseogrisum</i> <i>A. cremonium potonii</i> <i>Fusarium oxysporum</i>	
PSO	<i>T. rubrum</i> <i>T. richophyton megnini</i> <i>T. tonsurans</i> <i>T. mentagrophytes</i>				
Candidal			<i>C. albicans</i>		

Abbreviations: DSO, distal subungal onychomycosis; PSO, proximal subungal onychomycosis; WSO, white superficial onychomycosis.

Source: Adapted from Melody RVS, Hossain MA, Ghannoum MA. *Infect Dis Clin North Am* 2003; 17(1):87–112.



Figure 1 (See color insert) *Tinea cruris* in a transplant patient.

Pathogenesis

Dermatophytes use keratin as a nutrient source. Unlike most other fungi, dermatophytes produce keratinases allowing invasion into and subsequent colonization of the keratinized stratum corneum. They generally do not invade viable tissue and rarely extend below the epidermis. Involvement of underlying and surrounding tissues usually results from allergic or inflammatory host responses to the presence of the fungi. The circular morphology results from the inflammatory reaction forcing dermatophytes outward to inflammation-free areas. Outward migration continues as long as the infection persists (7).

In dermatophyte species infecting the hair (*tinea capitis* and *tinea barbae*), infection may occur in one of the two ways: (i) in ectothrix invasion (i.e., *M. canis*, *M. gypseum*, *T. equinum*, and *T. verrucosum*), the arthroconidia form on the outside of the hair shaft and destroy the cuticle; (ii) in endothrix infections (i.e., *T. tonsurans* and *T. violaceum*), the arthroconidia form within the hair shaft leaving the cuticle intact.

Transmission of dermatophytes to humans is classified into one of three categories: geophilic, zoophilic, and anthropophilic. Geophilic transmission involves transfer of fungal spores or hyphae from the soil to either a human or animal host, whereas zoophilic and anthropophilic transmission involves transfer of fungal spores and hyphae from animals to humans or humans to humans, respectively. Geophilic species survive by decomposing keratinaceous debris. Dermatophytes are often transmitted by either direct contact with infected humans or animals or indirectly by human contact with desquamated skin harboring the fungi (which can persist for months to years without an animal host). Human carriers occur when dermatophytes cause clinical or subclinical infections of the hair, skin, or nails. Subclinical infections usually occur within interdigital spaces or on the scalp and not only create a public health hazard but also act as reservoirs for autoinoculation, making eradication of dermatophytoses difficult.

Dermatophytes thrive in warm moist conditions. Conditions predisposing to fungal infections include immobility from increased age or medical comorbidities, immunosuppression, warm climates, poor hygiene, disruption of the skin barrier or skin maceration, and contact with infected individuals or surfaces (Fig. 1). Immunosuppression not only increases the risk for fungal infections but also may lead to more severe disease with increased rates of recurrence and possible direct deep dermal invasion (8).

Clinical Features

Tinea Pedis

Tinea pedis is the most common dermatophytosis with a lifetime incidence of up to 70% (4). The incidence of *tinea pedis* has been estimated at 3% in the United States but may be up to



Figure 2 (See color insert) Tinea pedis.

5% in the elderly and in excess of 20% in populations who use communal showers or locker rooms (9). *T. rubrum* causes the majority of infections. Risk factors for infection or conversion to symptomatic carriage include hyperhydrosis secondary to warm humid climates or occlusive footwear and walking barefoot on surfaces at risk for contamination, such as communal locker rooms and shower stalls.

Three clinical forms of tinea pedis predominate: (i) interdigital, (ii) moccasin, and (iii) vesiculobullous (1). Interdigital infection involves the web spaces, most commonly between the fourth and fifth toes. It is often malodorous because of bacterial superinfection and pruritic. The infection can be dry and scaly, resembling psoriasis, or moist and macerated (Fig. 2). Unlike interdigital infection, moccasin-type infections involve the heel, plantar, lateral, and medial aspects of the foot. It may present as mild asymptomatic scale or as silvery-white scale on a red, thickened base, resembling psoriasis or contact dermatitis. Moccasin-type tinea pedis infections can be recalcitrant to treatment and may be complicated by onychomycosis, tinea manuum, tinea cruris, or tinea corporis (10). Vesiculobullous tinea pedis usually occurs on the sole but may also be on the side of the foot. Vesicles or pustules are present, and the area may become macerated and secondarily infected with bacteria. This form may be misdiagnosed as dyshidrotic eczema, contact dermatitis, nummular eczema, or pustular psoriasis. Other conditions that may resemble tinea pedis include candidiasis, erythrasma, pyoderma, secondary syphilis, and pitted keratolysis.

Tinea Manuum

Tinea manuum is a much less prevalent infection than tinea pedis and involves the palmar, dorsal, or interdigital surface of the hands and fingernails. It may appear diffusely dry and hyperkeratotic or resemble tinea corporis (Fig. 3). Only one hand is usually involved; however, both may be affected. Bilateral tinea pedis is also frequently an accompanying feature. Differential diagnosis includes psoriasis, eczema, dyshidrotic eczema, callous formation, secondary syphilis, contact dermatitis, and infection with nondermatophytic fungi.

Tinea Corporis

Tinea corporis, or "ringworm," is an infection of the glabrous skin, usually on the trunk and extremities. The predominant pathogens are *T. rubrum* and to a lesser extent *T. tonsurans*. Infection in wrestlers is referred to as tinea corporis gladiatorum and is most often caused by *T. tonsurans*. In teams without known epidemics, prevalence rates ranged from 20% to 44% (11). Majocchi's granuloma, tinea imbricata, and tinea incognito are three variants of tinea corporis. Deep-seated tinea corporis, referred to as Majocchi's granuloma, is most often due to *T. rubrum* or *T. mentagrophytes*. It occurs most frequently in women and is typically located on



Figure 3 Tinea manuum.

the lower extremities (4) (Fig. 4). *Tinea imbricata* is caused by *T. concentricum* and is limited geographically to southwest Polynesia, Melanesia, Southeast Asia, India, and Central America. Lastly, *tinea incognito* is a term applied to atypical clinical lesions of tinea that have been treated with topical corticosteroids (Fig. 5).

Tinea corporis is characterized by an erythematous, round, annular plaque with central pallor and scaly, raised advancing border that may contain papules or pustules (Fig. 6). These infections are sometimes pruritic and can resemble nummular eczema, plaque psoriasis, granuloma annulare, erythema nodosum, contact dermatitis, cutaneous lupus erythematosus, drug eruptions, and erythema multiforme.

Tinea Faciei

Tinea faciei affects the nonbearded areas of the face. It may present as itchy, red poorly demarcated patches or may resemble *tinea corporis* with scaly, red annular plaques (Fig. 7). It should be considered in all erythematous eruptions on the face (1). The differential diagnosis includes rosacea, contact dermatitis, seborrheic dermatitis, lymphocytic infiltration, and discoid lupus erythematosus.



Figure 4 Majocchi's granuloma.

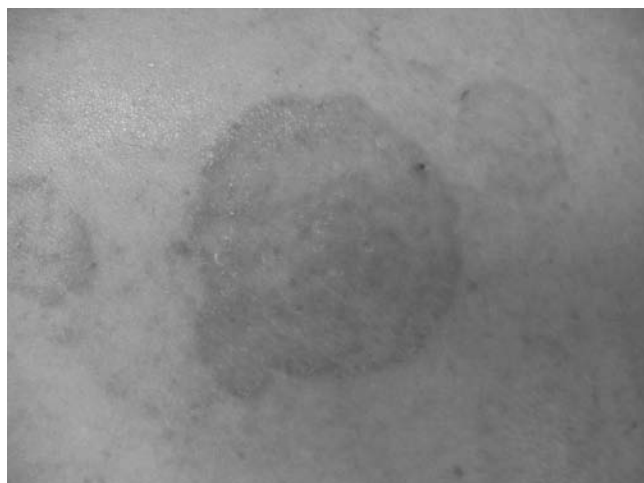


Figure 5 (See color insert) Tinea incognita.



Figure 6 Tinea corporis.



Figure 7 (See color insert) Tinea faciei.



Figure 8 Tinea cruris.

Tinea Cruris

Tinea cruris, or "jock itch," is an invasion of the hair follicles that can easily be confused with cutaneous candidal infection. It is most commonly caused by *T. rubrum* and often occurs during the summer, in young men, and in persons with tight-fitting clothing. Tinea cruris forms an erythematous, pruritic patch on the intertriginous inguinal folds and medial thighs with characteristic sparing of the scrotum and penis (Fig. 8). The patch spreads peripherally with partial central clearing and may have small follicular papules, pustules, or vesicles along the advancing border. Infections with *Candida* species may closely resemble tinea cruris; however, it may be distinguished by their moist appearance, presence of satellite lesions, and possible scrotal involvement. Mechanical intertrigo, or "chafing", may also be misdiagnosed as tinea cruris; however, it is usually sharply demarcated, tender, and lacking scale. Erythrasma can be distinguished by Wood's lamp examination, under which it fluoresces coral red. Lastly, inverse psoriasis and seborrheic dermatitis may be difficult to distinguish from tinea cruris without biopsy if other lesions outside the genital region are absent. Concurrent tinea pedis infection or onychomycosis can predispose to recurrence, suggesting possible transfer of organisms (4).

Tinea Capitis

Tinea capitis primarily affects preadolescent children, although adult carrier states have been reported (12). It can be transmitted via direct contact with infected persons and select animal vectors or indirectly through use of contaminated clothing, combs, and furniture. Prevalence in the United States is generally estimated to be between 3% and 8% of the pediatric population, with carriers occurring in as many as 34% of household contacts (13). Over the past 50 years, the primary etiologic agent of tinea capitis has changed from *Microsporum audouinii* to *T. tonsurans*. Recent studies report *T. tonsurans* as the predominant dermatophyte isolated in the United States, frequently achieving near exclusionary proportions (14–16). In contrast, *M. canis* is the most commonly isolated pathogen in Europe (8), and *T. violaceum* is the main infecting agent in children in India with tinea capitis (15).

The presentation of tinea capitis is highly variable. The primary lesions may be papules, pustules, plaques, or nodules on the scalp (Fig. 9). Inflammation and secondary infection lead to secondary processes, such as scaling, alopecia, erythema, exudate, and edema. The initial presentation may be subtle and asymptomatic. As the inflammatory response increases, inflammatory alopecia occurs with scaling and black dot alopecia due to breakage of weakened hairs at the scalp's surface (Fig. 10). A kerion may develop as a result of an intense cell-mediated immune response and is characterized by an inflamed, exudative, nodular, boggy swelling with associated hair loss and cervical lymphadenopathy (Fig. 11). An unusual scaling reaction, known as favus, confers a doughy or waxy appearance to the scalp by way of a saucer-shaped, yellow crust over hair follicles (called a scutula) with resultant scarring alopecia (Fig. 12). *T. schoenleinii* is responsible for this appearance (1,9).

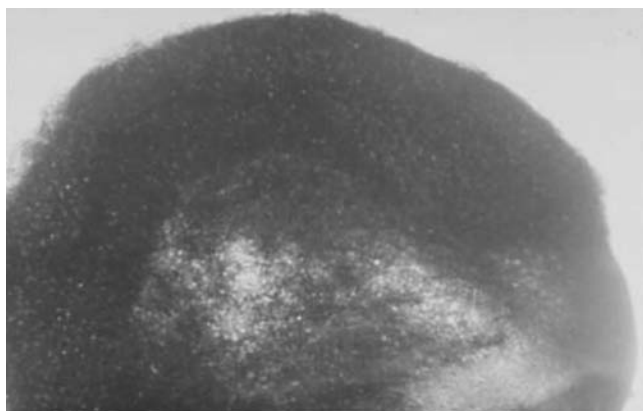


Figure 9 Tinea capitis.

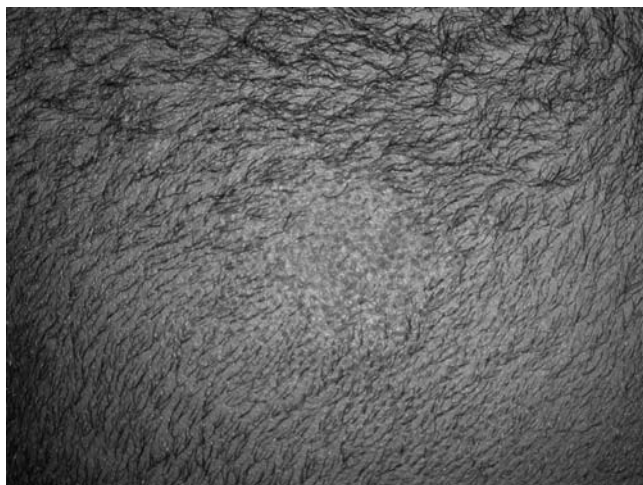


Figure 10 Tinea capitis with black dot alopecia.



Figure 11 (See color insert) Kerion.

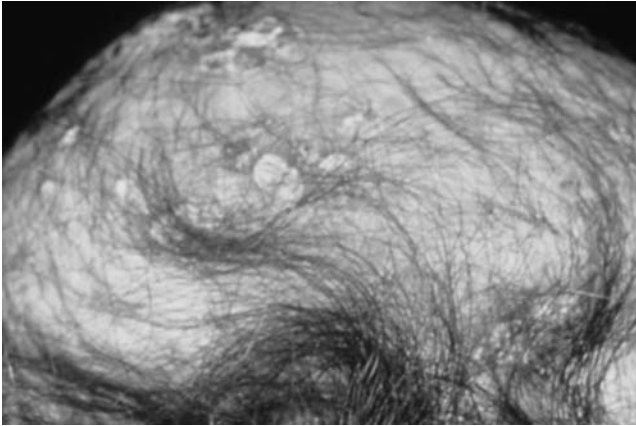


Figure 12 Flavus.

Tinea Barbae

Tinea barbae is a rare infection of the beard area. It most often occurs in men who are in close contact with farm animals (4,17). The fungi infect the hair shaft, forming either nodular, boggy, exudative lesions, or crusted patches with associated partial alopecia. Bacterial folliculitis and ingrown hairs may complicate the condition. *Tinea barbae* may be confused with folliculitis, sporotrichosis, candidiasis, pseudofolliculitis barbae, contact dermatitis, herpes labialis, acne, syphilis, and even malignant lymphoma.

Laboratory Diagnosis

Microscopic examination is the most efficient and cost-effective laboratory technique to diagnose dermatophyte infections. Diagnosis of *tinea pedis*, *tinea corporis*, *tinea cruris*, *tinea faciei*, *tinea barbae*, and *tinea manuum* can usually be made with 10% to 20% potassium hydroxide (KOH) preparation of scale scraped from the leading edge of the lesion. In vesicular or pustular lesions, the roof and contents of the vesicle or pustule should be examined. *Tinea capitis* can also be diagnosed by KOH via examination of extracted hairs. To speed the clearing of keratin, the slide is heated or dimethyl sulfoxide (DMSO) is added. Calcofluor is a stain specific for chitin, a fungal cell wall component, that may enhance the visualization of fungal elements. Under fluorescent microscopy, fungal elements fluoresce apple-green.

Fungal culture is used to confirm a diagnosis, especially in long-standing or recalcitrant disease, or when species identification is desired. Sabouraud dextrose agar with chloramphenicol and cycloheximide allows growth of dermatophytes while suppressing nondermatophytes and bacterial contaminants. The specimen (scale or extracted hair) is placed on the media and cultured at 26 °C to 28 °C for up to four weeks (9). In *tinea capitis*, cultures must involve extracted hairs, not simply scale, to provide accurate results. Any growth of dermatophytes is considered significant. Molecular sequencing is a more rapid alternative for species identification; however, its availability is limited. Lastly, a Wood's lamp can be used to diagnosis certain *tinea capitis* infections. Hair infected by ectothrix organisms, except for *T. verrucosum*, fluoresce bright green or yellow-green while endothrix organisms such as *T. tonsurans* do not fluoresce (Fig. 13).

Treatment and Prevention

Tinea Pedis

Tinea pedis usually responds to topical antifungal agents, many of which are available over the counter in a variety of formulations (creams, gels, powders, and aerosols). Antifungal powders (clotrimazole, miconazole, and tolnaftate) or gel formulations (naftifine, ciclopirox, and terbinafine) can be helpful, especially when there is appreciable moisture. Duration of topical treatment varies based on the antifungal agent and ranges from a single application with the new terbinafine film-forming solution to multiple applications daily for up to four



Figure 13 Kerion fluorescence due to ectothrix infection.

weeks. While the various topical antifungal agents and formulations have variable dosing regimens, their clinical and mycologic cure rates appear comparable (18). Recurrence is common, especially if there is concomitant untreated onychomycosis. Furthermore, topical antifungal treatments have been reported to fail in up to 33% of patients with tinea pedis (19). Oral therapy can be considered for extensive, severe, or recalcitrant disease. Some of the more common and effective oral treatments include terbinafine, itraconazole, and fluconazole. Considering relative cost-effectiveness, griseofulvin is also an option; however, current data suggests it may be significantly less effective than terbinafine (20). The recommended doses for the oral antifungal agents are as follows: griseofulvin 500 mg twice daily for four to eight weeks with higher doses used for relapse, itraconazole 400 mg daily for one week or 200 mg daily for two weeks, fluconazole 150 to 300 mg once weekly for four to six weeks, and terbinafine 250 mg daily for two to six weeks (21,22). Table 2 summarizes the dosing regimens for tinea pedis for the oral antifungal agents. The addition of products containing glycolic or lactic acids or urea may decrease the amount of hyperkeratosis.

Tinea Manuum

Tinea manuum, like tinea pedis, can usually be treated effectively with topical antifungal agents and keratolytics. Infections may recur if untreated tinea pedis or onychomycosis is present (1). Oral antifungal agents may be used for refractory disease and are dosed similarly to tinea pedis (17,23).

Tinea Corporis, Tinea Faciei, and Tinea Cruris

Tinea corporis, tinea faciei, and tinea cruris usually respond well to topical antifungal creams or powders. Treatment should be continued for 7 to 14 days beyond symptom resolution to minimize recurrence. Similar to tinea pedis and tinea manuum, oral medications are reserved for extensive or recalcitrant disease. Commonly prescribed doses of oral antifungals are listed in Table 2 and include itraconazole, 200 to 400 mg daily for one week; fluconazole, 150 to 300 mg once weekly for two weeks (tinea cruris) or four to six weeks (tinea corporis); terbinafine, 250 mg daily for 10 days; and griseofulvin, 500 mg daily for two to four weeks (24).

Tinea Capitis

Oral antifungals are currently the mainstay of treatment for tinea capitis. Griseofulvin has long been the standard of care for the treatment of tinea capitis and, until recently, had been the only

Table 2 Treatment of Cutaneous Mycotic Infections

Condition	Medication	Dose and regimen	Comments		
Tinea pedis	Topical	Econazole nitrate cream	Daily to bid 1–4 wk	Antifungal powders or gel formulations of ciclopirox or naftifine for moist webspaces	
		Ketoconazole cream	Daily to bid 1–4 wk		
		Terbinafine hydrochloride cream	Daily to bid 1–4 wk		Terbinafine film-forming solution → single use
	Oral	Ciclopirox cream	Daily to bid 1–4 wk	Higher doses for relapse	
		Griseofulvin	500 mg bid x 4–8 wk		
		Itraconazole	400 mg daily x 1 wk or 200 mg daily x 2 wk		
		Fluconazole	150–300 mg once weekly x 4–6 wk		
	Terbinafine	250 mg daily x 2–6 wk			
Tinea manuum	Topical	Lac-Hydrin 12% lotion	Bid		
	Oral	Griseofulvin	500 mg bid x 4–8 wk		
		Itraconazole	400 mg daily x 1 wk or 200 mg daily x 2 wk		
		Fluconazole	150–300 mg once weekly x 4–6 wk		
		Terbinafine	250 mg daily x 2–6 wk		
Tinea corporis	Topical	Miconazole nitrate cream	Bid	Continue topicals for 7–14 days beyond symptom resolution	
		Clotrimazole cream	Bid		
		Ketoconazole cream	Bid		
		Econazole cream	Bid		
	Oral	Terbinafine cream	Bid x 1 wk		
		Griseofulvin	500 mg daily x 2–4 wk		
		Itraconazole	200–400 mg daily x 1 wk		
		Fluconazole	150–300 mg once weekly x 4–6 wk		
			Terbinafine	250 mg qd x 10 days	
Tinea cruris	Topical	Econazole cream	Bid x 2–3 wk	7–14 days beyond symptom resolution; may help to add antifungal powders	
		Econazole cream	Bid x 2–3 wk		
		Miconazole cream	Bid x 2–3 wk		
		Ketoconazole cream	Bid x 2–3 wk		
		Terbinafine cream	Bid x 2–3 wk		
	Oral	Ciclopirox cream	Bid x 2–3 wk		
		Griseofulvin	500 mg daily x 2–4 wk		
		Itraconazole	200–400 mg daily x 1 wk		
		Fluconazole	150–300 mg once weekly x 2 wk		
		Terbinafine	250 mg daily x 10 days		
Tinea faciei	Topical	Econazole cream	Daily x 2 wk		
		Miconazole cream	Daily x 2 wk		
		Ketoconazole cream	Daily x 2 wk		

(Continued)

Table 2 Treatment of Cutaneous Mycotic Infections (*Continued*)

Condition	Medication	Dose and regimen	Comments		
Tinea capitis	Topical	Ketoconazole cream	Daily	Adjunct only; reduce fungal shedding Continue 2 wk beyond cure FDA approved in children	
	Oral	Ketoconazole shampoo	Daily		
		Griseofulvin	20–25 mg/kg/day x 8 wk or 10–15 mg/kg/day (ultra microsize)		
		Terbinafine	125 mg/day (<25 kg) 187.5 mg/day (25–35 kg) 250 mg/day (>35 kg)		FDA approved in children
		Ketoconazole Itraconazole Fluconazole	200 mg daily x 4 wk 5 mg/kg/day x 4–6 wk 3–6 mg/kg/day x 6 wk (oral solution)		Avoid oral solution
Tinea barbae	Oral	Griseofulvin	0.5–1 g/day x 2–4 wk	Combine with regular shaving and cleansing with antibacterial soaps	
		Itraconazole			
		Terbinafine			
		Fluconazole			
Tinea unguium	Topical	Ciclopirox 8% nail lacquer	daily, 8 hr before handwashing, remove weekly with alcohol	Mild to moderate disease only 5–8% cure rate	
		Oral	Griseofulvin		500 mg daily x 6 mo (fingernails) 500 mg daily x 12 mo (toenails)
	Terbinafine		250 mg daily x 6 wk (fingernails) 250 mg daily x 12 wk (toenails)		
			Itraconazole (continuous)	Fingernail 200 mg daily x 2 mo Toenail 200 mg daily x 3 mo	
	Itraconazole (pulsed)			400 mg daily x 7 days, repeat in 1 mo (fingernails) 400 mg daily x 7 days, repeat for 3–4 mo (toenails)	
			Fluconazole	150–300 mg/wk x 3–6 mo (fingernails) 150–300 mg/wk x 6–12 mo (toenails)	
	Tinea versicolor			Topical	Selenium sulfide shampoo
		Terbinafine 1% solution	Bid x 1 wk		
Econazole cream		Daily x 2 wk			
Oral		Ketoconazole shampoo	Daily x 3 days		
		Itraconazole	200 mg daily x 3–7 days		
		Ketoconazole	400 mg x 1 dose or 200 mg daily x 5–10 days		
		Fluconazole	400 mg x 1 dose		

Source: Adapted from Melody RVS, Hossain MA, Ghannoum MA. *Infect Dis Clin North Am* 2003; 17(1):87–112.

FDA-approved medication for tinea capitis in children. Griseofulvin continues to be widely used among pediatricians and dermatologists. Griseofulvin comes in both oral suspension and crushable tablet formulations. As it is poorly water soluble, absorption can be enhanced if taken with a fatty meal. Over the past few decades, higher doses have been required to eradicate infection. The current recommended dose is 20 to 25 mg/kg/day of the microsize formulation or 10 to 15 mg/kg/day of the ultramicrosize formulation for six to eight weeks, or two weeks beyond clinical cure (14). In 2007, terbinafine was approved by the FDA for treatment of tinea capitis in children older than four years of age. Results of a meta-analysis by Fleece et al. indicated that terbinafine is as effective as or more effective than griseofulvin for the treatment of tinea capitis caused by *T. tonsurans*, with equivalent side-effect profiles (25). Terbinafine is available in easy to use granules that can be sprinkled on nonacidic food. The approved pediatric dose is based on weight according to the following guidelines: 125 mg/day for children less than 25 kg, 187.5 mg/day for children weighing between 25 and 35 kg, and 250 mg/day for children over 35 kg. Therapy should be given once daily for six weeks. Serum transaminases are advised prior to treatment. While currently not FDA approved for tinea capitis, studies suggest that itraconazole and fluconazole may be effective alternative treatments (25,26). Additional studies are required to determine appropriate dosing of the azole antifungal agents, including pulse versus continuous scheduling, and to evaluate their safety in children. Currently available off label, itraconazole's oral suspension is not recommended due to concerns of potential carcinogenicity of its vehicle, hydroxypropyl-B-cyclodextrin.

Optimal treatment of asymptomatic carriers remains to be established. Topical therapy with antifungal shampoos such as ketoconazole or selenium sulfide may help to reduce shedding of viable spores. Combs, hats, pillows should also be frequently disinfected to prevent transmission of conidia to household contacts.

Tinea Barbae

Oral antifungal agents are also considered the first-line treatment for tinea barbae. Oral griseofulvin is conventionally used at a dose of 0.5 to 1 g/day for two to four weeks. Itraconazole, terbinafine, and fluconazole may also be used for difficult-to-treat cases. Regular shaving and cleansing with antibacterial soaps also aid in treatment.

ONYCHOMYCOSIS

Onychomycosis, or tinea unguium, is an infection of the nail plate or nail bed that interferes with normal nail function (Fig. 14). Eighty to ninety percent of infections are caused by dermatophytes such as *T. rubrum* and *T. mentagrophytes var interdigitale*. The remainder of infections is caused by a combination of nondermatophytic filamentous fungi (3–5%), molds, and yeasts (1–2%) (3,23,24). Patients with onychomycosis may experience pain, difficulty performing daily activities, and embarrassment over their condition due to associated nail disfigurement and limitation of mobility. Factors predisposing to onychomycosis infections include immunosuppression, diabetes, hemodialysis, concurrent dermatophyte infections at other locations, age greater than 60 years, obesity, male gender, poor peripheral circulation, smoking, genetic predisposition, and history of nail trauma (1,3,21,27,28). Conflicting data exists as to whether psoriasis, a condition that can be associated with nail deformity, is also a predisposing factor to onychomycosis (29). A recent prospective study of 2761 patients in Poland found 42.8% of patients with toenail onychomycosis had a concurrent second dermatophyte infection with tinea pedis being the most common concurrent fungal infection occurring in 33.8% of patients. Less common infections included fingernail onychomycosis (7.4%), tinea cruris (4.2%), tinea corporis (2.1%), tinea manuum (1.6%), and tinea capitis (0.5%) (30).

Epidemiology

Onychomycosis accounts for up to 50% of all nail disorders. The incidence of onychomycosis is increasing worldwide as a result of an aging population, occlusive footwear, and increasing numbers of immunocompromised patients (1). The prevalence of dermatophyte nail infections was found to be 8.7% in a 1997 cross-sectional survey conducted in a dermatology clinic waiting room in Cleveland. Prevalence ranged from 1.1% in children less than 18 years of age to as high

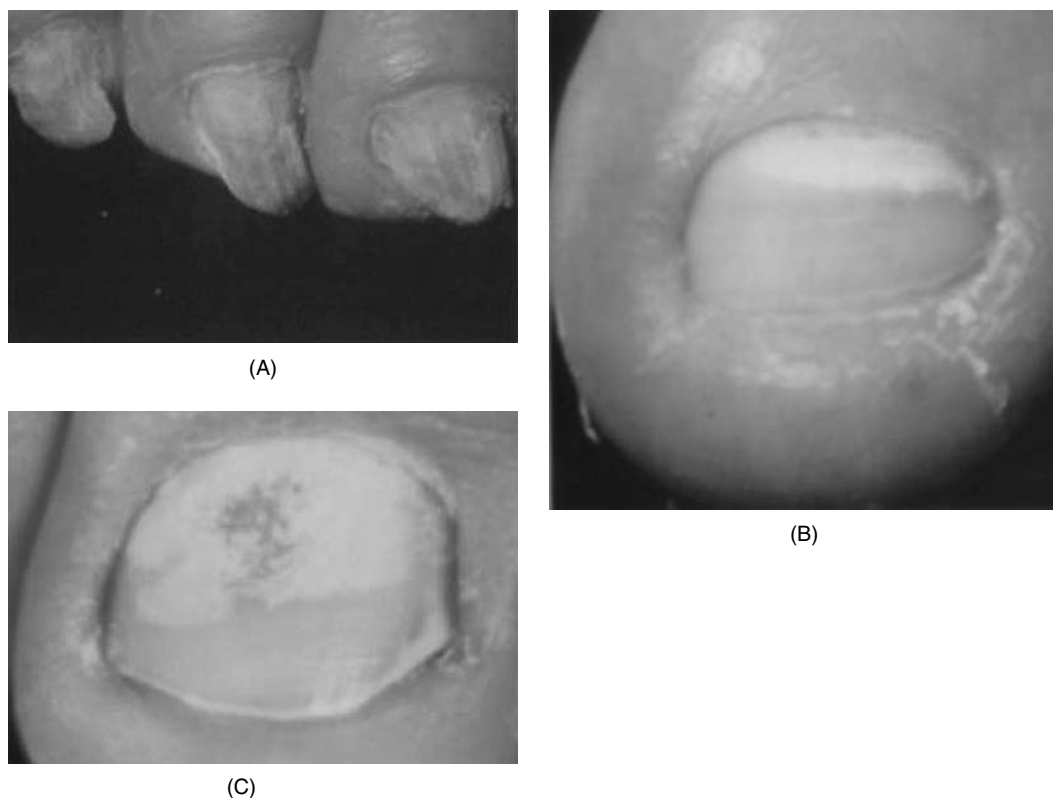


Figure 14 (A) Distal subungal (B) Proximal white subungal and (C) White superficial.

as 28.1% in adults over the age of 60 with an average prevalence of 6.5% in women and 13.3% in men (31). A more comprehensive multicenter study of 1832 patients reported a prevalence of 13.8% by microscopic examination with KOH and 7.2% by culture confirmation (3). The association of onychomycosis infection and increasing age may partially be attributed to an age-related decrease in immune function, lower vascular efficiency, and less efficient T cells and phagocytes.

Up to a third of diabetic patients may develop onychomycosis (32). Diabetic patients tend to have higher rates of complication including rapid spread to unaffected nails or the development of osteomyelitis and cellulitis, which may progress to necrosis and amputation if not aggressively treated (21,32). Patients with HIV are also at increased risk for complications including more clinically aggressive disease, higher incidence of atypical presentations, resistance to conventional therapy, and higher rates of recurrence. As many as 11% to 67% of patients with AIDS suffer from onychomycosis, with infection more likely when CD4 cell count is less than 450 cells/mm³ (33).

Clinical Features

Onychomycosis may be divided into four clinical types: (i) proximal subungal, (ii) distal subungal, (iii) white superficial, and (iv) mucocutaneous candidiasis. Each type differs in clinical presentation and causative etiologic agents. Dermatophytes are the principal causative agent for distal subungal, proximal subungal, and white superficial onychomycosis with *Candida* present in less than 1% of cases (1).

Proximal subungal onychomycosis is most commonly caused by *T. rubrum* but may also be caused by *T. megnini*, *T. tonsurans*, and *T. mentagrophytes* (34). Toenails are affected more often than fingernails. It is the least common clinical presentation among healthy individuals and may

be an early indicator of HIV infection. Infection enters the cuticle and involves the proximal nail bed. If left untreated, infection spreads distally eventually involving the entire nail plate. The nail plate is usually white in color with associated subungual hyperkeratosis and proximal onycholysis (1).

Distal subungual onychomycosis, in contrast, is the most common clinical presentation comprising more than 90% of all nail infections (1). Its most frequent etiologic agent is *T. rubrum*, but is also caused by *T. tonsurans*, *T. mentagrophytes*, and *E. floccosum*. It also preferentially affects the toenails. Fungal hyphae enter distally under the nail plate and spread proximally digesting the stratum corneum of the nail bed and nail plate. As the infection spreads, subungual hyperkeratosis, paronychia, and onycholysis can occur. Splinter hemorrhages can be seen secondary to mild inflammation compressing small vessels, and discoloration of the nail plate may occur as a result of secondary infection with mold or bacteria.

White superficial onychomycosis occurs in only 10% of cases. It is most commonly caused by *T. mentagrophytes* but may also be caused by nondermatophyte molds such as *Aspergillus terreus*, *Fusarium oxysporum*, *Acremonium potonii*, and *A. roseogrisum*. The dorsal surface of the nail plate is attacked. Minimal inflammation occurs as viable tissue is typically not involved. Clinically, the nail becomes crumbly and soft with a white discoloration and rough texture. If unchecked, the infection may spread through the nail plate to the cornified layer of the nail bed (1).

Candidal onychomycosis is usually found in patients with chronic mucocutaneous candidiasis. The typical pathogen is *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. guilliermondii*, and *C. parapsilosis* may also be found; although, the latter species are thought to be skin contaminants (3). *Candida* may cause a direct infection of the nail bed or plate, or it may indirectly involve the nail through infection of the nail folds, nail bed, or hyponychium after trauma or exposure to excessive moisture or irritants. Furthermore, other body sites may be involved including the face and arms. Candidal organisms causing onychomycosis have shown some resistance to oral antifungal agents (35).

Onychomycosis may resemble other disorders that involve the nails such as psoriasis, lichen planus, distal onycholysis, subungual tumor, verruca vulgaris, Reiter's syndrome, Hallopeau's acrodermatitis, yellow nail syndrome, paronychia congenita, contact dermatitis, traumatic onychodystrophies, and bacterial superinfection (1,3). Psoriasis can occasionally be clinically differentiated from onychomycosis by the presence of skin lesions and its symmetrical distribution compared to the frequently unilateral distribution of onychomycosis (36). Fungal culture is necessary in questionable cases; although, the two conditions may coexist. Notably, treatment for psoriasis may worsen onychomycosis; conversely, the presence of a fungal nail infection may inhibit response to psoriasis treatment. Approximately 10% of patients with lichen planus develop nail abnormalities that may also be difficult to differentiate from onychomycosis. These abnormalities usually consist of onychorrhexis, longitudinal ridging of the nail plate, and "angel-wing" deformity. Additional findings of lichen planus—including violaceous polygonal papules on the extremities and mucous membranes, wickham's striae, and pterygium—may help to differentiate the nail findings of lichen planus from onychomycosis (1,3). A culture, however, may be required to distinguish these conditions. Lastly, distal onycholysis caused by formaldehyde in nail products, repetitive nail trauma, and contact dermatitis may cause hyperkeratosis of the nail bed with resultant onycholysis. History, culture, or patch testing may distinguish these conditions from onychomycosis.

Laboratory Diagnosis

Culture is the definitive diagnostic tool for onychomycosis (1). Microscopic preparation of debris underneath the nail plate in 10% to 15% KOH with calcofluor and gentle heating may aid in diagnosis, with sensitivity of 92% and specificity of 95% (37). Culture is considered the mainstay of diagnosis because it not only isolates the organism but also allows for identification of the etiologic agent allowing treatment to be tailored appropriately. Other diagnostic tests include nail unit biopsy, immunochemistry, polymerase chain reaction, restriction enzyme analysis, and flow cytometry. Most of these tests, however, are more difficult or more expensive (although probably faster) than culture and are currently only available as research tools.

Treatment and Prevention

Systemic therapy is the mainstay of treatment for onychomycosis as less than 20% of cases respond to topical treatment alone due to poor penetration of the nail bed (1). Topical treatment with ciclopirox 8% nail lacquer may be effective in mild-to-moderate (early) *T. rubrum* onychomycosis infections without lunula involvement when used every day, eight hours before washing hands, with removal once a week with alcohol. Treatment may take six months to one year to be effective with a clinical cure rate of 5–8% and a mycological cure rate of 29–36% (38). Terbinafine, itraconazole, and fluconazole (not FDA approved) are the most frequently used oral antifungal agents for onychomycosis. Oral griseofulvin may also be effective for infections caused solely by dermatophytes. Dose and length of treatment differ for toenail and fingernail involvement. The recommended dose of terbinafine is 250 mg daily for six weeks (fingernails) or 12 weeks (toenails) (1,31,39). Itraconazole and fluconazole may be given in a pulsed dosing regimen. Itraconazole (pulsed dose) is given at 400 mg daily (or 200 mg twice a day) for seven days repeating in one month for fingernails and continuing for three to four months in toenail infections (27,31,39,40). Itraconazole (continuous dosing) may also be given at 200 mg daily for two months (fingernails) or three months (toenails) (40). Fluconazole (pulsed dosing) is usually effective at 150 to 300 mg once weekly for three to six months (fingernails) or 6 to 12 months (toenails). Several recent studies suggest an added benefit of adjuvant topical therapy with ciclopirox nail lacquer to systemic antifungal treatment (27,41). When treating onychomycosis it is important to realize that visible clearance of infection occurs after the process of nail-plate turnover is complete, which typically takes 12 to 18 months for toenails and 4 to 6 months for fingernails. Some investigators recommend continuation of treatment until the diseased nail is replaced by normal growth; however, this issue remains controversial (42).

Despite treatment, recurrence of onychomycosis is not uncommon, with reported rates ranging from 10% to 53% of cases (43).

TINEA VERSICOLOR

Tinea versicolor, otherwise known as *pityriasis versicolor*, is a common superficial mycotic infection of otherwise healthy young and middle-aged adults caused by lipophilic yeast of the *Malassezia* genus. Recent evidence has suggested *Malassezia* species are not only the causative agent of tinea versicolor but also seborrheic dermatitis and possibly atopic dermatitis (44).

Epidemiology

Malassezia species are found worldwide. Affected individuals are typically young adults; however, people of all ages may develop the disease. Use of new molecular techniques recently allowed for better classification of the *Malassezia* genus including the identification of seven species: *M. furfur*, *M. pachydermatitis*, *M. sympodialis*, *M. globosa*, *M. slooffiae*, *M. restricta*, and *M. obtuse* with possibly five additional species awaiting validation. While tinea versicolor had long been attributed to *M. furfur*, the aid of newer molecular techniques recently uncovered *M. globosa* as the predominant species of tinea versicolor reaching incidences of up to 90% (45).

Pathogenesis and Host Defense

Malassezia species may be part of the normal human flora. Tinea versicolor occurs when the yeast form transforms into the mycelial form. This transformation has been reported to occur in the presence of high temperature or high humidity, oily skin, excessive sweating, immunodeficiency, malnutrition, pregnancy, and hereditary predisposition. The change from the saprophytic to mycelial phase, however, is not completely understood. *Malassezia* species have an oil requirement for growth likely accounting for the increased incidence of disease in adolescents and preference for sebum-rich areas of the skin. The yeast does not cause an inflammatory response in the host but may lead to decreased pigmentation. The reduction in pigmentation has been hypothesized to result from inhibitory effects of dicarboxylic acids and lipoperoxidases, produced by the metabolism of surface lipids by yeast, on melanocytes (45). Overall, the condition causes minimal morbidity with most patients seeking treatment for cosmetic reasons.



Figure 15 Tinea Versicolor.

Clinical Features

Tinea versicolor causes erythematous, hypopigmented, or hyperpigmented macules or patches which may or may not be pruritic (Fig. 15). Fine scaling may be elicited with scratching of the skin surface. Sebum-rich areas, in particular the upper chest, upper back, and shoulders, are frequent sites of involvement. In temperate climates, facial involvement occurs primarily in children, whereas in tropical and subtropical regions, it is common in both adults and children (45). Tinea versicolor may be confused with tinea corporis, vitiligo, pityriasis rosea, pityriasis alba, and secondary syphilis, among others (17).

Laboratory Diagnosis

Clinical diagnosis of tinea versicolor is easily confirmed with direct microscopic examination. Microscopic examination with KOH demonstrates characteristic short, angular hyphae and budding yeast commonly referred to as “spaghetti and meatballs” appearance. Wood’s lamp can also be used to diagnose tinea versicolor; however, the yellow fluorescence is only elicited in cases caused by *M. furfur*, as it is the only *Malassezia* species that produces fluorochromes. As a result, fluorescence is not appreciated in up to two-thirds of cases. Culture is usually not necessary for diagnosis but can be used for species identification. Special media (mDixon or Leeming-Notman) with an exogenous lipid source is needed as only *M. pachydermatis* grows in routine mycologic media such as Sabouraud dextrose agar. In addition, as several species are inhibited above 37°C, cultures should be incubated between 30°C and 35°C (45).

Treatment and Prevention

Tinea versicolor does not clear spontaneously and alterations in pigment may take years to normalize despite treatment. Due to the organism’s lipophilic nature, patients are instructed to avoid oils applied to the skin or bath. For eradication of the *Malassezia*, both topical and oral medications have been found to be effective. Topical treatments include azole antifungal agents, terbinafine, and selenium sulfide preparations. Various dosing regimens have been suggested. Possible regimens include azole creams daily for one to two weeks, ketoconazole 2% shampoo daily for one week, or selenium sulfide 2.5% shampoo daily (left on the skin for 3–5 minutes and then rinsed off) for seven days. Oral therapy may be preferred in patients with extensive skin involvement or frequent recurrences. Ketoconazole, itraconazole, and fluconazole are the three main oral therapies used. Recommended dosing regimens include ketoconazole 400 mg once, itraconazole 200 mg daily for five to seven days, or fluconazole 400 mg once or 300 mg weekly for two weeks (45). Oral terbinafine and griseofulvin are ineffective in the treatment of tinea versicolor. Periodic retreatment or prophylactic treatment, i.e., ketoconazole shampoo

or selenium sulfide 2.5% shampoo once weekly, may be needed due to tinea versicolor's high recurrence rate reaching up to 80% after two years (45).

SIDE EFFECTS OF ORAL ANTIFUNGALS

Newer, oral antifungal agents have greatly improved the management of dermatomycoses but not without consequence. Some are quite expensive (although this is changing with some of the oral agents becoming generic), have side effects and organ toxicity that may or may not be tolerable, or have significant drug interactions (23,46–48). As a result, treatment selection should be based on a patient's likelihood of compliance, age, concomitant medical conditions, and potential drug interactions.

Griseofulvin is an effective inexpensive treatment option for dermatophyte infections; however, it is ineffective against yeast and nondermatophyte organisms. Hepatotoxicity is a concern especially with long-term use, as in the treatment of onychomycosis. Other less severe but more common side effects include rash, headache, photosensitivity, nausea, and vomiting. Less commonly, arthralgia, peripheral neuritis, memory lapse, confusion, and insomnia have been reported (1,4,49). Griseofulvin has interactions with barbiturates, alcohol, cyclosporine, oral contraceptives, aspirin, and warfarin (47,48).

Hepatotoxicity is also a concern with ketoconazole occurring in one out of 10,000 to 15,000 people. Other side effects include nausea, vomiting, abdominal pain, diarrhea, headache, pruritus, insomnia, leukopenia, hemolytic anemia, decreased libido, and impotence (1,4,49). A key concern with the use of ketoconazole and the other azole antifungal agents are their numerous drug–drug interactions. Use of H₁ blockers (astemizole, terfenadine) as well as cisapride and triazolam are absolute contraindication for use of ketoconazole. A complete list of interactions can be found in a review on drug interactions by Brodell and Elewski (47).

Similar to ketoconazole, itraconazole is associated with numerous medication interactions. Use of itraconazole is contraindicated in patients taking terfenadine, astemizole, diazepam, oral triazolam, oral midazolam, cisapride, and HMG-CoA reductase inhibitors (lovastatin, simvastatin). Numerous additional interactions exist (1,47). Absorption is optimized with an acidic environment so it should also be taken one hour before or two hours after antacid use. Similarly, ingestion of a cola beverage can improve absorption in patients with achlorhydria. Absorption is also improved if taken with food (11). Side effects of itraconazole include diarrhea, headache, rhinitis, dyspepsia, nausea, dry skin, rash, weakness, pruritus, dizziness, hypertension, and loss of libido. Hepatotoxicity has only rarely been reported (1,4,49).

Similar to ketoconazole and itraconazole, fluconazole is associated with numerous medication interactions. Significant reactions have been associated with terfenadine, tacrolimus, astemizole, rifabutin, oral hypoglycemics, coumarin derivatives, phenytoin sodium, cyclosporine, theophylline, cisapride, and rifampin (1,4,47). Side effects occur with fluconazole, more often in HIV-positive patients (4) and include nausea, vomiting, headache, rash, diarrhea, dizziness, abdominal pain, dyspepsia, and taste perversion. Rare cases of hepatotoxicity, anaphylaxis, and exfoliative skin disorders have been reported (1,4,47).

Terbinafine has been associated with diarrhea, pruritus, dyspepsia, rash, taste disturbance, urticaria, abdominal pain, headache, visual disturbance, and neutropenia. Rare cases of cholestatic hepatitis and fulminant hepatic failure have been reported (50–52). Terbinafine should be avoided in patients with renal impairment or hepatic cirrhosis, as terbinafine clearance is reduced by 50%. In addition, terbinafine levels are potentiated by cimetidine and antagonized by rifampin. Cyclosporine levels should be monitored if taken concurrently with terbinafine (1,4,49).

Laboratory monitoring is recommended during treatment with the oral antifungal medications especially for prolonged therapy. Baseline liver function tests should be obtained for terbinafine and itraconazole. When itraconazole is prescribed to patients on concurrent cyclosporine, cyclosporine levels should be closely monitored in addition to serum creatinine concentration. Similarly, in patients on oral hypoglycemic agents, blood glucose levels should be closely monitored if concurrent fluconazole is prescribed. Renal, hepatic, and hematopoietic functions should be obtained for patients on prolonged griseofulvin therapy. Lastly, ketoconazole should be avoided for durations greater than 7 to 10 days. With this duration, monitoring

is not needed. It is generally agreed that treatment with oral antifungals should be avoided during pregnancy as the risk of use does not outweigh the benefits.

SUMMARY

Cutaneous fungal infections cause significant morbidity for healthy and ill patients. The incidence of some dermatomycoses is increasing despite availability of newer and better topical and systemic treatments. Fungal remnants last months to years under ideal conditions allowing continued spread of infection. Mycoses treated in one area may recur because of organism travel from concomitant areas of infection. Failure of patients and physicians to recognize a fungal etiology early may lead to more extensive, severe, or difficult-to-treat infections. Finally, a patient's concurrent illnesses may play a part in susceptibility and ability to manage fungal infections. For these reasons, scientists have studied and developed newer antifungal agents with better efficacy and great convenience in dosing. These drugs, however, still have side effects and drug-drug interactions that may limit their use in some patients. Better efforts to educate patients and physicians alike may aid in faster recognition, diagnosis, and treatment of dermatophytoses. More research is needed to continue to develop drugs suitable for use in a broader range of patients and diagnostic tests that may be quicker or more specific than conventional ones.

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16 Invasive Candidiasis

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INTRODUCTION

The term “invasive candidiasis” (IC) encompasses a wide variety of severe and invasive disorders that include candidemia, disseminated candidiasis, endocarditis, meningitis, endophthalmitis, and other deep organ involvement; it excludes more superficial and less severe diseases, such as oropharyngeal and esophageal candidiasis. Emerging trends in IC are notable for a dramatic increase in infections due to non-*albicans* *Candida* spp. Changes in the epidemiology of this disorder have been widely documented (1). The extensive use of prophylactic antifungal agents, mainly fluconazole, the wide use of broad-spectrum antibacterial agents, the more aggressive management of patient with leukemia and malignancy, the widespread use of transplant practice, and importantly the extensive use of invasive medical devices (e. g., chronic indwelling intravascular catheters) have contributed toward the changing epidemiology of IC. *Candida* spp. now rank as the fourth leading cause of hospital acquired bloodstream infection (BSI) in the United States, accounting for 8% to 10% of all BSIs acquired in the hospital (2). One of the major concerns with IC is that it is associated with an excess attributable mortality rate of up to 49% (3–7) and an excess length of hospital stay of 3 to 30 days (8,9). Furthermore, the estimated additional cost for each episode of IC in adults is approximately \$40,000 (5). Similar impacts of candidal infections have been seen in the pediatric population (8,10).

This chapter addresses the epidemiology and risk factors associated with IC, with particular emphasis on the common clinical presentations, diagnostic modalities, and the new treatment guidelines.

EPIDEMIOLOGY AND RISK FACTORS

Most IC infections are caused by one of five *Candida* spp. (*albicans*, *parapsilosis*, *tropicalis*, *glabrata*, and *krusei*), with *C. albicans* accounting for approximately 40% to 50% of infections in most reports (11). However, recent reports have noted an increasing trend in the isolation of non-*albicans* spp. in some clinical settings (1,12–14). What is more, each of the non-*albicans* *Candida* spp. has been associated with a specific patient population and risk factors (Table 1).

Candida glabrata ranks second to *C. albicans* as a cause of BSI in the United States accounting for 20% to 24% of all *Candida* BSIs (11). Trick et al. have demonstrated that among the *Candida* spp., *C. glabrata* alone has increased as a cause of BSI in US intensive care units (ICUs) since 1993 (15). On a global scale, the frequency of *C. glabrata* as a cause of BSI varies from 22% in North America to 4–6% in Latin America (16,17), 10.5–8.8% in Europe, and 12.1–7.2% in the Asia-Pacific region (18,19). The prevalence of this species is related to different factors, including geographic characteristics (16,17,19), patient age (20,21), characteristics of the patient population studied (22–24), and the frequent use of fluconazole (22–24). For instance, numerous studies have demonstrated that both colonization and infection with *C. glabrata* are rare among infants and children and increase significantly with increasing patient age (25–29). More than one-third of *Candida*-associated BSIs among patients >60 years of age are due to *C. glabrata* (20,21,30). In addition to age, the use of broad-spectrum antibiotics, particularly piperacillin/tazobactam and vancomycin, the frequent use of central venous catheters, the receipt of parenteral nutrition, and a prolonged stay in ICU have all been associated with an increase risk of *C. glabrata* BSI (21,31). Importantly, the widespread use of fluconazole has contributed to the emergence of resistant non-*albicans* spp., including *C. glabrata* (22,32). In one study investigating the impact of fluconazole prophylaxis on *Candida* spp. in the pre- and post-fluconazole era, the incidence of infections caused by *C. albicans* isolates decreased by 50% (decreasing from 2.1% to 1.6%), with a corresponding significant increase in infections caused by *C. glabrata* spp. (33).

Table 1 Risk Factors Associated with Candidemia by Species

<i>Candida</i> species	Risk factors
<i>C. glabrata</i>	Age, azole exposure, HIV/AIDS, surgery, urinary, and vascular catheter
<i>C. guilliermondii</i>	Polyene use
<i>C. krusei</i>	Azole exposure, bone marrow transplant, neutropenia
<i>C. lusitanae</i>	Polyene use
<i>C. parapsilosis</i>	Central venous catheter, neonates, parenteral nutrition, hematologic malignancy
<i>C. tropicalis</i>	Neutropenia, hematologic malignancy, bone marrow transplantation

Source: From Refs. 13, 14, 39, and 41.

In contrast to the situation in the United States, *C. parapsilosis* is the most common non-*albicans* spp. of *Candida* recovered from blood cultures in other parts of the world (19). It is an important species to consider in hospitalized patients with vascular catheters. In the neonatal population, *C. parapsilosis* accounts for more than 30% of all *Candida* bloodstream isolates, compared with only 10% to 15% of *Candida* bloodstream isolates in adults (2,6,34,35). With the exception of prematurity and congenital abnormalities, risk factors for the development of IC in infants and children are similar to those in adults. *C. parapsilosis* infections are especially associated with hyperalimentation solutions (TPN), prosthetic devices, and indwelling catheters. Moreover, *C. parapsilosis* is the most common species found on the hands of healthcare workers, which has been associated with nosocomial infections (36). A number of nosocomial outbreaks of catheter-associated candidemia due to *C. parapsilosis* have been reported in the literature, underscoring the importance of hand hygiene and proper catheter care in hospital settings (34,37–39). Finally, recent data show that the incidence of *C. parapsilosis* resistance has increased nearly tenfold (Pfaller MA, data presented at the 19th Focus on Fungal Infections meeting in Florida March 4–6, 2009).

C. tropicalis is the fourth most frequent *Candida*-associated BSI isolate recovered in North America (7% of BSIs). It ranks second in Latin America (20%) and is more common than *C. glabrata* (13% vs. 10%, respectively) in the Asia-Pacific region (16,19). *C. tropicalis* is an important fungal pathogen in patients with chemotherapy-induced neutropenia and those with hematologic malignancies, especially leukemia, and in bone marrow transplant recipients (22,23,40–42). Among patients with neutropenia who are found to be colonized with *C. tropicalis*, 60% to 80% develop invasive infection (41–45). *C. tropicalis* has remained highly susceptible to fluconazole, and prophylaxis with fluconazole in patients with neutropenia is protective against the development of infections caused by this pathogenic yeast (23).

C. krusei causes 2% to 4% of all *Candida*-associated BSIs (16,25,28) and is best known for its propensity to emerge in the setting of fluconazole prophylaxis due to its innate resistance to this azole. Colonization and infection with *C. krusei* were noticeable in certain medical centers among stem cell transplant recipients and patients who had leukemia and received fluconazole as part the prophylaxis regimen (40,42,46–48). Interestingly, it was observed that patients exposed to piperacillin/tazobactam and vancomycin are more likely to develop *C. krusei* BSI (31). It should be noted that *C. krusei* organisms are inherently resistant to fluconazole (24,25,27). Such characteristics have a considerable impact on patient outcome, as BSIs due to *C. krusei* were reported to be associated with a high mortality rate (59–80% crude mortality and 40% attributable mortality), primarily related to its poor response to standard antifungal therapy (24,49).

Other important risk factors for IC among adults are listed in Table 2 and include the development of acute renal insufficiency, hemodialysis (acute or chronic), severe pancreatitis, diabetes mellitus, immunosuppressive therapy, and any surgery that requires general anesthesia (especially upper gastrointestinal tract surgeries).

CLINICAL MANIFESTATIONS OF INVASIVE CANDIDIASIS

Candidemia

The isolation of *Candida* spp. in one or more blood cultures is the most commonly recognized manifestation of IC, and occurs in 50% to 70% of patients with this disorder. A positive blood

Table 2 Risk Factors Associated with Invasive Candidiasis

Prolonged length of stay in an ICU
High acute physiology and chronic health evaluation II score
Central venous catheter
Parenteral nutrition
Broad spectrum of antibiotics
Prolonged antibiotics use
Malignancy
Neutropenia
Bone marrow transplant recipients
Solid organ transplant recipient
HIV/AIDS
Diabetes mellitus
Liver disease
Hemodialysis within 3 months
Renal failure
Autoimmune disease
Immunosuppressive therapy
Surgery in the last 3 months
Candida colonization at multiple sites
Very low birth weight neonate
Extensive burns
Malnutrition
Severe pancreatitis

Source: From Refs. 12, 27, and 125.

culture for *Candida* spp. should never be regarded as merely a contaminant, and should trigger immediate intervention with effective antifungal therapy and other appropriate management strategies.

A wide spectrum of clinical manifestations has been described for this disease, ranging from "transient" or self-limited candidemia to sepsis, multiorgan failure, and rapid demise. Also, clinical presentation of candidemia varies in different patient populations. Respiratory distress and apnea are the prominent clinical signs of candidemia in neonates. Spread to multiple organs including the skin (66%), the central nervous system (64%), and the retina (54%) is commonly reported (50).

Fever is the most common clinical presentation of candidemia in adults. Three patterns of fever unresponsive to broad-spectrum antibiotic therapy that should always raise the clinical suspicion of acute disseminated candidiasis in adults are (i) sudden onset of fever with sepsis, (ii) insidious onset of fever, and (iii) fever with a progressive deterioration of the general condition (51).

Acute disseminated candidiasis is usually seen in patients who are neutropenic as a result of cytotoxic chemotherapy for an underlying hematologic malignancy. Discrete erythematous or hemorrhagic palpable rash, which is consistent with small vessel vasculitis, is a major clinical manifestation of this form of IC and has been described essentially in neutropenic hosts (52). A prompt recognition and management of candidemia is crucial, as the overall crude 12-week mortality rate is high reaching 35.2% in one study (1).

Candida Endophthalmitis

Candida chorioretinitis or endophthalmitis are two of the most serious complications of untreated candidemia, underscoring the ability of *Candida* organism to attach and invade endothelial cells (53). Ocular presentations may be the first clinical manifestation of hematogenous spread and can develop after the diagnosis of candidemia, leading to permanent blindness if not identified and treated adequately (54,55). *Candida* endophthalmitis begins as a choroidal lesion that progresses to an area of retinal necrosis followed by full-blown vitreitis and endophthalmitis. The most common initial symptoms are eye redness, pain, and diminished or blurry vision. Unilateral involvement is the rule, although bilateral endophthalmitis leading to total blindness has been reported. Endophthalmitis can present up to two weeks after the diagnosis of candidemia,

and some authors have suggested that patients should have a dilated retinal exam at baseline and two weeks after the documentation of candidemia. A recent consensus document recommends that all patients with candidemia should have at least one careful retinal examination, preferably by an ophthalmologist (56).

Candida spp. may also cause meningitis, septic arthritis, tenosynovitis, isolated involvement of the kidney, and pneumonia, which are less common manifestations of IC and are not discussed in detail in this chapter.

Chronic Disseminated Candidiasis (Hepatosplenic Candidiasis)

Chronic disseminated candidiasis (previously known as hepatosplenic candidiasis) is almost exclusively described in patients who have undergone myeloablative chemotherapy and have developed IC during the period of neutropenia (57). It is usually associated with recovery from neutropenia, and may occur after the treatment of an episode of candidemia. It is worth mentioning that the frequency of chronic disseminated candidiasis has decreased dramatically during the last decade. This decrease has been noted at large leukemia and bone marrow transplant centers, such as M. D. Anderson Cancer Center and Fred Hutchinson Cancer Center, where fluconazole prophylaxis has been used extensively (58). Clinical signs and symptoms include fever unresponsive to antibiotics, abdominal pain, and hepatosplenomegaly. Laboratory examination might reveal negative blood cultures and an elevated serum alkaline phosphatase. Imaging studies show multiple focal lesions in the liver, spleen, kidneys, or rarely, the lungs. In the absence of candidemia to guide the diagnosis, a liver biopsy is often recommended.

DIAGNOSTIC ASSAYS

Recovery of *Candida* from blood is commonly achieved by using standard bacterial blood culture media in automated blood culture systems (59,60). Generally, blood cultures are positive in one to two days for *C. albicans*, *C. parapsilosis*, and *C. tropicalis*, whereas *C. glabrata* and *C. krusei* may require longer incubation times (61). One of the major drawbacks of blood cultures is that they give false-negative data in nearly 50% of cultures in spite of biopsy-proven infection.

Once isolated, *C. albicans* can be presumptively identified in 90 minutes by germ tube formation (62). Other species may require 72 hours to be identified by morphology and carbohydrate metabolism determined by many available commercial kits (e. g., API 20 C BioMerieux, Durham, NC). Several agar-based systems are now available to more rapidly differentiate *Candida* spp. (63). In addition, a recent test known as Peptide Nucleic Acid Fluorescence In Situ Hybridization (PNA FISH) that targets rRNA of *Candida* was recently approved by the Food and Drug Administration (FDA), which can provide clinicians with a very quick (in 2.5 hours) answer of as to what species is infecting the patient (64,65). One of the major advantages of this test is its ability to be performed directly using aliquots from positive blood culture bottles, providing a rapid and accurate diagnosis at the species level.

Aside from culture, nonculture methods have been developed to assist the clinician with an early diagnosis of IC. The FDA has recently approved the 1,3 β -glucan (Fungitell™ assay) as an adjunct test for the diagnosis of invasive pan fungal infections including candidiasis. The assay has a sensitivity and specificity of 70% and 87%, respectively, among patients who have proven IC (66,67). Studies are in progress to confirm the clinical utility of the β -glucan in antifungal treatment initiation (preliminary data shows that this test can detect fungal infections seven days prior to positive blood culture), and its usefulness for treatment follow-up (67). The assessment of the efficiency of combining β -glucan monitoring with traditional diagnostic tools in preemptive management of IC is still under investigation.

Detection of *Candida* mannan antigenemia and anti-*Candida* antibodies has been described, but studies to support their use are not convincing to date (68–71). Also, polymerase chain reaction assays are largely home-brewed and, although they have potential, they are still investigational.

PREDICTION RULES

Several authors have tried to develop models to identify independent factors that are predictive of IC, and to use these factors to build clinically relevant scores that may help clinicians to identify, implement, and adapt an optimal therapeutic approach (72,73). In this regard,

Table 3 Minimum Inhibitory Concentration (MIC) Breakpoints of Approved Antifungal Agents Against *Candida* spp.

Antifungal agent	Susceptible (S)	Susceptible-dose dependent (S-DD)	Intermediate (I)	Resistant (R)	Nonsusceptible
Anidulafungin	≤2	–	–	–	≥2
Caspofungin	≤2	–	–	–	≥2
Fluconazole	≤8	16–32	–	≥64	–
Flucytosine	≤4	–	8–16	≥32	–
Itraconazole	≤0.125	0.25–0.5	–	≥1	–
Micafungin	≤2	–	–	–	≥2
Voriconazole	≤1	2	–	≥4	–

Source: From Refs. 78 and 79.

Ostrosky-Zeichner et al. attempted to identify patients at high risk for IC in the ICU. The best-performing rule in this case was ≥1 day of systemic antibiotic therapy or presence of a central venous catheter, and at least two of the following: total parenteral nutrition, any form of dialysis, any major surgery, pancreatitis, any use of steroids, or use of an immunosuppressive agent. The rate of IC among patients meeting the rule was 9.9%, capturing 34% of cases of IC, with the following performance: relative risk 4.36, sensitivity 0.34, specificity 0.90, positive predictive value 0.01, and negative predictive value 0.97 (74).

More over, Faiz et al. used this risk-based approach, combined with fluconazole prophylaxis, in a medical ICU with a high incidence of *Candida* bloodstream infection and demonstrated that it was safe and cost-effective and produced a significant decrease in the incidence of *Candida* bloodstream infection (75).

ANTIFUNGAL SUSCEPTIBILITY TESTING

Antifungal susceptibility testing is a tool that aids the physician in selecting the most appropriate antifungal agent, especially when managing serious forms of candidiasis and in situations in which there is a failure to respond to initial antifungal therapy. Routine use of antifungal susceptibility testing is generally uncommon. Expert opinion suggests that laboratories perform routine antifungal susceptibility testing against fluconazole for *C. glabrata* isolates from blood and sterile sites, for other *Candida* spp. that have failed to respond to antifungal therapy, or in which azole resistance is strongly suspected (56,76,77). Minimum inhibitory concentration (MIC) breakpoints have been established for *Candida* spp. against fluconazole, itraconazole, voriconazole, and flucytosine, enabling selection of a suitable therapeutic agent (78,79). Interpretive MIC breakpoints for echinocandins (anidulafungin, caspofungin, and micafungin) were recently added (80,81) (Table 3).

Among the five most common *Candida* spp., *C. albicans*, *C. parapsilosis* and *C. tropicalis* are usually susceptible to azoles, polyenes, flucytosine, and the echinocandins (82–84), while *C. parapsilosis* exhibits higher MICs than other species of *Candida* for the echinocandins. The correlation of this in vitro finding to clinical outcome is uncertain, since this species generally responds well clinically to echinocandin therapy (Table 4).

C. glabrata is inherently less susceptible to fluconazole than are most other species of *Candida*. Both voriconazole and posaconazole (the new third-generation triazoles) are active against the vast majority of *C. glabrata* isolates. Although cross-resistance within the azole class is documented for this species, certain strains remain susceptible to voriconazole (therefore, it is recommended that prior to using voriconazole, *C. glabrata* antifungal susceptibility should be performed). *C. glabrata* is very susceptible to the activity of the echinocandins (81,85,86).

In addition to its intrinsic resistance to fluconazole, *C. krusei* shows decreased susceptibility to both amphotericin B and flucytosine. In contrast, this species is very susceptible to both the extended-spectrum triazoles (posaconazole and voriconazole) and the echinocandin antifungal agents (82,85).

Table 4 Antifungal Susceptibility of *Candida* spp. to Approved Antifungal Agents

Species	Amphotericin B	5-FC	Fluconazole and		Echinocandins
			itraconazole	Voriconazole	
<i>C. albicans</i>	S	S	S	S	S
<i>C. glabrata</i>	S to I	S	S-DD to R	S to S-DD	S
<i>C. krusei</i>	S to I	I to R	R	S to S-DD	S
<i>C. lusitanae</i>	S to R	S	S	S	S
<i>C. parapsilosis</i>	S	S	S	S	S to I
<i>C. tropicalis</i>	S	S	S	S	S

Abbreviations: S, Susceptible; I, Intermediate; R, Resistant; S-DD, Susceptible dose dependant.

Source: From Ref. 56.

TREATMENT

The armamentum of anticandidal agents has expanded during the past decade to include the new echinocandins (anidulafungin, caspofungin, and micafungin), voriconazole, and posaconazole. The Infectious Disease Society of America (IDSA) has recently updated the guidelines for the treatment of candidemia and IC (56). As a rule, treatment of IC depends on several factors including the clinical status of the patient, the species of *Candida* isolated, the susceptibility patterns of *Candida* spp. in a healthcare setting, the patient's prior antifungal exposure, and the organ involved (CNS, cardiac valves, and/or visceral organs) (87,88) (Table 5).

Fluconazole remains the standard therapy in selected patients who do not have a previous history of azoles exposure, have mild-to-moderate illness, and are not at high risk for *C. glabrata* (elderly patients, patients who have neoplasia, diabetics) (56,89–92). Although, itraconazole has a spectrum of antifungal activity comparable to fluconazole, it has a limited role in the treatment of IC because of its pharmacokinetics properties. Fluconazole is not advisable as primary therapy for proven or suspected IC in patients with neutropenia, severe illness [e. g., Acute Physiology and Chronic Health Evaluation (APACHE)>20], and in special clinical scenarios when involvement of cardiac valves, myocardium, or the central nervous system is suspected. Fluconazole is a reasonable “step-down therapy” for patients who are improving on more aggressive initial antifungal therapy (e. g., echinocandin or amphotericin B), who are infected with a susceptible organism, and who are ready for transition to oral therapy (56).

Although voriconazole has a broad activity against most *Candida* spp. especially *C. krusei* and most *C. glabrata* isolates, it is not indicated as primary therapy in IC. Voriconazole is considered an excellent oral alternative to intravenous amphotericin B or an echinocandin in clinically stable patients with *Candida* isolates resistant to fluconazole and are ready for transition to oral therapy (93). An underlying liver disease is a contraindication for voriconazole therapy. A number of points should be considered when prescribing voriconazole: its unpredictable pharmacokinetics, drug–drug interactions, and poor tolerance (94).

Despite an excellent in vitro activity against most *Candida* spp., posaconazole has no role in the treatment of candidemia except for selected patients for whom transition to an expanded-spectrum azole is an acceptable option.

The echinocandins have become the agents of choice for primary therapy of proven or suspected IC because of their ease of administration, safety profile, few drug–drug interactions, and in general broad fungicidal activity against most *Candida* spp. The safety, tolerability, and efficacy of these compounds in the treatment of candidemia and other forms of IC have been well studied in large, controlled, randomized phase III clinical trials (91,95,96). Successful outcome was achieved in 75% of patients in each of these trials. Each agent demonstrates diminished in vitro activity, expressed by a higher MIC against *C. parapsilosis*. Recent data from Vazquez group and ours showed that there is a difference in the activity among echinocandins against certain *C. parapsilosis* isolates, in that susceptibility was observed to anidulafungin but not to caspofungin or micafungin (97,98). The Expert Panel favors fluconazole over the three available echinocandins for treatment of candidemia due to *C. parapsilosis* on the basis of the decreased in vitro activity of echinocandins against *C. parapsilosis* (99,100) and reports of echinocandin resistance among selected isolates (97,101).

Table 5 Treatment Recommendation for Candidemia and Other Forms of Invasive Candidiasis

Condition	Therapy		Duration	Comments
	Primary	Alternative		
Candidemia				
Nonneutropenic adults	Fluconazole 800 mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily or an echinocandin ^a	LFAmB 3–5 mg/kg daily; or AmB-d 0.5–1 mg/kg daily; or voriconazole 400 mg (6 mg/kg) bid for 2 doses, then 200 mg (3 mg/kg) bid	14 days after last positive blood culture and resolution of signs and symptoms	Ophthalmological examination is recommended in all patients with candidemia. Remove all intravascular catheters, if possible
Neutropenic patients	An echinocandin ^a or LFAmB 3–5 mg/kg daily	Fluconazole 800 mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily; or voriconazole 400 mg (6 mg/kg) bid for 2 doses then 200 mg (3 mg/kg) bid	14 days after last positive blood culture and resolution of signs and symptoms and resolved neutropenia	Removal of all intravascular catheters is controversial. Gastrointestinal source is common.
Suspected candidiasis treated with empiric antifungal therapy				
Nonneutropenic patients	Treat as above for candidemia. An echinocandin or fluconazole is preferred	LFAmB 3–5 mg/kg daily or AmB-d 0.5–1 mg/kg daily	Uncertain but treatment should be stopped if work-up is negative	Echinocandin is preferred for patients with severe illness and recent azoles exposure
Neutropenic patients	LFAmB 3–5 mg/kg daily, caspofungin 70 mg loading dose, then 50 mg daily, or voriconazole 400 mg (6 mg/kg) bid for 2 doses then 200 mg (3 mg/kg) bid	Fluconazole 800 mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily; or itraconazole 200 mg (3 mg/kg) bid		Avoid azoles in patients with recent azoles exposure
Chronic disseminated candidiasis	Fluconazole 400 mg (6 mg/kg) daily for stable patients; LFAmB 3–5 mg/kg daily or AmB-d 0.5–0.7 mg/kg daily for severely ill patients; after patient is stable, change to fluconazole	An echinocandin ^a for several weeks followed by fluconazole	Several months till resolution of lesions. Treatment should continue through period of immunosuppression	
CNS candidiasis	LFAmB 3–5 mg/kg with or without 5-FC 25 mg/kg qid for several weeks, followed by fluconazole 400–800 mg (6–12 mg/kg) daily	Fluconazole 400–800 mg (6–12 mg/kg) daily for patients unable to tolerate LFAmB	Till resolution of all signs and symptoms including CSF and radiologic abnormalities	

(Continued)

Table 5 Treatment Recommendation for Candidemia and Other Forms of Invasive Candidiasis (*Continued*)

Condition	Therapy		Duration	Comments
	Primary	Alternative		
<i>Candida</i> endophthalmitis	AmB-d 0.7–1 mg/kg with 5-FC 25 mg/kg qid; or fluconazole 6–12 mg/kg daily; surgical intervention for patients with severe endophthalmitis or vitritis	LFAmB 3–5 mg/kg daily; voriconazole 6 mg/kg q12 h for 2 doses, then 3–4 mg/kg q12 h; or an echinocandin ^a		
<i>Candida</i> infection of the cardiovascular system				
Endocarditis	LFAmB 3–5 mg/kg with or without 5-FC 25 mg/kg qid; or AmB-d 0.6–1 mg/kg daily with or without 5-FC 25 mg/kg qid; or an echinocandin ^a	Step-down therapy to fluconazole 400–800 mg (6–12 mg/kg) daily for susceptible organism in stable patient with negative blood culture results		Valve replacement is recommended Chronic suppression with fluconazole is recommended if surgery not possible
Pericarditis or myocarditis	LFAmB 3–5 mg/kg daily; or fluconazole 400–800 mg (6–12 mg/kg) daily; or an echinocandin ^b	After stable, step-down therapy to fluconazole 400–800 mg (6–12 mg/kg) daily	No data available	Pericardial window or pericardiectomy is recommended
Suppurative thrombophlebitis	LFAmB 3–5 mg/kg daily; or fluconazole 400–800 mg (6–12 mg/kg) daily; or an echinocandin ^b	After stable, step-down therapy to fluconazole, 400–800 mg (6–12 mg/kg) daily	14 days after candidemia has cleared	Surgical incision and drainage is recommended
Infected pacemaker, ICD, or VAD	LFAmB 3–5 mg/kg with or without 5-FC 25 mg/kg qid; or AmB-d 0.6–1 mg/kg daily with or without 5-FC 25 mg/kg qid; or an echinocandin ^b	Step-down therapy to fluconazole 400–800 mg (6–12 mg/kg) daily for susceptible organism in stable patients with negative blood culture results	4–6 wk after device removal	Removal of pacemakers and ICDs is recommended. Chronic suppression with fluconazole if VAD remains in place
Neonatal candidiasis	AmB-d 1 mg/kg daily; or fluconazole 12 mg/kg daily for 3 wk	LFAmB 3–5 mg/kg daily	At least 3 wk	A lumbar puncture and dilated retinal examination is recommended on all neonates. Intravascular catheter should be removed

^aEchinocandin dosing in adults is as follows: anidulafungin, 200 mg loading dose, then 100 mg/day; caspofungin, 70-mg loading dose, then 50 mg/day; and micafungin, 100 mg/day.

^bFor patients with endocarditis and other cardiovascular infections, higher daily doses of an echinocandin may be appropriate (e. g., caspofungin 50–150 mg/day, micafungin 100–150 mg/day, or anidulafungin 100–200 mg/day).

Source: From Ref. 56.

The echinocandins are indicated as first-line therapy in patients who have a recent history of exposure to azoles, ongoing neutropenia, are hemodynamically unstable, have a history of allergy or intolerance to azoles or amphotericin B, or patients at high risk for infection with *C. krusei* or *C. glabrata*. Echinocandins should not be used to treat central nervous system (CNS) infections because of their poor CNS penetration.

With the availability of alternatives and less toxic antifungal agents, amphotericin B deoxycholate or lipid formulation amphotericin B is only recommended for the treatment of IC as initial therapy in special circumstances: when alternative therapy is unavailable or unaffordable, when there is a history of intolerance to echinocandins or azoles, when the infection is refractory to other therapy, when the organism is resistant to other agents, or when there is a suspicion of infection due to non-*Candida* yeast such as *Cryptococcus neoformans* IC (89,90,95,96,102).

The use fluconazole or an echinocandin is recommended for infection caused by *Candida lusitanae*, since in vitro data show that this species tends to exhibit polyene resistance.

A dilated funduscopic examination (preferably by an ophthalmologist) along with blood cultures is strongly recommended for all patients with candidemia within the first week after initiation of therapy. Such strategy has an influence on the duration of treatment. For instance, if there are no metastatic complications such as endophthalmitis, meningitis, or endocarditis, the duration of antifungal therapy is 14 days after resolution of signs and symptoms attributable to infection and clearance of *Candida* spp. from the bloodstream (56) (Table 5)

PROPHYLAXIS

Antifungal prophylaxis has led to a tremendous decrease in mortality related to fungal infections in critically ill and in cancer patients (103,104). Several antifungal agents have proved to reduce the rate of IC in clinical trial in population at high risk to develop IC. A dramatic reduction in the rates of invasive fungal infection was observed when fluconazole or liposomal amphotericin B was used as prophylactic regimen in liver transplant recipients (105–109). Prophylactic fluconazole therapy after liver transplantation prevented invasive fungal infections caused by most *Candida* spp., but not *C. glabrata* (108).

Other at risk population who benefit from fluconazole prophylaxis include pancreas and small bowel transplant recipients (110,111). The risk of IC after transplantation of other solid organs, such as kidneys and hearts, is too low to warrant routine prophylaxis (112).

A meta-analysis of randomized, placebo-controlled trials has shown that prophylactic antifungal agents reduced the number of superficial and invasive fungal infections in patients with chemotherapy-induced neutropenia (104). In patients undergoing chemotherapy for acute myelogenous leukemia or the myelodysplastic syndrome, posaconazole prevented invasive fungal infections more effectively than fluconazole or itraconazole and improved overall survival (113). In addition, intravenous itraconazole and caspofungin provided similar protection against invasive fungal infection during induction chemotherapy in patients with hematologic malignancies (114). Although a meta-analysis of 13 randomized, controlled trials showed that itraconazole administered orally and/or intravenously as antifungal prophylaxis is effective in reducing the rate of fungal infection in neutropenic patients with hematologic malignancies, itraconazole has little advantage over more tolerable antifungal agents (115).

Micafungin administered at 50 mg daily before engraftment was effective in reducing the rate of candidiasis in stem cell transplant recipients, compared with fluconazole administered at a dosage of 400 mg daily (116). Because of its broad spectrum of activity, posaconazole was shown to be more effective than fluconazole in preventing invasive fungal infections in stem cell transplant recipients who had severe graft-versus-host disease (117). The use of other agents, such as itraconazole and amphotericin B, in stem cell transplant recipients is limited by side effects, drug–drug interactions, and bioavailability (118). The optimal duration of prophylaxis is not known but should usually include the period of neutropenia risk.

Patients in ICU settings are among the population at high risk for IC that might benefit from antifungal prophylaxis. Three randomized, placebo-controlled trials have shown a reduction in the incidence of candidal infection in selected high risk patients given fluconazole prophylaxis in ICU settings (119–121). It is important to note that these studies have all failed to show a survival benefit associated with this strategy (122,123). Furthermore, a recent randomized, placebo-controlled trial did confirm this finding (124).

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17 | Invasive Aspergillosis

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INTRODUCTION

Invasive aspergillosis (IA) has emerged as a leading cause of death in severely immunocompromised patients, particularly patients with leukemia and recipients of hematopoietic stem cell transplant (HSCT) (1). Its incidence has significantly increased over the past two decades due to the development of new intensive chemotherapy regimens, increased use of high-dose corticosteroids, worldwide increase in solid organ and bone marrow transplantation, and increased use of immunosuppressive regimens for autoimmune diseases (2,4). Despite a high incidence, a significant decrease in mortality in patients with a diagnosis of IA following HSCT was noticed in recent years coinciding with multiple changes in transplantation practices, including the use of nonmyeloablative conditioning regimens, receipt of peripheral blood stem cells, more prompt diagnosis of IA and, importantly, the use of voriconazole (5). This chapter addresses the risk factors associated with IA, the common clinical presentation, novel approaches for early detection of subclinical infection and diagnosis modalities, and updated treatment strategies.

EPIDEMIOLOGY AND RISK FACTORS

Invasive Aspergillosis in HSCT

Invasive aspergillosis is usually caused by *A. fumigatus* and to a lesser extent by *A. flavus*, *A. niger*, and *A. terreus* (6). Transplant recipients are among the most significant subgroups of immunosuppressed hosts at risk for IA. The cumulative incidence of IA for the two highest populations at risk, HSCT and solid organ transplant (SOT), has been reported from a recent multicenter study in the United States (7). In the HSCT population, *Aspergillus* now exceeds *Candida* as the most common invasive fungal pathogen and the cumulative incidence is higher at 12 months in patients with allogeneic unrelated donors (3.9%) than in those with allogeneic HLA-mismatched (3.2%), HLA-matched (2.3%), and autologous (0.5%) donors (7). The rates were similar for myeloablative and nonmyeloablative conditioning regimen before allogeneic HSCT (3.1% vs. 3.3%). Prolonged and profound neutropenia secondary to the administration of antineoplastic chemotherapy and secondary neutropenia associated with failure of hematopoietic stem cell engraftment represent a well-known risk factor for IA in the HSCT population (8,9). Typically, IA occurs in the preengraftment period where neutropenia is the predominant immune defect associated with early IA. In the nonneutropenic host, however, IA occurs as a late infection (more than three months after engraftment) when recipients are receiving immunosuppressive drugs (especially high doses of corticosteroids) for the treatment of graft-versus-host disease (GVHD) (1,8). In addition to neutropenia, other risk factors for IA in HSCT include acute and chronic GVHD, a prior history of fungal infection, corticosteroids therapy for GVHD, HLA mismatch, unrelated donor, receipt of T-cell depleted or CD34 selected transplant, and higher age of the recipients (10,11). Cytomegalovirus (CMV) and respiratory virus infection, lymphopenia (1), the use of ganciclovir and alemtuzumab therapy (8,12), as well as an increased bone marrow iron stores secondary to repeated transfusion (13) have been recently identified in various studies as potential risk factors for IA.

Invasive Aspergillosis in SOT

Although the net state of immunosuppression and intensity of the immunosuppressive regimen is a major determinant for the development of IA in SOT recipients, the incidence of IA and risk factors varies by the type of SOT. The highest incidence among SOT recipients occurs in lung transplant recipients with 2.4% reported cumulative incidence at 12 months following

transplantation (7). The cumulative incidence of IA is 0.8% after heart transplantation, 0.3% after liver transplantation, and 0.1% after kidney transplantation (7).

Invasive Aspergillosis in Lung Transplant Recipient

Invasive fungal infections occur in 15% to 35% of lung transplant recipients, with *Aspergillus* spp. accounting for one-half of these (14–16). Lung transplant recipients have a unique predisposition and clinical manifestation for *Aspergillus* infection. Impairment of local host defenses (e.g., mucociliary clearance and cough reflex), ischemic airway injury, altered alveolar phagocytic function, direct communication of the transplanted organ with the environment and an overall higher intensity of immunosuppression, render these patients uniquely susceptible to airway colonization with *Aspergillus* and invasive disease (17). Up to 4% of patients develop isolated tracheobronchitis and 6% to 8% develop invasive pulmonary or disseminated aspergillosis. Anastomotic complication and airways or graft ischemia, during the surgery, predispose lung transplant recipients to IA. In the posttransplant period, *Aspergillus* infection has been reported in patients with reperfusion injury and poor allograft function. CMV infection and requirement of a higher degree of immunosuppressive therapy for rejection or bronchiolitis are also described to be a risk factor for IA following lung transplant (18,19). It is important to mention that a higher incidence of invasive pulmonary aspergillosis occurs in recipients with single lung transplant than in those with bilateral lung transplant (20). IA affects the native lung more frequently than the transplanted lung (21). The mortality rate of IA in lung transplant recipients varies between 20% and 83% depending on the study (7,17,22).

Invasive Aspergillosis in Heart Transplant Recipient

Heart transplantation has been reported as the only type of solid organ transplantation in which fungal infection is caused predominantly (i.e., in 68% of cases) by *Aspergillus* spp. (23). The cumulative incidence of IA at 12 months after heart transplantation is 0.8% (7). Independent risk factors for IA following heart transplantation includes reoperation (RR:5.8; 95% CI = 1.8–18; $P = 0.002$), CMV disease (RR:5.2; 95% CI = 2–13.9; $P = 0.001$), requirement of hemodialysis posttransplant (RR:4.9; 95% CI = 1.2–18; $P = 0.02$), and the existence of an episode of IA in the institutional transplant program two months before or after the date of transplantation (RR:4.6; 95% CI = 0.3–0.8; $P = 0.007$) (24,25). The mortality rate of IA following heart transplant ranges between 66% and 78% (7,17).

Invasive Aspergillosis in Liver Transplant Recipient

A number of well-characterized risk factors have been shown to carry a high risk of IA after liver transplantation. Retransplantation and renal failure are among the most significant risk factors for IA in these patient populations (26). The median time of onset of IA after renal replacement therapy and retransplantation was 13 and 28 days, respectively, in one study (27). Other factors associated with IA in liver transplant recipients include transplantation for fulminant liver failure, CMV infection, and prolonged intensive care stay (28–30). Hepatitis C and CMV are independent risk factors for late onset IA (>3 months) in liver transplant recipients.

Invasive Aspergillosis in Kidney Transplant Recipient

Renal transplant recipients are at lower risk for IA than other transplant recipients. The reported cumulative incidence of IA at 12 months following kidney transplantation is only 0.1% (7). High dose and prolonged duration of corticosteroids, graft failure requiring hemodialysis, and potent immunosuppressive therapy have been associated with increased risk of IA (17,31,32). Despite a low incidence compared to other solid transplant recipients, IA in kidney transplant recipients carry a high mortality rate ranging from 67% to 75% (17,22).

Invasive Aspergillosis in Hematologic Malignancy

Patients with hematologic malignancies are at an increased risk for developing IA. Acute myeloid leukemia is the most frequent underlying condition (33), but in recent years, IA has been increasingly diagnosed in patients with multiple myeloma. In a study of HSCT recipients, the risk of IA was 4.5 times greater in patients with multiple myeloma, compared with the risk for patients with chronic myeloid leukemia in the chronic phase (1).

Corticosteroid Therapy

It was shown that the risk of IA is a function of the dose and duration of steroid therapy (1,11,34). Corticosteroids affect the host immune response to *Aspergillus* by preventing killing of phagocytosed *A. fumigatus* conidia by alveolar macrophages (35) and by blunting alveolar macrophage production of proinflammatory cytokines (IL-1 α and TNF- α) and chemokines (macrophage inhibitory factor-1 α) that are pivotal for recruiting neutrophils and monocytes (36). Corticosteroids also affect the type of T helper cell response to IA. Corticosteroids therapy is associated with induction of TH2 cell responses and poor outcome (37). It was shown that hydrocortisone enhances the doubling time and the hyphal extension rate of *A. fumigatus* grown in vitro (38). Such characteristics have a clinical impact on patients receiving corticosteroids therapy for various indications. From a clinical perspective, the administration of 0.5 mg/kg of body weight/day for a period of more than 30 days or >1 mg/kg for a period of more than 21 days (3,39,40) has been associated with an increased risk of IA, which starts within two weeks of steroid administration (>1 mg/kg/day of prednisone or equivalent) and has a dose response extending to more than six weeks. Also, lower doses (0.25–1 mg/kg/day) for 2 to 10 weeks have shown to impact the risk of IA (12).

Other Risk Factors

Other at-risk groups for IA include persons who require chronic corticosteroid therapy, have been hospitalized for a prolonged time in intensive care units, have poorly controlled diabetes mellitus or AIDS, and those with primary immunodeficiency (e.g., chronic granulomatous disease) (41–43).

SPECTRUM OF DISEASE

Invasive Pulmonary Aspergillosis

Invasive pulmonary aspergillosis (IPA) is the most common form of invasive *Aspergillus* disease. Clinical presentation is highly dependent on the host immune status and the risk factors for disease development. Patients usually present with respiratory symptoms that are consistent with bronchopneumonia, fever, cough, and dyspnea. Two other symptoms that are significant and raise the possibility of IPA in the appropriate clinical setting are pleuritic chest pain (due to small pulmonary infarctions secondary to vascular invasion) and hemoptysis, which is usually mild but could be massive. IPA is one of the most common causes of hemoptysis in neutropenic patients and has been reported to be associated with cavitation that occurs with neutrophil recovery (44). Recently, a pulmonary immune reconstitution inflammatory syndrome has been described in patients with pulmonary aspergillosis, who present with a worsening of respiratory clinical features and imaging changes in the absence of dissemination to other organs (45). This clinical picture was observed after neutrophil recovery and coincided with microbiological and clinical response in 84% of the subjects (46–48). The clinical presentation in other hosts, such as SOT recipients and patients on high dose corticosteroids, tend to be less fulminant than that seen in HSCT recipients or patients with hematologic malignancies (49,50). Other pulmonary manifestations of IA include isolated tracheobronchitis with severe inflammation of the airways that is associated with ulcerations and plaque formation leading to airway obstruction and secondary atelectasis. This form of IPA has been most commonly reported in patients with AIDS and in lung transplant recipients (51,52).

Cerebral Aspergillosis

With the predilection of *Aspergillus* to invade blood vessels, IPA commonly leads to areas of infarction and hemorrhage in the primary organs (usually the lungs), and the organism spreads hematogenously to other organs, most commonly the brain and less commonly the skin, kidneys, pleura, heart, esophagus, liver, or any other site (53). Cerebral aspergillosis may also arise by continuous invasion from adjacent anatomical sites such as the paranasal sinuses (54,55). Cerebral aspergillosis has also been reported following neurosurgical procedure or vascular intervention or in association with endocarditis (56–58). It should be noted that this form of IA almost exclusively occurs in severely immunocompromised patients and carries a particularly poor prognosis and high mortality rate approaching 100% (59,60). Cerebral aspergillosis has

been observed in patients with absent or a lesser degree of immunosuppression (e.g., diabetes and short course of corticosteroids) (61,62). It has also been reported in patients with inherited defects of phagocytes (63).

Clinical manifestations of cerebral aspergillosis parallel the vascular tropism of the fungus. Following hematogenous spread, *Aspergillus* hyphae lodge and obstruct cerebral vessels, often large and intermediate-sized vessels, causing arterial thrombosis and infarction, which is typically hemorrhagic (64). Although angioinvasion is a common finding in IA, true mycotic aneurysms due to *Aspergillus* spp. have been infrequently observed (65). Abscess formation is the most common finding in a patient with cerebral aspergillosis (66). Clinical symptoms in cerebral aspergillosis are variable and nonspecific. Altered mental status, focal neurologic deficits, and seizures are the most common symptoms reported.

Invasive *Aspergillus* Sinusitis

Acute

Aspergillus spp. are the most frequently identified pathogens in patients with fungal sinusitis (67) with *A. flavus* being the most common isolated organism (68). Aspergillosis of the paranasal sinus is virtually always acquired by inhalation of conidia. The disease occurs almost exclusively in immunocompromised hosts, including patients with profound neutropenia, AIDS patients, and after HSCT (69,70). It is interesting to note that acute invasive *Aspergillus* sinusitis is less common than invasive pulmonary aspergillosis with a frequency of sinus infections of only 5% compared to a frequency of pulmonary infections of >56% among immunocompromised patients (71,72). Acute IA is characterized by an abrupt onset with rapid progression and a tendency of destructive invasion into neighboring structures resulting in orbital cellulitis, retinitis, palate destruction, or brain abscess formation. Patient at risk usually present with fever, facial pain, nasal discharge or congestion, epistaxis, and periorbital swelling (69,73,74). Involvement of the ethmoid sinus carries a particularly high risk of extension to the cavernous sinus and potentially the internal carotid artery. Early signs of ophthalmoplegia may precede radiologic evidence of cavernous sinus thrombosis.

Chronic

Unlike acute invasive *Aspergillus* sinusitis, the chronic form of the disease tends to occur in patients carrying a low level of immunosuppression, such as patients with poorly controlled diabetes mellitus and patients on chronic steroids therapy (75). Patients usually present with the orbital apex syndrome are characterized by decreasing vision and ocular immobility resulting from orbital mass. Chronic invasive sinus aspergillosis carries a poor prognosis and should be managed similar to acute invasive disease.

DIAGNOSIS

The diagnosis of invasive aspergillosis relies on a combination of clinical, radiographic, microbiologic, serologic, and histological parameters. A high index of clinical suspicion is required especially in the setting of severe immunosuppression and uncommon risk factors. Early identification of patients at risk who require antifungal therapy is an important goal, as a prompt diagnosis and institution of the right antifungal agent has been associated with improved patient outcome and increased survival (5,76).

When a pulmonary IA is suspected, a high-resolution **computed tomography (CT) scan is recommended** (77,78). Pulmonary nodules are the most common findings in early IPA in neutropenic patients and HSCT recipients and can be easily missed by a regular chest radiograph (77).

The "halo sign," a haziness surrounding a nodule or infiltrate, is a characteristic chest CT feature of angioinvasive organisms and is highly suggestive of IA in patients with prolonged neutropenia. Analysis of the high-resolution CT images of patients with probable and proven IA showed that those with a halo sign at initiation of antifungal therapy had a significantly better response and greater survival than those presenting with other CT images (77). In patients with cerebral or sinus IA, a CT or magnetic resonance imaging (MRI) scan allows early detection of inflammatory soft tissue edema, bone destruction, or invasion into adjacent structures and guide the subsequent diagnostic approach and surgical management (79).

Although radiological features are characteristic, they are not diagnostic of IA. For instance, infections due to other angioinvasive filamentous fungi such as *Zygomycetes*, *Fusarium* spp., and *Scedosporium* spp., as well as due to *Pseudomonas aeruginosa* and *Nocardia* spp., may cause a halo sign and other radiologic patterns of IA. Therefore, culture confirmation is important to differentiate *Aspergillus* from other filamentous fungal infections. However, recovery of *Aspergillus* spp. from nonsterile sites is only suggestive of infection, but is not diagnostic. In this regard, the host immune status is critical in culture interpretation. The positive predictive value of finding *Aspergillus* spp. in immunocompromised host with neutropenia was 64% in one study compared to 1% in patients with cystic fibrosis (6). Definitive diagnosis often requires an invasive procedure with tissue biopsy (e.g., thoracoscopic biopsy, endoscopy for sinusitis, video-assisted thoracoscopic biopsy). Fluid and tissue specimens from these procedures may reveal characteristic angular dichotomously branching septate hyphae on direct microscopic examination and/or *Aspergillus* spp. on culture. However, lack of positive culture or direct smear results do not rule out the diagnosis of IA. Moreover, clinical conditions in severely immunocompromised patients, such as thrombocytopenia, often preclude invasive procedure for tissue diagnosis. Thus, other markers are often used in the assessment of IA.

LABORATORY MARKERS

Laboratory markers that detect *Aspergillus* antigen can be used as a diagnostic adjunct, as a surveillance tool in high-risk patients (e.g., allogeneic HSCT recipients) to detect subclinical infection, and as a monitoring response to antifungal therapy. A double sandwich ELISA serum test for detection of the fungal cell wall constituents and galactomannan was recently approved in the United States as an adjunct tool for the diagnosis of IA (80,81). The combination of host factors predisposition to IA (e.g., prolonged neutropenia), a compatible clinical and radiologic finding (e.g., pulmonary nodule), and two consecutive positive serum galactomannan assays is equated with “probable invasive aspergillosis” (82) and avoids the need for an invasive procedure and tissue biopsy when the clinical status of the patient precludes invasive intervention. Galactomannan antigen has been detected in the CSF of patients with CNS aspergillosis (83–85) and the bronchoalveolar lavage fluid specimens of patients with IPA. However, the use of the galactomannan assay in these contexts is still investigational (86,87).

It should be noted that the sensitivity and the specificity of the assay is affected by host factors and medications (81,88). Most notably, the sensitivity of the assay is reduced by concomitant use of antifungal agents with activity against molds (89). False-positive galactomannan results testing have been reported in several contexts, including in patients who had received certain antibiotics (piperacillin/tazobactam and amoxicillin/clavulonate), in cases of neonatal colonization with bifidobacterium and in patients with other invasive mycoses (*Penicillium*, histoplasmosis, and blastomycosis) (90–92).

The value of surveillance serum galactomannan monitoring is still unclear. Many studies yielded conflicting results. In the best scenario, 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 IA (93). In another study, the sensitivity was only 64.5% in cases of definite IA (94). The positive predictive value was poor when used as a surveillance tool in patients with persistent neutropenic fever (positive predictive value = 7.1%) and in HSCT (mostly autologous) recipients (positive predictive value = 10%). In a study of 170 patients at high risk for invasive mold infection from North American cancer centers, the galactomannan assay identified 25 of 31 patients with IA (81% sensitivity) and had a specificity of 89%. Further studies are required to demonstrate whether surveillance galactomannan testing in targeted high-risk patients will translate into improved outcomes.

Detection of serum β -glucan, a fungal cell wall constituent, has recently received Food and Drug Administration approval for the diagnosis of invasive mycosis, including aspergillosis (95,96). In patients with acute myeloid leukemia and myelodysplastic syndrome, the assay was highly sensitive and specific in detecting early invasive fungal infections, including candidiasis, fusariosis, trichosporonosis, and aspergillosis (96). The database for this assay in other populations at risk is limited. More research is required to define the utility of this assay in nonneutropenic patients at high risk for invasive mold infections, most notably, in allogeneic HSCT recipients with GVHD.

Polymerase chain reaction–based detection of aspergillosis applied to blood (97,98) and bronchoalveolar lavage specimens (99,100) is another investigational tool for early diagnosis of IA. However, these systems have not been standardized and are not commercially available.

PHARMACOLOGIC TREATMENT OF INVASIVE ASPERGILLOSIS

The Infectious Disease Society of America (IDSA) has recently updated the guidelines for the treatment of various forms of aspergillosis (101) including IA, chronic (and saprophytic) forms of aspergillosis, and allergic forms of aspergillosis. This chapter focuses on the different strategic treatment for invasive aspergillosis, summarized in Table 1. Pharmacologic description of each antifungal agent is beyond the scope of this chapter and can be found elsewhere (101).

Voriconazole and deoxycholate amphotericin B (D-AMB) are now the only compounds licensed in the United States for primary treatment of IA. The lipid formulations (AMB lipid complex, liposomal-AMB, and AMB colloidal dispersion), itraconazole, and caspofungin are

Table 1 Recommendations for the Treatment of Aspergillosis (See IDSA Guidelines for Specific Parameters) (101)

Presentation	Primary therapy	Alternative/salvage therapy
Invasive pulmonary	Voriconazole	L-AMB ^a
Invasive sinus		Caspofungin
Tracheobronchial		Micafungin
Subacute invasive pulmonary		Posaconazole
Central nervous system		Itraconazole
Chronic cavitary pulmonary	Voriconazole	L-AMB
	Itraconazole	Caspofungin
		Micafungin
		Posaconazole
Endocarditis, pericarditis, myocarditis	Voriconazole	L-AMB
Osteomyelitis		Caspofungin
Septic arthritis		Micafungin
		Posaconazole
		Itraconazole
Endophthalmitis	Intraocular AMB	L-AMB
Keratitis		Caspofungin
		Micafungin
		Posaconazole
		Itraconazole
Cutaneous	Voriconazole	L-AMB
		Caspofungin
		Micafungin
		Posaconazole
		Itraconazole
Peritonitis	Voriconazole	L-AMB
		Caspofungin
		Micafungin
		Posaconazole
		Itraconazole
Aspergilloma	Surgical resection	Voriconazole
		L-AMB
		Caspofungin
		Micafungin
		Posaconazole
		Itraconazole
Allergic bronchopulmonary	Itraconazole	Voriconazole
		Posaconazole
Invasive pulmonary prophylaxis	Posaconazole	Itraconazole
		Micafungin

^aLipid formulations of amphotericin B.

approved for salvage therapy of IA. Posaconazole is licensed for prophylaxis of IA in neutropenic patients with leukemia and myelodysplasia and in allogeneic HSCT recipients with GVHD. Posaconazole also was approved in the European Union for treatment of IA that is refractory to an AMB formulation or to itraconazole. Micafungin and anidulafungin, which are members of the class of echinocandins, have in vitro, in vivo, and clinical activity against aspergillosis but are not licensed in the United States for this indication.

There are few randomized trials on the treatment of IA. The largest landmark randomized, controlled trial demonstrates that voriconazole is superior to D-AMB as primary treatment for IA (72). In this study, a total of 277 patients with definite or probable aspergillosis were randomized to receive voriconazole or D-AMB for 12 weeks. In most of the patients, the underlying condition was allogeneic hematopoietic-cell transplantation, acute leukemia, or other hematologic diseases. Partial (significant clinical improvement and at least 50% decrease in size of radiological lesions) or complete responses were considered a successful outcome. Patients given voriconazole had higher successful outcome rates with a 53% response (complete 21%, partial 32%) compared to 32% response rate in those given amphotericin B (complete 17%, partial 15%). Furthermore, the survival rate at 12 weeks was 70.8% in the voriconazole group compared to only 57.9% in the amphotericin B group (hazard ratio, 0.59; 95% CI = 0.40–0.88). Because of better survival and improved responses of initial therapy with voriconazole, primary therapy with D-AMB is no longer recommended for the treatment of IA (level of evidence A-I) (101).

Parenteral or oral voriconazole is currently the treatment of choice for various forms of IA. Because of its excellent bioavailability and excellent CNS penetration, voriconazole in conjunction with a surgical intervention is recommended for patients with CNS and invasive sinus aspergillosis (72,102,103).

For patients who are intolerant or refractory to voriconazole, a formulation of AMB is an appropriate alternative. Lipid formulation of amphotericin B is used as an alternative for primary therapy in patients with underlying liver disease or other contraindications to voriconazole (104–107).

Echinocandins have not been evaluated as initial monotherapy for IA in clinical trials. However, caspofungin was evaluated as salvage therapy in patients with IA. A favorable response was seen in 37 (45%) of the 83 patients refractory to or intolerant to conventional therapy (108).

Orally or intravenously administered itraconazole has also been used to treat patients with IA who are refractory to or intolerant to D-AMB (109,110). Measurement of itraconazole serum levels is generally required when the oral form is used because of its erratic bioavailability.

Posaconazole was approved in Europe for salvage treatment of patients with IA who are refractory to AMB or itraconazole. In the United States, posaconazole is not recommended for primary therapy of IA, but is generally recommended at the same level of strength as the lipid formulations of AMB, caspofungin, and itraconazole (101).

There has been significant interest in combination therapy pairing an echinocandin with either an amphotericin B preparation or a mold-active azole. The rationale is that echinocandins target the β -glucan constituent of the fungal cell wall, a site distinct from the fungal cell membrane targeted by polyenes and azoles. The combination of an echinocandin with an azole or amphotericin B has neutral to synergistic activity in vitro. Enhanced efficacy of combination regimens pairing an echinocandin with either an azole or an amphotericin B formulation occurred in some animal models of aspergillosis (111–113), but not in other models (114–116).

IMMUNE AUGMENTATION STRATEGIES

Augmentation of Neutrophil Number

Colony-Stimulating Factors

Colony-stimulating factors (CSFs) are used to accelerate myelopoiesis in neutropenic patients. Several in vitro studies have shown that CSFs enhance the host defense against *Candida* and *Aspergillus* spp. by affecting neutrophil and macrophage function for which the host immune

defense is dependant (117,118). Granulocyte colony-stimulating factor (G-CSF) influences survival, proliferation, and differentiation of all cells in the neutrophil lineage and augments the function of mature neutrophils. Macrophage CSF increases phagocytosis, chemotaxis, and secondary cytokine production in monocytes and macrophages (119). Granulocyte macrophage CSF (GM-CSF) stimulates various neutrophil effectors functions and prolongs neutrophil survival in vitro, accelerates the proliferation of the monocyte–macrophage system, and is a potent activator of monocytes and macrophages (119).

Despite a potent in vitro activity, the clinical database on CSFs as adjunctive therapy for fungal infections is inadequate to assess efficacy, and clinical trials have led to conflicting results. Multiple randomized clinical trials of prophylactic recombinant G-CSF and GM-CSF have shown the benefit of CSF in reducing the time to neutrophil recovery and the duration of fever and hospitalization in patients with acute myelogenous leukemia (120). In one randomized study in patients receiving chemotherapy for AML, prophylaxis with GM-CSF led to a lower frequency of fungal infections and overall mortality compared to placebo (121). No other randomized, controlled trial of prophylactic CSF has demonstrated a survival advantage compared to placebo in patients with hematological malignancies (122).

The American Society of Clinical Oncology has established authoritative guidelines related to the use of prophylactic CSF in standards practice (123).

Granulocyte Transfusions

Granulocyte transfusions provide supportive therapy for patients with neutropenia who have a life-threatening infection by augmenting the number of circulating neutrophils until neutrophil recovery occurs. This strategy is recommended for patients with prolonged neutropenia and life-threatening infections refractory to conventional therapy (124). The Transfusion Medicine and Hemostasis Network of the National Heart Lung and Blood Institute are currently in the planning stages of a randomized study of adjunctive granulocyte transfusions among neutropenic patients with severe bacterial and fungal infections. This study is expected to evaluate the benefits and risks of adjunctive granulocytes transfusion.

Recombinant Interferon- γ

Studies in vitro and from animal models and limited patient data provide a rationale for adjunctive IFN- γ for the treatment of IA (125). Recombinant IFN- γ augmented the human neutrophil oxidative response and killing of *A. fumigatus* hyphae in vitro and acted additively with G-CSF (126). It also prevented corticosteroid-mediated suppression of neutrophil killing of hyphae (127). It enhances killing of *A. fumigatus* by human monocytes (117). IFN- γ confers protection against a variety of experimental fungal infections in animals. Recombinant IFN- γ is licensed as a prophylactic agent in patients with congenital granulomatous disease, an inherited disorder of phagocyte NADPH oxidase characterized by recurrent life-threatening bacterial and fungal infections (128). Adjunctive IFN- γ showed promising results in a pilot study of AIDS associated cryptococcal meningitis (129). Due to the lack of randomized clinical trial supporting robust recommendation, recombinant IFN- γ is currently reserved for patients with life-threatening mold infection refractory to standard antifungal therapy.

PROPHYLAXIS AGAINST INVASIVE ASPERGILLOSIS

Primary prophylactic strategic approach with the aim to prevent IA is recommended in patients at high risk to develop IA, including patients with prolonged neutropenia and severe GVHD, lung transplant recipients, patients receiving long-term high-dose corticosteroid therapy, some liver transplant recipients, and those with certain inherited immunodeficiency disorders (e.g., CGD). Posaconazole has recently received FDA approval for primary prophylaxis in HSCT recipients with GVHD and in patients with acute myelogenous leukemia or myelodysplastic syndrome, who are at high risk for IA. Posaconazole was superior to fluconazole and itraconazole in preventing IA in patients with acute myeloid leukemia and myelodysplasia (130). In this study, 602 patients with prolonged neutropenia following intensive chemotherapy regimen for acute myelogenous leukemia and myelodysplastic syndrome received posaconazole at 200 mg three times a day or fluconazole or itraconazole prophylaxis with each cycle of chemotherapy. Prophylaxis was continued until recovery from neutropenia and complete remission or until

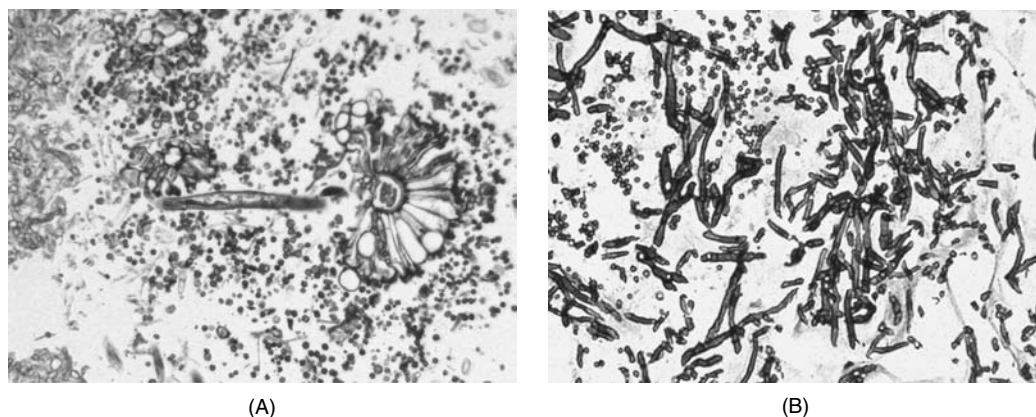


Figure 1 (See color insert) Paranasal sinus biopsy showing (A) the *Aspergillus* fruiting head (GMS stain) and (B) branching septate hyphae (H&E stain). Source: Courtesy of Dr. Michael Jacobs, University Hospitals of Cleveland Case Medical Center.

occurrence of an invasive fungal infection or for up to 12 weeks. Probable or proven invasive fungal infection was reported in 2% of patients who received posaconazole compared to 8% in the control group. Moreover, significantly fewer patients in the posaconazole group had IA [2 (1%) vs. 20 (7%), $P < 0.001$]. Importantly, the overall survival rate was significantly higher in the posaconazole prophylactic arm. A separate study of posaconazole prophylaxis during GVHD in HSC T recipients also found a significant reduction in proven and probable invasive fungal infections compared with those receiving fluconazole (131). Itraconazole has been evaluated in several prospective trials, but conclusions regarding its efficacy in preventing IA have been limited (132–134). Oral itraconazole in capsular form was ineffective for prophylaxis because of erratic bioavailability, while parenteral or oral solution of itraconazole was partially effective in reducing the incidence of IA in neutropenic patients with hematological dysfunction, with a mean hazard ratio of 0.52 (range, 0.3–0.91) (135). Voriconazole has not been studied in this context, although clinical trials are in progress.

Patients with a previous history of IA are at high risk for recurrence during subsequent immunosuppression (136,137). Therefore, an antifungal therapy with a mold activity is a reasonable approach during the entire period of immunosuppression as secondary prophylaxis against IA (137). Several studies indicate that secondary prophylaxis against IA can be successful when an anti-*Aspergillus* azole (voriconazole, posaconazole, or itraconazole) or lipid formulation of amphotericin B is given to patients receiving ongoing immunosuppressive therapy following treatment of a documented episode of IA (136,138). Prophylactic regimen and indications are discussed in length in other chapters in this book.

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18 Management of Cryptococcosis

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INTRODUCTION

Cryptococcosis is a global invasive mycosis with significant morbidity and mortality (1). Although the widespread use of highly active antiretroviral therapy (HAART) has lowered the incidence of cases in medically developed countries (2), the incidence of this infection is extremely high in areas where large numbers of HIV disease still persist and there is limited access to HAART and/or health care. It has recently been estimated that the global burden of HIV-associated cryptococcosis approaches a million cases per year with over 700,000 deaths (3). Even in medically developed countries, this infection continues to find new risk groups such as those receiving high doses of corticosteroids, monoclonal antibodies such as alemtuzumab (4) and infliximab (5), or other immunosuppressive agents used with transplantation. Therefore, modern medicine from the severely immunosuppressed patients with HIV infection or organ transplantation to the apparently normal host must deal with the management of this encapsulated yeast with a propensity to invade the central nervous system.

Antifungal drug regimens for the management of cryptococcosis represent some of the best-characterized for invasive fungal disease (1,6,7). However, despite access to advanced medical care with availability to HAART, the three-month mortality rate during management of acute cryptococcal meningoencephalitis still approximates 20%, and without treatment certain HIV-infected populations have reported mortality rates of 100% within two weeks of presentation to health care facilities (8). It is apparent that there is a wide spectrum of needs for successful management of this life-threatening infection. It revolves around access to antifungal drugs and diagnostic strategies, but there are also complex clinical scenarios in which correcting host immunodeficiency and immune reconstitution can be extremely difficult and requires careful clinical strategies one patient at a time.

This chapter attempts to provide information and insights into the management of this infection. The 2000 and updated 2009 IDSA guidelines for management of cryptococcosis have been presented (9,10) and form the foundation for this chapter.

CRYPTOCOCCAL MENINGOENCEPHALITIS

The treatment strategies for the patient with cryptococcal meningoencephalitis will be divided up into three risk groups (HIV-infected, transplant recipient, and non-HIV, nontransplant host). Although the principles are similar, some risk groups have different demands on management. However, several important issues carry across all patient groups. First, the treatment goal is to rapidly and consistently reduce the burden of yeasts. This goal is primarily achieved with a polyene-driven induction therapeutic regimen and then patients are placed on consolidation/maintenance regimens. Second, there are a series of complications and confounders that need to be addressed in many patients from increased intracranial pressure to immune reconstitution inflammatory syndrome. Third, control of the underlying disease will be essential to improve final outcome. For instance, an underlying disease of HIV and cryptococcosis has a better prognosis than disseminated cryptococcosis in a patient with severe liver disease because of limited abilities to treat end-stage liver disease.

HIV-Infection with Cryptococcal Meningoencephalitis

Treatment of cryptococcal meningoencephalitis in an HIV-infected individual is based on the principles of a high burden of CSF yeasts and a severely depressed immune system (profound CD4 lymphocytopenia). In medically advanced countries the treatment strategy has been formalized into the concept of induction, consolidation (clearance), and maintenance (suppression) phases. Table 1 gives the details from the new (2009) IDSA guidelines. It places an emphasis on combination therapy with amphotericin B and flucytosine, which has been shown to be the most

Table 1 Treatment Recommendations for Cryptococcal Meningoencephalitis in HIV-Infected Individuals

Initial antifungal regimen induction and consolidation	Duration
AmBd ≥ 0.7 –1.0 mg/kg/day + 5FC 100 mg/kg/day ^a	2 wk
then fluconazole 400 mg/day	8 wk
Liposomal AmB 3–4 mg/kg/day or ABLC 5 mg/kg/day (with renal concerns)	2 wk
then fluconazole 400 mg/day	8 wk
AmBd 0.7–1.0 mg/kg/day or liposomal AmB 3–6 mg/kg/day or	4–6 wk
ABLc 5 mg/kg/day (flucytosine intolerant) then fluconazole 400 mg/day	8 wk
^b Alternatives: AmBd + fluconazole, fluconazole + flucytosine, fluconazole; itraconazole	
Maintenance	
Fluconazole 200 mg/day ^a	≥ 1 yr ^d
Alternatives:	
Itraconazole 400 mg/day ^c	≥ 1 yr ^d
AmBd 1 mg/kg \times wk ^c	

^aBegin HAART 2–10 wk after start of initial antifungal treatment.

^bAlternatives: Because of unique clinical situations, primary recommendations are not available, then consideration of alternative regimens may be made but not encouraged as substitutes.

^cInferior to primary recommendation.

^dWith successful introduction of HAART: CD4 count ≥ 100 cells/ μ L and negative viral load for ≥ 3 months with minimum of 1 year of antifungal therapy.

Abbreviations: AmBd, amphotericin B deoxycholate; 5FC, flucytosine.

Source: Adapted from Ref. 122.

fungicidal regimen (6,11–13). It is also noted that with the Van der Horst et al. study, the use of a higher amphotericin B dose (0.7 mg/kg/day) and lower flucytosine dose (100 mg/kg/day) provided the best regimen to reliably sterilize the CSF, and most but not all patients receiving this regimen will be CSF culture negative after two weeks of induction therapy (14). This substantial reduction in the burden of yeasts is a much better position clinically to start with fluconazole treatment in the consolidation and maintenance phases of treatment. It has been shown in multiple studies that fluconazole acts in a fungistatic manner in cryptococcal meningitis (15–17) and for a significant impact with this azole there needs to be either a small burden of yeasts at the site of infection or very high doses of fluconazole must be administered (18,19). Therefore, a polyene-based regimen when available is first-line induction therapy and not an azole. In the general guidelines, it is recommended that induction therapy last at least two weeks, but for very high burden CSF yeast infections and those anticipated to have prolonged fungal CSF cultures, a longer induction may be necessary and this is a bedside decision. In patients with developing renal dysfunction, the substitution of lipid formulations of amphotericin B for amphotericin B is appropriate at the doses recommended in Table 1 (20–24). There are now several studies that robustly support this strategy. There are a series of other alternate induction regimens that might be necessary to consider for a variety of reasons (25–28) but not using the standard induction regimen will need to be defended with clinical considerations since they may be inferior to amphotericin B and flucytosine. The consolidation phase allows fluconazole exposure in relatively high doses for approximately eight weeks and this azole was superior to itraconazole (29). If the patient continues to be stable, then the patient is placed on maintenance (suppressive) therapy.

Prior to HAART, the relapse rate after initial therapy for cryptococcal meningoencephalitis was high (15%) and therefore several studies validated that fluconazole dramatically reduced relapses and was actually superior to itraconazole and weekly intravenous amphotericin B (1 mg/kg) for this suppression strategy (29–31). The fluconazole regimen became standard policy in HIV-infected patients and was continued for indefinite periods of time. With the immune reconstitution associated with HAART, several studies have now supported discontinuing suppressive therapy with fluconazole when CD4 cell count returns above 100 cell/ μ L and an undetectable HIV RNA level is sustained for ≥ 3 months (32–36). Therefore, clinicians may

consider stopping suppressive therapy when the previous conditions of immune reconstitution are met and the patient has received at least a minimum of 12 months of therapy.

There are several clinical issues related to cryptococcal meningoenzephalitis and coinfection with HIV infection.

1. In resource-limited environments, intravenous amphotericin B deoxycholate may not be available or combination therapy with flucytosine nonexistent. It should be emphasized that if at all possible, amphotericin B deoxycholate in high doses (1 mg/kg/day) be encouraged for ideally two weeks and at least one week. It allows CSF yeast counts to drop before fluconazole is administered. If fluconazole is the only drug available for treatment in some countries or certain geographical areas then doses of ≥ 1200 mg of fluconazole are recommended.
2. When does a clinician start HAART during management of meningitis? There have been suggestions that early HAART administration can be both helpful (37) and deleterious. It can help immune reconstitution but could add to IRIS or drug–drug interaction toxicities. On a practical basis, the institution of HAART at present is recommended to be started between 2 and 10 weeks after beginning antifungal therapy.
3. Asymptomatic cryptococcal antigenemia can occur in HIV-infected individuals (38–42). If it is detected, generally clinicians will use this biomarker as a strategy for preemptive therapy. It is recommended that blood and CSF cultures are obtained to rule out proven disease but if cultures are negative most clinicians still would consider fluconazole therapy until immune reconstitution occurs with HAART in cryptococcal antigenemia patients.
4. Primary antifungal prophylaxis for cryptococcosis (43,44) is not routinely recommended in HIV-infected patients in developed countries but in areas with limited HAART availability, high antiretroviral drug resistance, and high burden of cryptococcal disease, consideration of prophylaxis or a preemptive strategy using the serum cryptococcal antigen might be advisable.

Organ Transplant Recipient

Cryptococcosis has been found in approximately 3% of solid organ transplant recipients and the majority will occur after the first year posttransplant and most have disseminated disease on presentation (45–48). In many respects, the strategies in the highly immunosuppressed transplant recipient are similar to HIV-infected patients (Table 2). However, an important emphasis is that these patients have potentially compromised renal functions or are receiving nephrotoxic agents, such as calcineurin inhibitors, and therefore lipid formulations of amphotericin B are the preferred polyenes for induction therapy (49). Following the recommendations in Table 2, there is a good chance for success but there are several issues with transplant recipients and their cryptococcal disease management.

1. Transplant recipients with disseminated disease are generally treated for a total of at least one year.

Table 2 Treatment Recommendations for Cryptococcal Meningoenzephalitis in Transplant Recipients

Initial antifungal regimen ^a (induction and consolidation)	Duration
Liposomal AmB 3–4 mg/kg/day or ABLC 5 mg/kg/day + 5FC 100 mg/kg/day	2 wk
then fluconazole 400–800 mg/day	8 wk
Alternative: Liposomal AmB 6 mg/kg/day or ABLC 5 mg/kg/day then	4–6 wk
fluconazole 400–800 mg/day	8 wk
AmBd use (must weigh risk of renal dysfunction) ^b	(See lipid products of AmB)
Maintenance	
Fluconazole 200 mg/day	6 mo–1 yr

^aImmunosuppressive management may require sequential or step-wise reductions.

^bMany transplant recipients have been successfully managed with AmBd; however, issues of renal dysfunction with calcineurin inhibitors are important.

Abbreviations: AmBd, amphotericin B deoxycholate; 5FC, flucytosine; ABLC, amphotericin B lipid complex.

Source: Adapted from Ref. 122.

2. Immunosuppressive management should include sequential or step-wise reduction of immunosuppressants with corticosteroids being considered first since calcineurin inhibitors may have some antifungal activity (50).
3. It is important to recognize IRIS in these patients since its appearance may be diagnosed as failure and/or associated with organ rejection.
4. If patient received one year of antifungal treatment and off antifungal drug for another year with normal CSF, consideration for transplantation could be re-initiated (e. g., renal transplants).
5. Occasionally, transplanted organs contain *Cryptococcus* at the time of transplantation. If this is detected, it will require that the recipient is aggressively treated because risk of disseminated disease is substantial in these cases.

Non-HIV, Nontransplant Hosts

This is a heterogeneous group of patients from liver to connective tissue diseases to sarcoidosis and many of these disease processes are linked to the use of corticosteroids (51,52). There is also the “presumably immunocompetent hosts” in which it is difficult to define their immune state. However, it is clear that those with apparently much less severe or variable immune defects compared with HIV infection or transplant recipients can present with therapeutic challenges (53). There have been few studies in this group since the classical comparative studies of Bennett and Dismukes (54,55). In these studies, the combination of amphotericin B and flucytosine was validated as an effective regimen, and the length of induction therapy for six weeks appeared better than four weeks. However, despite the guidance of these studies, the use of higher doses of the polyene today and the integration of fluconazole in the therapeutic strategy has transformed the management of cryptococcal meningoencephalitis in this risk group without robust studies for the present recommendations. In Table 3, there is a set of guidelines for the treatment of cryptococcal meningoencephalitis in this group and they represent reasonable starting points for management but future studies are needed to precisely determine best strategies and as can be observed these guidelines have been co-opted from the severely immunosuppressed populations. Relapse of cryptococcal meningoencephalitis in the non-HIV-infected patient ranged from 15% to 25% prior to the AIDS epidemic and fluconazole use and thus there has been a focus to treat (suppress) patients for at least a year with fluconazole after induction and consolidation phases of treatment.

There are a series of complications which may occur during management of cryptococcal meningoencephalitis and the following issues are examined: (i) persistence/relapse,

Table 3 Treatment Recommendations for Cryptococcal Meningoencephalitis in Non-HIV-Infected and Nontransplant Patients

	Duration
Initial antifungal regimen	
AmBd ≥ 0.7 –1.0 mg/kg/day + 5FC 100 mg/kg/day	≥ 4 wk ^{a,b}
AmBd ≥ 0.7 –1.0 mg/kg/day (flucytosine intolerant)	≥ 6 wk ^{a,b}
If (AmBd intolerant), liposomal AmB 3–4 mg/kg/day or ABLC 5 mg/kg/day combined with 5FC when possible	≥ 4 wk ^{a,b}
In low-risk patients for therapeutic failure ^c , AmBd 0.7 mg/kg/day + 5FC 100 mg/kg/day	2 wk
Consolidation	
Fluconazole 400 mg/day	8 wk
Maintenance	
Fluconazole 200 mg/day ^b	6–12 mo

^aFour weeks are reserved for patients with meningitis without neurological complications; no significant underlying diseases or immunosuppression and with end of two weeks treatment CSF sample without viable yeasts on culture; second two weeks may substitute LF AmB for AmBd.

^bFluconazole at 200 mg daily to prevent relapse following induction and consolidation therapy is recommended.

^cLow risk = early diagnosis by history, no uncontrolled underlying or immunocompromised state, excellent response to initial two weeks combination course.

Abbreviations: AmBd, amphotericin B; 5FC, flucytosine.

Source: Adapted from Ref. 122.

(ii) increased intracranial pressure, (iii) immune reconstitution inflammatory syndrome, (iv) cryptococcomas.

Persistence/Relapse

The definition of persistent infection is arbitrary but a reasonable starting point is persistently positive CSF cultures after four weeks of proven antifungal therapy at a proper dose. Relapse has three features: recovery of viable yeasts from CSF, previous cultures at the site were negative, and recrudescence of signs and symptoms. It is important to note that positive India ink exams, changing antigen titers (56), and abnormal cellular reactions or chemistries are insufficient to alter direct antifungal treatment strategies. Most cases of relapse are due to inadequate primary induction therapy or failure of compliance with outpatient regimens. In these cases it is important to check relapse isolates for direct in vitro drug resistance since there are direct drug resistance strains (57–59). Although there are no formal in vitro susceptibility breakpoints established for *Cryptococcus*, for fluconazole MICs $\geq 16 \mu\text{g/mL}$ may be associated with treatment failure (60–62). Furthermore, most primary isolates of *C. neoformans* and *C. gattii* do not have high MICs by direct in vitro susceptibility testing (57). In most cases there will be a need to reintroduce an induction regimen with at least a polyene for a longer period of time and then ensure compliance. In some cases, voriconazole or posaconazole might be considered for the suppressive phase of treatment (63,64) but in most cases these agents will not likely improve outcome over readministration of high doses of fluconazole. Recombinant interferon-gamma as adjunctive therapy has been used in cryptococcal meningitis induction therapy without conclusive results regarding efficacy (65). However, in difficult cases to clear the cerebrospinal fluid of viable yeasts, it might be considered on an individual case with careful monitoring. GM-CSF has rarely been used (66) and there is too little data to make any comment on its usefulness in cryptococcosis.

Elevated Cerebrospinal Fluid (CSF)/Pressure

One of the most critical acute determinants of cryptococcal meningoencephalitis outcome is control of CSF pressure (67,68). Approximately half the HIV-infected patients with cryptococcal CNS disease have elevated baseline intracranial pressure ($>25 \text{ cm of CSF}$) and this CSF pressure can rise further as treatment is started with a subsequent increased morbidity and mortality. Most patients will receive CT/MRI scan on their initial evaluation and any patients without mass lesions with persistent symptoms and increased pressure should have repeated daily lumbar punctures and if symptoms persist after several days of CSF withdrawal strategy, then lumbar drains can be considered (69). Ventriculo-peritoneal (VP) shunts are a more permanent solution and are used in subacute/chronic conditions of hydrocephalus (70). Generally, these VP shunts can be placed during active infection if appropriate antifungals have been started (71,72). Increased intracranial pressure in cryptococcosis is not helped by medical treatments like acetazolamide (73), corticosteroids (68), and mannitol. If there is a careful effort to control intracranial pressure, outcomes of these CNS infections appear to be improved (74).

Immune Reconstitution Inflammatory Syndrome (IRIS)

In cryptococcosis, IRIS can occur in two forms: (i) unmasking IRIS in which cryptococcal symptoms first appear after the start of HAART, or (ii) paradoxical IRIS during the treatment of cryptococcosis and administration of HAART (75–79). It is important to emphasize that cryptococcosis-associated IRIS has also been reported as a complication in both solid organ transplant recipients (80) and normal hosts (53). It is the classic Goldilock's immunity paradigm of "not too much or not too little immunity but need to get it just right" (81). Many times, cryptococcosis occurs when immunity is depressed and during treatment the goal is to improve immunity but subsequently the host overshoots. In many cases, IRIS just needs to be identified and observed but in the central nervous system the excess inflammation can cause new, prolonged and even life-threatening symptoms. With major complications such as CNS inflammation with an associated increased CSF pressure, consideration of corticosteroid therapy may be necessary for IRIS (82). Generally, a taper of corticosteroids is considered over two to six weeks. It is important to realize that IRIS may present early in the management of meningitis or after several months of treatment. There are a series of criteria reported to diagnose IRIS but in

sum, it is increased inflammation with all biomarkers or cultures for yeasts either reducing or negative. It must be considered in all patients when new symptoms suggest failure. In transplant patients, IRIS can lead to graft loss (80).

Cerebral Cryptococcomas

Cerebral cryptococcomas can cause significant short- and long-term sequela. They are more commonly observed in *C. gattii* infections but may be detected in *C. neoformans* infections. It is essential in severely immunosuppressed patients with a nonresponding parenchymal brain mass(s) that the mass may also be considered as caused by a second pathogen and a brain biopsy or aspirate will be necessary. Brain lesions detected by CT scan are seen in apparently normal hosts (14%), AIDS patients (4–5%), or those with other risk factors (10%). Although there are no definitive studies in CNS cryptococcoma management, recommendations are based on case reports, retrospective and prospective observational studies, and expert opinions. In general, induction regimen with amphotericin B and flucytosine is prolonged and the suppressive phase with fluconazole is also extended from one to two years. Of course, these guidelines can be adjusted depending on the patient's response and other underlying conditions. However, radiographically it may take a long time for these lesions to disappear (83). In very large cryptococcomas (>3 cm) there have been successful cases managed in which surgically the parenchymal lesion was debulked.

CRYPTOCOCCAL MENINGOENCEPHALITIS TREATMENT IN SPECIAL CLINICAL SITUATIONS

1. Pregnancy (84–87)

Goal is to use polyene regimen and avoid fluconazole especially early in pregnancy (first trimester) (88). The use of flucytosine must be a bedside decision. It is important to watch for IRIS during postpartum period (89).

2. Children (90–95)

Cryptococcal disease occurs less frequently in children than adults. There are some unique childhood conditions such as hyper IgM syndrome, Severe Combined Immunodeficiency syndrome, Acute Lymphoblastic Leukemia, and Sarcoma associated with cryptococcosis. In general, pediatric meningoencephalitis is treated like adults except that higher doses of amphotericin B and lipid formulations can be used (96) and similarly fluconazole dosing is increased to approximate children metabolism and clearance (97).

Cryptococcosis in a Resource-Limited Health Environment

Sub-Saharan Africa is a common area for cryptococcal disease and it is primarily related to HIV infection. Details of treatment issues have been addressed but specifically, more aggressive induction therapy is needed with either short courses of high doses of intravenous amphotericin B (1 mg/kg/day for 1–2 weeks) (98–101) and/or high doses of fluconazole (≥ 1200 mg/day) (102).

***Cryptococcus gattii* Infections (103)**

Infections caused by this species predominantly occur in apparently normal hosts although occasionally there are HIV-infected cases (104,105). Despite some very difficult cases because of IRIS and variable drug susceptibility (106), at present most clinicians still treat *C. gattii* infections similar to *C. neoformans* infections (107).

The appropriate treatment strategies for patients with nonmeningeal cryptococcosis have been much less studied and only pulmonary cryptococcosis has enough cases reported to make robust recommendations (Table 4).

Pulmonary Cryptococcosis

Pulmonary cryptococcosis includes clinical presentations ranging from asymptomatic colonization to pneumonia to severe acute respiratory distress syndrome (108–112). Once *Cryptococcus* is isolated from the lung or airway of the host, there is an assessment of whether the patient is immunosuppressed or not. An immunosuppressed patient needs consideration of a lumbar puncture to rule out CNS disease even in a patient who is asymptomatic. In the

Table 4 Recommendations for Nonmeningeal Cryptococcosis

Initial antifungal regimen	Duration
Immunosuppressed and immunocompetent patient with pulmonary cryptococcosis (mild-to-moderate), fluconazole 400 mg/day	6–12 mo
Immunosuppressed ^a and immunocompetent patients with pulmonary cryptococcosis (severe), same as CNS disease	12 mo
Nonmeningeal, nonpulmonary cryptococcosis	
1. Cryptococemia, same as CNS disease	12 mo
2. CNS disease ruled out and no fungemia, single site, and no immunosuppressive risk factor, fluconazole 400 mg/day	6–12 mo

^aMust directly rule out CNS disease with lumbar puncture.

Source: Adapted from Ref. 122.

immunocompetent patient who has no CNS symptoms or signs, it is less critical to determine whether CNS involvement has occurred since it is very unlikely to present in the CNS in this group without symptoms (113,114). Of course, it is important to identify CNS cryptococcosis since it has a different induction therapeutic regimen. Pneumonia associated with CNS or documented dissemination and/or severe pneumonia (ARDS) should be treated like CNS disease. However, for those with mild-to-moderate symptoms or simply positive cultures or histopathology from a pulmonary lesion, fluconazole 400 mg/day for 6 to 12 months is a reasonable starting strategy (114–116). It can be adapted depending on patient's response, condition, and the impact of other therapies like starting HAART or stopping monoclonal antibodies like anti-TNF or anti-CD54. Although some clinicians have not treated what is diagnosed as asymptomatic colonization or nodule in normal host, others recommend treating all patients with *Cryptococcus* isolated. Since there are no randomized, prospective studies, the dose and duration of fluconazole is not precise and represents clinical experience and opinions more than robust evidence-based studies.

The nonmeningeal, nonpulmonary cryptococcosis is not common (117–121). There are occasional cases of primary (inoculation) skin infections (118) but most skin infections are primarily the consequence of dissemination even if only a single site is identified. In these cases one should check HIV test and work-up symptoms/signs and body sites such as CSF and blood. If cryptococemia or high-level dissemination (at least two noncontiguous sites) is involved or if there is evidence of high fungal burden based on cryptococcal antigen $\geq 1:512$, then treat as CNS disease. If CNS disease is ruled out, no fungemia, single site, and no major immunosuppressive risk factor(s), then consider fluconazole 400 mg/day for 6 to 12 months.

Cryptococcal infection can involve any body site or structure from liver, bone, lymph nodes, peritoneum, kidney, adrenal, or eyes. In the ocular infections, there will be a need for individualized strategy depending on the extent of ocular involvement. Consulting an ophthalmologist will be necessary since antifungal drugs like fluconazole and flucytosine penetrate eye tissue well but the patient may also need intravitreal amphotericin B.

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19 Management of Endemic Mycoses

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INTRODUCTION

The endemic mycoses are the classical invasive fungal pathogens that have restricted geographical barriers and generally a dimorphic fungal life cycle. From serological and skin test studies it is clear that the spectrum of illnesses ranges from subclinical infection to acute or chronic pneumonia, and in certain hosts the disease can disseminate to other organs in the body such as the central nervous system (CNS). Whenever you have this breadth of clinical presentations, it is clear that the individual host responses are critical to final outcome of the infection. These endemic mycoses have developed the ability to infect human hosts and produce disease. Although, there are described hypovirulent strains or mutants, in most cases after exposure, which is primarily by inhalation of spores or direct traumatic implantation into the skin, the individual immune response determines the disease presentation. The management of each of these endemic mycoses is discussed separately, but the principles for management with HIV coinfection are similar. Patients who received induction and then suppression therapy treatments should continue until immune reconstitution has occurred with HAART.

HISTOPLASMOSIS

Primary lung infection and severity of illness for *Histoplasma capsulatum* depends on the intensity of inhalation exposure to conidia as well as the immune status of the host and the underlying lung architecture (1).

Acute Pulmonary Disease

In a healthy host, asymptomatic infection or mild pulmonary disease from low aerosol exposure typically requires no therapy. However, if the patient remains symptomatic for three to four weeks, some clinicians might treat this acute infection with itraconazole (200 mg po bid for three months). If there is a large inoculum exposure, this may cause severe pulmonary infection with mediastinitis, hypoxia, and respiratory failure. In this setting of severe disease, amphotericin B (0.7 mg/kg/day) or liposomal amphotericin B (5 mg/kg/day) would be considered primary therapy until the patient clinically improves, and then followed by itraconazole (200 mg po bid for 6 to 12 months).

Pulmonary Nodules

Asymptomatic pulmonary nodules detected by radiographic studies and due to recent or previous infection can be confused with malignancy. Therefore, lesions are biopsied or excised and the tissue stains positive for *Histoplasma*. If the cultures from these lesions are negative, then generally no treatment is given. On the other hand, symptomatic pulmonary nodules and associated mediastinal adenopathy may be considered a recent infection and itraconazole therapy may be considered.

Broncholithiasis and Fibrosing Mediastinitis

When calcified lymph nodes erode into the airway producing respiratory symptoms or hemoptysis, patients may cough up broncholiths and conservative measures are appropriate. However, for difficult cases these broncholiths may need to be removed during bronchoscopic evaluation (2,3) or even require surgery but antifungal drugs are not necessary for treatment. Fibrosing mediastinitis can be fatal and is another complication of chronic *Histoplasma* infection. Although there has been no robust evidence that antifungal treatment makes any impact on outcome of fibrosing mediastinitis (4), some clinical experts would consider itraconazole therapy for three months. The use of adjuvant therapies like corticosteroids and antifibrotics such as tamoxifen

(5) are not certain to be of benefit. The use of stenting for mediastinal vessels will require consultation with surgeons specializing in mediastinal disease but may be necessary for the compressive disease of the airways and vascular structures which may occur with this disease process (6,7).

Chronic Histoplasmosis

In patients with mucosal membrane involvement (ulcers) or cavitary lung disease, itraconazole (200 mg po bid for 6 to 12 months) has been standard therapy (8). Chronic histoplasma cavitary lung disease (9), which is generally seen in those with underlying lung disease, can at times be refractory to initial treatment and therefore recommendations for itraconazole therapy may be extended for longer periods (12 to 24 months).

Disseminated Histoplasmosis

For patients with disseminated disease, which primarily occurs in immunocompromised hosts, the treatment strategy will primarily depend on severity of the illness but generally most clinicians will start initial therapy with a polyene regimen. In fact, one clinical study has supported the use of liposomal amphotericin B (5 mg/kg/day) over amphotericin B deoxycholate in a group of seriously ill patients with AIDS and disseminated histoplasmosis (10). After induction therapy for four to six weeks, patients may receive itraconazole for six months to one year (11,12). In AIDS patients, itraconazole therapy is prolonged for at least a year and maintenance therapy can be stopped when effective immune reconstitution occurs during HAART (CD4 count > 200/ μ L and low or undetectable viral load) (13). Immunosuppressed patients with disseminated disease may have detection of urine and/or serum histoplasma polysaccharide antigen. For this serology which can help in diagnosis, change in antigen measurements may also help judge effectiveness of treatment and serial measurements are encouraged. With effective treatment, antigen levels are reduced and/or eliminated. The *Histoplasma* antigen levels both for diagnosis and treatment may also be helpful in histoplasma meningitis. In meningitis, there are no definitive treatment studies but liposomal amphotericin B is probably the formulation of choice for CNS disease and this induction therapy may need to be prolonged compared to other sites of infection.

Although previous studies have shown that fluconazole and ketoconazole can be used to treat chronic and acute histoplasmosis, they are probably inferior to itraconazole which is the primary azole for most endemic mycoses. Alternative azoles to itraconazole, when it is not tolerated or has poor absorption, would likely be voriconazole or posaconazole. These azoles have excellent in vitro activity against *Histoplasma* and in animal models (14,15). Their use at present has primarily been in salvage or open-labeled protocols (16–18) but successes have been high for both drugs in histoplasmosis. Caspofungin and presumably the other echinocandins have not been found to be effective treatment for *Histoplasma* infections (19).

General Issues in Histoplasmosis Treatment

There are several issues with *Histoplasma* infection management. First, if itraconazole is used it should probably have a serum drug level measured at steady state with a goal of ≥ 0.5 μ g/mL and this is particularly important in patients with serious infections. The capsule formulation can be used but if absorption problems occur or are anticipated the suspension formulation may be more appropriate with its better absorption profile. However, it has higher gastrointestinal intolerance. Second, the use of corticosteroids for severe hypoxemia and diffuse pulmonary infiltrates and the development of acute respiratory distress syndrome becomes a bedside decision and should be considered. Third, the use of itraconazole prophylaxis in high-risk patients like AIDS patients in endemic areas with high likelihood of occupational or recreational exposure has been considered (20) but is not routinely recommended. Although it has been shown to be successful in prevention, the outcomes and costs are not well established in this era of HAART. Also, another area of risk has been the introduction of anti-TNF- α agents for treatment of certain underlying diseases in areas with histoplasmosis (21). Clinicians should be aware of the association between the use of these agents and *Histoplasma* disease. However, at present there are no formal recommendations for screening, prophylaxis, or exactly how to

manage patients after treatment for *Histoplasma* infection in regards to restarting the anti-TNF- α agents, although they are generally discontinued during the initial *Histoplasma* treatment.

SPOROTHRICOSIS

Sporothricosis is a disease caused by *Sporothrix schenckii*. It is found in decaying vegetation like sphagnum moss and generally is either directly inoculated into host tissue or possibly in the minority of cases, spores are inhaled. The primary presentation of this disease is either the lymphocutaneous or ulcerative forms of the disease but with inhalation, pulmonary sporothricosis can occur. *Sporothrix* can disseminate to other body sites with primarily bone and large joints or rarely meningitis as specific body sites. The therapeutic use of heat to skin lesions or the oral use of supersaturated potassium iodide has generally been replaced by itraconazole. Itraconazole is the drug of choice for most forms of sporothricosis and the general dose is 200 mg po bid. Amphotericin B or its lipid formulations is used for meningeal disease and may need to be used for difficult-to-treat cases of pulmonary and osteoarticular disease. There have been few reports of voriconazole or posaconazole use in sporothricosis, but these drugs should be effective and terbinafine use has not demonstrated superiority over the azoles.

BLASTOMYCOSIS

Blastomyces dermatitidis causes blastomycosis and this fungal infection occurs most often in persons living in the midwestern, south central, or southeastern United States or Canadian provinces that border the Great Lakes. Blastomycosis generally occurs as localized disease of the lungs but 25% to 40% of cases can present as disseminated disease such as cutaneous, osteoarticular, genitourinary, or CNS disease. As with most endemic mycoses, disseminated blastomycosis occurs more frequently in immunosuppressed individuals such as organ transplant recipients and/or those infected with HIV. A 2008 Update of Infectious Disease Society of America Clinical Practice Guidelines for management of blastomycosis has recently been published (22).

Pulmonary Blastomycosis

Acute (primary) blastomycosis can mimic community-acquired bacterial pneumonia. It is clear from clinical experience that this acute infection in normal hosts can spontaneously clear, and therefore it is not certain what exactly is the impact of antifungal treatment on the outcome of infection. However, some clinical experts will treat well-documented symptomatic blastomycete (acute) pneumonia with itraconazole despite its potential for spontaneous clearing of infection, in an attempt to improve more rapid clearance of symptoms or prevent chronic disease or dissemination.

Chronic pulmonary blastomycosis presents as a persistent pneumonia with alveolar infiltrates or masses that appear like lung cancer. Also, there are patients who have diffuse pulmonary infiltrates associated with acute respiratory distress syndrome (ARDS) which is associated with a substantial mortality. Generally, the diagnosis is made with histopathology of tissue or cytology demonstrating wide-based budding yeasts, cultures, or a *Blastomyces* antigen (urine, blood, or other fluids). In patients with mild-to-moderate pulmonary symptoms, itraconazole 200 mg po bid for 6 to 12 months has a high yield of success (>90% success) and this antifungal drug is probably better than ketoconazole or fluconazole. Although there were similar outcomes in 200 mg versus 400 mg and 400 mg/day versus 800 mg/day fluconazole studies (23,24), the higher dose study (400 mg vs. 800 mg) had higher success rates and thus if fluconazole is used, most would recommend starting with higher daily doses. There are simply too few cases treated with the newer triazoles, such as voriconazole or posaconazole, to make any robust recommendations but in vitro and animal studies support their activity against *Blastomyces* (25). Voriconazole has the most human clinical experience with *Blastomyces* and with its excellent CNS penetration it has been used to successfully treat CNS blastomycosis (26,27). In moderate-to-severe pulmonary disease, it is recommended that induction therapy starts with a polyene. Although amphotericin B deoxycholate has had many years of successful clinical experience, it is likely that the lipid formulations of amphotericin B would be similarly effective with less treatment toxicity. For instance, success rates with amphotericin B deoxycholate using >1g total dose can approach 90% and lipid formulations with higher doses have less reports

but should have similar efficacy. A common strategy in severely ill patients is to use induction polyene therapy for two to four weeks and then with clinical improvement, patients are switched to finish out six months to one year with itraconazole. An important area is ARDS in pulmonary blastomycosis. This presentation is a life-threatening event and although not common, it occasionally occurs and clinicians need to be prepared for its presentation. In these cases, corticosteroids may be of benefit to help control excess, damaging inflammation in combination with high dose polyene therapy.

Disseminated Disease

For mild-to-moderate disease such as osteoarticular (28–30), skin, and prostate, most patients can be managed with itraconazole and ensuring that the drug is being absorbed. In patients with severe symptoms or immunosuppression, polyene (either lipid formulation or deoxycholate) should be used for induction therapy for at least two weeks and then itraconazole. Specifically, for CNS disease, lipid formulations of amphotericin B at ≥ 5 mg/kg/day for four to six weeks should be given then followed by itraconazole (or voriconazole) for one year. For certain immunosuppressed patients, length of therapy will depend on how well the immunosuppression is reversed.

Specific Issues

Several issues associated with blastomycosis management need to be emphasized. First, the clinician must ensure that itraconazole levels are adequate. Second, itraconazole treatment is probably better than fluconazole and ketoconazole while voriconazole and posaconazole are alternative agents. Third, the echinocandins have no clinical activity for treatment of blastomycosis. Fourth, combination antifungal therapy or immunomodulating strategies have not been well established for blastomycosis and are rarely used. Fifth, corticosteroid therapy may be necessary in ARDS. Sixth, polyene induction therapy is used for severe cases of blastomycosis and then once it is controlled the patient can be shifted to azole therapy. Seventh, since underlying diseases less often complicate cases of blastomycosis, success rates in management of this infection are relatively high.

COCCIDIOIDOMYCOSIS

Coccidioidomycosis is caused by a soil fungus, which through molecular studies has been divided into two geographically separated species: *Coccidioides immitis* and *Coccidioides posadaei*. From a clinical and treatment standpoint, they are considered together. This fungus has strict geographical boundaries in North America from the arid regions of the southern portion of the San Joaquin Valley of California to south central Arizona and northwestern Mexico. It may also be found in South America. Like many of the inhalational endemic mycoses, there is both an acute pulmonary coccidioidomycosis, which presents like a community-acquired pneumonia. It may have some unique features such as hilar adenopathy, peripheral eosinophilia, and/or appearance of erythema multiforme or erythema nodosum. Diagnosis is made by culture, histopathology that shows spherules, or complement-fixation antibodies in serum or cerebrospinal fluid (CSF) (31).

Acute Infection

Primary pulmonary coccidioidomycosis in endemic regions without identified immunosuppressive risk factors is generally considered self-limited and does not require treatment. A retrospective study supports this observation. However, this present strategy continues to suffer from lack of evidence-based prospective, randomized studies and a study is needed to determine outcome of primary antifungal treatment versus placebo for total impact on patients such as time to clearing of symptoms. While nonimmunosuppressed patients with acute pulmonary coccidioidomycosis are not generally treated, treatment is given to those with impaired cellular immunity such as solid organ transplant recipients, HIV-infected patients with low CD4 count, comorbid conditions such as cardiac or pulmonary disease, and underlying conditions (i.e., diabetes) and those receiving potent immunosuppressive agents such as anti-TNF therapy (32). Chronic pulmonary coccidioidomycosis can be defined as symptoms for more than three months and these patients should be treated especially with cavitary lung disease and

hemoptysis. The use of surgery in cases with hemoptysis will need expert opinions regarding its need and implementation.

All patients should be followed for a year after acute pulmonary coccidioidomycosis for complete resolution since dissemination primarily occurs in this time frame. Clinicians should be able to diagnose disseminated coccidioidomycosis relatively early and particular emphasis needs to be placed on cell-mediated immune defective patients and certain racial types like African and Filipino American males, who have a relatively high rate of disseminated infections. The most common sites for dissemination of infection are skin and soft tissue, bones and joints, and the CNS. Since CNS invasion may occur and be manifested within the first one to three months after acute pulmonary infection, if CNS symptoms occur, it will be necessary to rule out coccidioidal meningitis.

Chronic Infection Management

Although antifungal treatment for acute pulmonary infection is not used routinely, chronic coccidioidomycosis is treated with antifungal agents for 12 to 18 months and longer courses in immunosuppressed individuals. In CNS involvement with coccidioides infection, treatment is considered for a life time. Available agents for treatment of coccidioidomycosis include azoles and polyenes. In skin and pulmonary infections, outcomes seem similar with fluconazole or itraconazole at a minimum dose of 400 mg/day and in bone/joint infections there is one report that suggests itraconazole may be superior to fluconazole (33). There is a rich history of clinical trials for this chronic infection stage and despite excellent responses there are still a number of patients who relapse after treatment. There are some reports which suggest that voriconazole (34) and posaconazole (35,36) are active in chronic and/or recalcitrant cases but there remains a need for their performance to be compared to the gold standard, itraconazole. In patients with severe disseminated disease, more consideration should be given to initial induction therapy with polyene (either amphotericin B deoxycholate or lipid formulation of amphotericin B). There are no definitive data suggesting that one polyene preparation is better than another and the precise length of induction treatment is empirical before then switching to azole in the consolidation or suppressive phases of treatment.

Patients with coccidioidal meningitis (cellular reaction in CSF such as eosinophils and either culture or complement fixation antibody positive with evidence of inflammation) are a special group since the goal appears to be suppression of infection rather than consistent cure. These specific patients with present therapeutic regimens have substantial failure rates if antifungals are stopped (37). The most common drug for initial treatment of meningitis is fluconazole at ≥ 800 mg/day indefinitely and a report of successful treatment with voriconazole has been made (38). In very difficult to control patients, there is still the intermittent use of intrathecal amphotericin B (0.25 mg IT) to control symptoms (39). However, there are substantial neurological side effects with intrathecal amphotericin B and these may arise and need management.

The use of prophylaxis for high-risk patients in highly endemic areas for coccidioidomycosis could be considered, but rates of disease remain too uncertain and the arena for prophylaxis or prevention may be best controlled with an effective vaccine. There has been a history of interest in coccidioidal vaccines but none have made it to clinical practice.

PENICILLIOSIS

Penicillium marneffe is a fission yeast in tissue and red mold on agar plates at room temperature. It has a specific geographical niche and this is primarily in certain parts of southeast Asia. During the AIDS epidemic, this endemic mycosis rose to prominence as a disseminated opportunistic infection associated with HIV infection. The clinical presentation is similar to histoplasmosis in AIDS patients but it does have unique propensity for skin involvement.

Treatment of Disseminated Infection

In the management of disseminated penicilliosis, the primary focus is on both treatment and control of the underlying HIV infection. Both itraconazole and amphotericin B are active against this fungus and have been used in treatment. At present, itraconazole is probably the initial drug of choice but for seriously ill patients, amphotericin B may be necessary for induction

therapy before conversion to azole. Voriconazole and posaconazole would probably be effective in treatment but there is very limited clinic experience with these new extended-spectrum azoles. Importantly, like all endemic invasive mycoses, in the presence of underlying HIV infection, the use of HAART and documented immune reconstitution will be necessary before antifungal drugs are discontinued, but at a minimum patients should receive one year of therapy if available.

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20 Human Hyalohyphomycoses: A Review of Human Infections Due to *Acremonium* spp., *Paecilomyces* spp., *Penicillium* spp., and *Scopulariopsis* spp.

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INTRODUCTION

In recent years, there has been an increase in the incidence of opportunistic fungal infections. This has been directly related to advances in modern medical technology including the use of broad-spectrum antibiotics, immunosuppressant drugs for organ and bone marrow transplantation, cytotoxic chemotherapy for cancer, corticosteroids, and the increased use of indwelling medical devices. Besides the commensal organisms *Candida albicans* and *Malassezia furfur*, the majority of the opportunistic mycoses are caused by exogenous fungi that exist in nature as free-living saprophytes or plant parasites (1). Historically, these fungi have been treated as contaminants when isolated from clinical specimens, however, a significant number of these saprophytic molds have become opportunistic pathogens in the immunocompromised host (2).

These molds, which form mycelia in tissue, can be divided into two groups based on their cell-wall pigmentation. Hyalohyphomycoses represent molds with hyaline or light colored cell walls, while the phaeohyphomycoses are those molds with dark colored cell walls due to the presence of melanin (1). This review will focus on the hyalohyphomycoses. Table 1 lists the genera and species of the organisms classified as hyaline molds. Since *Fusarium* and *Scedosporium* are covered elsewhere in this supplement and much has already been written about *Aspergillus*, this article will focus on four other hyaline molds that cause significant clinical disease. These include *Acremonium* spp., *Paecilomyces* spp., *Penicillium* spp., and *Scopulariopsis* spp. Within each genus, we will discuss the organisms' mycology, epidemiology, clinical presentation, and treatment.

ACREMONIUM SPECIES

Epidemiology and Mycology

The genus *Acremonium*, formerly known as *Cephalosporium*, contains about 100 different species that are commonly found in our environment in soil, insects, sewage, plants, and other environmental substrates (3). The first recorded isolation of this species was *Acremonium cephalosporium* in 1939 (4). In 1945, *A. cephalosporium* was isolated in the sewage effluent during a search for antibiotic-producing organisms of the coast of Sardinia (4). By 1953, the active antimicrobial compound cephalosporin C was isolated from *A. cephalosporium* by investigators at Oxford University leading to the development of the cephalosporin class of antibiotics (4).

Most early mycologists felt *Acremonium* species represented laboratory contaminant until the middle of the 20th century when the first evidence of pathogenicity was reported (4). To date, only seven species of *Acremonium* have been reported to cause infection in humans. Of these, 80% are caused by *A. falciforme*, *A. recifei*, and *A. kiliense* while the remaining 20% are caused by *A. potronii*, *A. roseo-griseum*, *A. strictum*, and *A. alabamensis* (4).

This genus is distinguished by formation of narrow hyphae bearing solitary, slender, unbranched needle-shaped phialides with some species having shallow collarettes forming conidia in slimy masses (Fig. 1) (3). *Acremonium* species grow within five days on Sabourand dextrose agar forming white, salmon, or yellowish-green colonies that are usually velvety,

Table 1 Current Agents of Hyalohyphomycoses

<i>Acremonium</i>	<i>A. glaucus</i> grp.	<i>C. tropicum</i>	<i>F. tabacinum</i>	<i>O. Canadensis</i>	<i>Scopulariopsis</i>
<i>A. alabamensis</i>	<i>A. nidulans</i> grp.	<i>C. zonatum</i>	<i>F. ventricosum</i>	<i>Ovadendron</i>	<i>S. brevicaulis</i>
<i>A. blochii</i>	<i>A. niger</i>	<i>Colletotrichum</i>	<i>Gymnascella</i>	<i>O. sulphureo-ochraceum</i>	<i>S. brumptii</i>
<i>A. curvulum</i>	<i>A. ochraceus</i>	<i>C. atramentum</i>	<i>G. hyalinospora</i>	<i>Paecilomyces</i>	<i>S. candida</i>
<i>A. fatifforme</i>	<i>A. oryzae</i>	<i>C. gloeosporioides</i>	<i>Metarhizium</i>	<i>P. farinosus</i>	<i>S. chartarum</i>
<i>A. hyalinum</i>	<i>A. parasiticus</i>	<i>Coprinus</i>	<i>M. anisopliae</i>	<i>P. fumosoroeus</i>	<i>S. flava</i>
<i>A. kilianse</i>	<i>A. restrictus</i>	<i>C. cinereus</i>	<i>Microascus</i>	<i>P. javanicus</i>	<i>S. fusea</i>
<i>A. potronii</i>	<i>A. sydowii</i>	<i>Engyodontium</i>	<i>M. cirrosus</i>	<i>P. lilacinus</i>	<i>Trichoderma</i>
<i>A. roseogriseum</i>	<i>A. terreus</i>	<i>E. album</i>	<i>M. manginii</i>	<i>P. marquandii</i>	<i>T. harzianum</i>
<i>A. strictum</i>	<i>A. ustus</i>	<i>Fusarium</i>	<i>Microhilum</i>	<i>P. varioti</i>	<i>T. longibrachiatum</i>
<i>Aphanoascus</i>	<i>A. versicolor</i>	<i>F. aquaeductum</i>	<i>M. oncoperae</i>	<i>P. viridis</i>	<i>T. viride</i>
<i>A. fulvescens</i>	<i>Beauveria</i>	<i>F. chlamydosporum</i>	<i>Microsphaeropsis</i>	<i>Penicillium</i>	<i>Tritirachium</i>
<i>Arthrographis</i>	<i>B. alba</i>	<i>F. dimerum</i>	<i>M. olivacea</i>	<i>P. chrysogenum</i>	<i>T. oryzae</i>
<i>A. kalrae</i>	<i>B. bassiana</i>	<i>F. farinosus</i>	<i>Monoascus</i>	<i>P. citinum</i>	<i>Tubercularia</i>
<i>Aspergillus</i>	<i>Cephalophora</i>	<i>F. incarnatum</i>	<i>M. rubber</i>	<i>P. commune</i>	<i>T. vulgaris</i>
<i>A. candidus</i>	<i>C. irregularis</i>	<i>F. moniliforme</i>	<i>Myriodontium</i>	<i>P. decumbens</i>	<i>Verticillium</i>
<i>A. carneus</i>	<i>Cerinoaterus</i>	<i>F. napiforme</i>	<i>M. keratinophilum</i>	<i>P. expansum</i>	<i>V. serrae</i>
<i>A. deflektus</i>	<i>C. cyanescens</i>	<i>F. oxysporum</i>	<i>Nannizziopsis</i>	<i>P. marneffei</i>	<i>Volutella</i>
<i>A. fischeri</i> grp	<i>Chrysonilia</i>	<i>F. pallidroseum</i>	<i>N. vriesii</i>	<i>Pseudallescheria</i>	<i>V. cinerescens</i>
<i>A. flavipes</i>	<i>C. sitophila</i>	<i>F. proliferatum</i>	<i>Neocosmospora</i>	<i>P. boydii</i>	
<i>A. flavus</i>	<i>Chryso sporium</i>	<i>F. sacchari</i>	<i>N. vasinflecta</i>	<i>Scedosporium</i>	
<i>A. fumigatus</i> grp.	<i>C. pannicola</i>	<i>F. solani</i>	<i>Onychocolla</i>	<i>S. commune</i>	

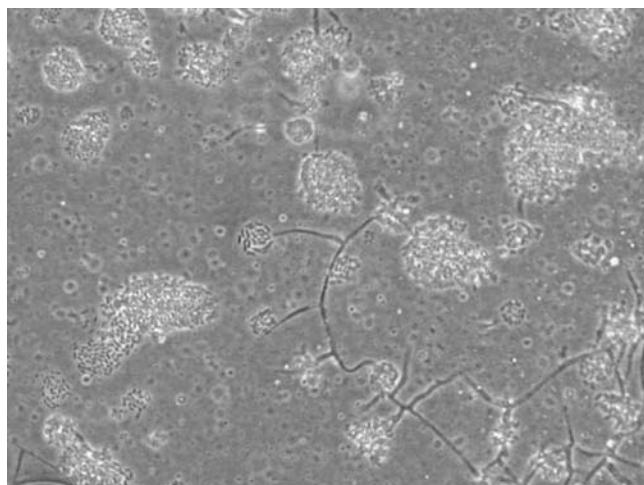


Figure 1 *Acremonium* species. Microscopy characterized by narrow hyphae with thin walled, slightly tapered phialides and ellipsoidal conidia forming slimy masses.

cottony, or fasciculate with flat or slightly raised centers (Fig. 2) (3). On oatmeal agar, the most medically important species, *A. kiliense*, forms chlamydospores (3).

Clinical Presentation

The most common infections due to *Acremonium* species are mycetomas or ocular infections, although the presence of the organisms in the soil, water, and air can lead to superficial infections after traumatic inoculation, onychomycosis, and complications in burn patients (5). In more recent years, the incidence of infections with this genus, including localized or disseminated infections, has been on the rise. This trend is probably related to advances in medical care leading to organ transplantation, prolonged neutropenia, and the increased use of indwelling medical devices and broad-spectrum antibiotics.

Mycetoma is the most common human infection caused by *Acremonium* species. This chronic infection of the distal extremities is marked by the triad of swelling, sinus tract formation, and fungal granules, and causes only mild discomfort with prolonged infection leading to



Figure 2 (See color insert) Colony of *Acremonium* species. Moderately fast growing with pinkish color, velvety texture, and slightly raised center.

underlying bone destruction (4). Although this infection is most commonly seen in young, immunocompetent men living in tropical/subtropical climates, *Acremonium* mycetomas have been reported in Africa, Asia, Europe, and North and South America and have been seen in a few immunocompromised patients following penetrating skin wounds (4,6).

Ocular infections represent the second most common infection caused by *Acremonium* species. Multiple cases of keratitis (4,7,8) and endophthalmitis (4,9) have been reported in the medical literature. The majority of these infections occur following trauma, surgery, or weakening of local eye defenses with topical antibiotics or steroids (5). The colonization of soft contact lenses with *Acremonium* species leading to the invasion of the cornea has also been reported (5) and has led to the recommendation that contact lenses be disinfected after exposure to high concentrations of environmental fungi (i.e., following raking leaves) (4).

Several cases of locally invasive *Acremonium* infections have been reported in the literature. These reports include esophagitis in a 5-year-old girl with mixed connective tissue disease (10), a chronic painless lesion on the cheek of an immunocompetent woman (11), bilateral nodular pulmonary infiltrates in a neutropenic patient with chronic lymphocytic leukemia (12), pleural empyema following a therapeutic pneumothorax (4), septic arthritis of the knee (4), and osteomyelitis of the calvarium following trauma (4). Several cases of *Acremonium* infection in patients with end-stage renal disease have also been reported. Interestingly, these infections have all been associated with the dialysis access site. Fincher described two cases of infected arteriovenous (AV) grafts and two cases of peritonitis secondary to infected peritoneal dialysis catheters in his review and an additional three cases of CAPD-related peritonitis have since been reported (4,13,14).

Disseminated infections have become more frequent over the past decade, which is related to the increasing immunocompromised population in our hospitals. Fincher reported only three cases of disseminated infection in his 1991 review, which included cases of endocarditis, meningitis, and diffuse cerebritis (4). Since 1991, there have been 10 case reports of bloodstream infection with *Acremonium* species. These cases have occurred mostly in patients with profound neutropenia (5,15–19), but have also been seen in patients with severe combined immunodeficiency (20), and Addison's disease (6). Disseminated infection has also been reported in immunocompetent patients as illustrated in case reports of right-sided pacemaker-associated endocarditis with secondary endophthalmitis (21) and a gentleman with a splenectomy who developed lumbar diskitis following *Acremonium* endophthalmitis (22). The severity of this increasing incidence of disseminated infection is confounded by the frequent difficulty in diagnosis and the high mortality rate ranging from 30% to 50% (15).

Treatment

Treatment of *Acremonium* infections presents a challenge to those involved. The optimal therapy for such infections remains unknown due to the small number of infections and a lack of clinical data including speciation of the *Acremonium* isolates. Clearly, in patients with neutropenia, the best hope of recovery is the return of granulocytes. This is well demonstrated in a case report of a woman with line-associated *Acremonium* fungemia that resolved with resolution of her neutropenia despite no antifungal therapy and retention of the catheter only to recur with her next bout of chemotherapy-related neutropenia (18).

There is little data supporting drug susceptibility of various *Acremonium* species. Guarro et al. published antifungal susceptibilities to 33 clinical isolates and Table 2 is a compilation of the drug susceptibilities of the isolates from published case reports. Unfortunately, many of the case reports did not speculate their *Acremonium* isolates. In general, these organisms are resistant to available antifungal agents. Susceptibility appears to be species dependent, but some generalizations can be made. Amphotericin B appears to be the most effective agent although there has been at least one published case of amphotericin B failure (12,23). Fluconazole and 5-fluorocytosine resistance are uniform amongst all isolates while ketoconazole and itraconazole susceptibility appeared to be species dependent. Terbinafine showed promising in vitro activity against one strain of *A. strictum* but there has been no other in vitro testing done with this drug or case reports of its use (15).

There is very little data concerning the use of newer antifungal agents in the treatment of *Acremonium* infections. One case report describes the successful use of liposomal amphotericin B

Table 2 Antifungal Susceptibility Testing of *Acremonium* Clinical Isolates from Published Case Reports

Ref	Organism	MIC ($\mu\text{g/mL}$)									
		AMB	KETO	FLU	ITRA	5-FC	TBF	VOR	POS	CSP	ANU
4	<i>Acremonium sp</i>	1.0	32	32	16	—	—	—	—	—	—
4	<i>Acremonium sp</i>	1.0	0.25	16	2.0	—	—	—	—	—	—
4	<i>Acremonium sp</i>	1.0	8.0	32	32	—	—	—	—	—	—
4	<i>Acremonium sp</i>	1.0	32	0.5	16	—	—	—	—	—	—
4	<i>Acremonium sp</i>	2.0	0.5	64	2.0	—	—	—	—	—	—
4	<i>Acremonium sp</i>	1.0	64	>64	16	—	—	—	—	—	—
18	<i>Acremonium sp</i>	<0.5	—	1024	1024	—	—	—	—	—	—
15	<i>A. strictum</i>	2.0	—	>64	32	>64	0.125	0.5	—	—	—
21	<i>Acremonium sp</i>	31	—	50	3.1	—	—	0.39	—	—	—
16	<i>A. falciforme</i>	0.5	>32	>256	>32	—	—	—	—	—	—
12	<i>A. strictum</i>	8.0	—	64	8	—	—	—	>8.0	—	—
54	<i>A. strictum</i>	—	—	—	—	—	—	—	>16	0.5	0.06
85	<i>A. alabamensis</i>	1.0	—	16	2.0	—	—	0.25	—	—	—
85	<i>A. strictum</i>	16	—	>128	>32	—	—	2.0	—	—	—

Abbreviations: AMB, amphotericin; KETO, ketoconazole; FLU, fluconazole; ITRA, itraconazole; 5-FC, flucytosine; TBF, terbinafine; VOR, voriconazole; POS, posaconazole; CSP, caspofungin; ANU, anidulafungin.

in two children with severe neutropenia, but there is no available in vitro susceptibility data (19). Information concerning the use of the new triazoles is limited only to case reports (21,24,25). In this regard, Mattei et al. described two patients with disseminated *Acremonium* infection who experienced failure of deoxycholate amphotericin B, but were successfully treated with voriconazole (24). The efficacy of voriconazole in the treatment of *Acremonium* infection was further highlighted in a recent report (25). The author presented a patient with *Acremonium* vertebral osteomyelitis that relapsed despite surgical debridement along with six weeks of liposomal amphotericin B and eight weeks of oral itraconazole. The patient responded well to a prolonged course of voriconazole therapy suggesting that voriconazole might be a promising agent in the treatment of *Acremonium* infection. In addition to the observed clinical success, in vitro data are also encouraging for voriconazole with MICs against different *Acremonium* spp. ranging from 0.25 to 2.0 $\mu\text{g/mL}$. Although in vitro data have been less encouraging for posaconazole with MICs of two isolates >8 $\mu\text{g/mL}$, there is one case report of successful treatment of bilateral nodular pulmonary infiltrates in a neutropenic patient who failed therapy with amphotericin B (12). The effectiveness of echinocandins remains unknown at this time. One in vitro isolate revealed susceptibility to both caspofungin and anidulafungin. In another report, the organism appeared resistant to micafungin with MIC of 0.125 $\mu\text{g/mL}$ (26). In the former study, a patient developed a breakthrough disseminated *Acremonium* infection while on micafungin therapy suggesting that micafungin is ineffective against *Acremonium* infection and therefore not recommended.

Anecdotal reports of combination therapy with amphotericin B and an azole (fluconazole, ketoconazole, or itraconazole) exist but these regimens remain empirical until further study is completed (15,19,20).

PAECILOMYCES SPECIES

Epidemiology and Mycology

The genus *Paecilomyces* contains 31 species that occur worldwide as saprophytes in soil, decomposing organic matter, and occasionally as insect parasites (2). The first species isolated was *Paecilomyces variotii* by Bainier in 1907, which was closely followed by the identification of *P. lilacinus* by Thom in 1910 (27). *Paecilomyces* are rare human pathogens but the most pathogenic strains are *P. lilacinus* and *P. variotii* while *P. marquandii*, *P. javanicus*, and *P. viridis* have also been documented to cause infections in humans (2). *Paecilomyces* are common contaminants of sterile solutions since they are resistant to most commercial sterilization techniques, which explain why infections with this organism are often associated with surgical procedures (27).

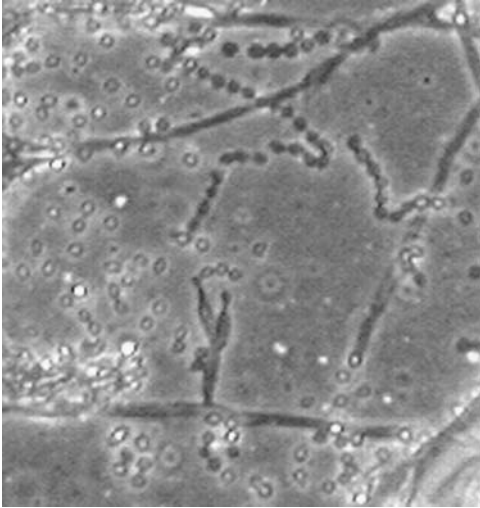


Figure 3 *Paecilomyces lilacinus*. Microscopy is notable for phialides that are swollen at the base with long tapered necks and long chains of elliptical conidia.

Microscopically the genus *Paecilomyces* is similar to *Penicillium*. The most distinguishing feature is the shape of the phialides, which are swollen at the base and terminate in long slender tubes frequently bent away from the main axis with long chains of elliptical to fusiform conidia on the tips (Fig. 3) (27). The lack of bright green or blue-green colonies and the characteristic phialides distinguish *Paecilomyces* from *Penicillium*. *Paecilomyces* species grow well on blood, chocolate, potato dextrose, and Sabouraud dextrose agar, but the color, colony size, and growth rate vary depending on the species (28). *P. lilacinus* grows rapidly forming white cottony colonies that gradually become lilac or vinaceous in color (Fig. 4) while *P. variotii* forms yellowish-brown, powdery colonies with a raised center and brown reverse producing a sweet smell (27,29).

Clinical Presentation

Human infections by *Paecilomyces* species remain rare but, like other opportunistic fungi, seem to be on the rise. This is supported by the 32 case reports that have been published in the last decade while only 39 cases had been reported in the preceding 30 years. Risk factors for infection by this organism include immunosuppression, surgery, foreign body implants, and

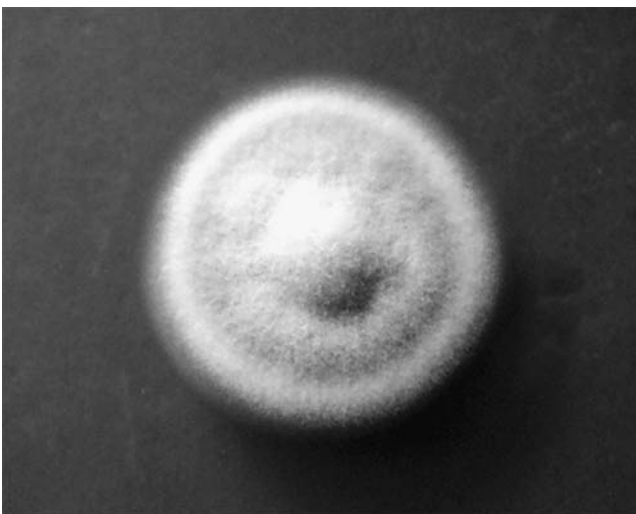


Figure 4 Colony of *Paecilomyces lilacinus*. Cottony colonies that gradually become lilac in color.

trauma, and the most common areas infected are the eye, skin and soft tissue, heart, sinuses, and lung (27,30).

All reported eye infections with *Paecilomyces* have been preceded by trauma, surgery, foreign body implant, or corticosteroid therapy (27). The most common eye infections include keratitis and endophthalmitis. An outbreak of *P. lilacinus* endophthalmitis occurred in 1975 in 13 patients who underwent cataract extraction and lens implantation. The source of this outbreak was traced to a contaminated neutralizing solution that was used to rinse the lenses during surgery (27).

Immunosuppression places patients at risk for infection with *Paecilomyces* species. Case reports range from soft tissue infections and osteomyelitis in two children with chronic granulomatous disease (31,32), to catheter-associated fungemia (33,34), sinusitis (35), and disseminated fungemia in bone marrow transplant patients (36) and those with AIDS (37,38). There are multiple reports of cutaneous *Paecilomyces* infection in patients with solid organ transplants (39,40) and lymphoma (41). In 1993, 12 patients on a bone marrow transplant ward in Switzerland developed infections with *P. lilacinus* that was traced to a contaminated skin lotion (42).

The association of *Paecilomyces* infections with surgery and medical implants may be explained by this organism's resistance to most commercial sterilization equipment and the possibility that foreign bodies may enhance the invasive potential of this pathogen (27). The first documented human infection with *Paecilomyces* in 1963 was a fatal case of prosthetic mitral-valve endocarditis and eight other cases of prosthetic-valve endocarditis have since been reported (27). Case reports of postoperative complications include mediastinitis following lung transplantation (43), abdominal infection following cesarean section (44), and contamination of a saline-filled breast implant (45). Besides prosthetic heart valves, the other most commonly infected indwelling medical devices are intravenous (33,34) and peritoneal dialysis catheters (29,46,47).

Paecilomyces infections in immunocompetent individuals are rare, but do occur. The majority of the reports in the literature describe cutaneous infections (30,48–50), but there are isolated cases of lung abscess (51), pneumonia (52), sinusitis (28,53), and prepatellar bursitis (54).

Treatment

Like other hyalophycomycoses, the optimal antifungal therapy for *Paecilomyces* infections remains uncertain. However, there are two important steps that should be taken in all *Paecilomyces* infections: (1) early removal of any infected indwelling device, and (2) speciation of the isolate since in vitro antifungal susceptibility is highly species dependent (27). *P. lilacinus* and *P. marquandii* are highly resistant to amphotericin B, fluconazole, and flucytosine while almost universally susceptible to ketoconazole and miconazole (27,55,56). Itraconazole susceptibility varies for these two isolates. *P. lilacinus* susceptibility to the new triazoles appears very favorable in vitro with MICs from one study for voriconazole, posaconazole, and ravuconazole reported as 0.25, 0.125, and 0.5 $\mu\text{g}/\text{mL}$, respectively (48). Furthermore, in vitro combinations of voriconazole and terbinafine was shown to be particularly effective against *P. lilacinus* organisms (57–61). There are also multiple case reports of favorable outcome when *P. lilacinus* infections were treated with voriconazole (38,40,56,62,63). Voriconazole was effective in the treatment of complicated keratitis due to *P. lilacinus* using either the topical (64) or systemic voriconazole formulation (62) or in combination with terbinafine (65). Oral voriconazole has been successful in eradicating multiple skin lesions that failed previous therapy with itraconazole and amphotericin B (38) in an AIDS and in a postrenal transplant patient (40). *Paecilomyces* infections including *P. lilacinus* and *P. marquandii* may require higher concentrations of voriconazole over an extended period to achieve inhibition of the organism (40,63). Of note, the new antifungal posaconazole has shown consistent efficacy in vitro, but no clinical data or case reports of its use with *Paecilomyces* species have been published to date (59,61).

The effect of echinocandins on *P. lilacinus* is still uncertain. In vitro data suggest that caspofungin may not be effective with MICs ranging from 1.0 to $>100 \mu\text{g}/\text{mL}$ in two studies (48,66), but one case report describes the successful treatment of progressive cutaneous *P. lilacinus* infection with a combination of caspofungin and itraconazole (48). To date, there is no conclusive evidence that echinocandins should be used clinically for invasive *Paecilomyces*

infection (57,59). Other combinations that have been used with various successes against *P. lilacinus* include amphotericin B/itraconazole and amphotericin B/flucytosine (33,35).

Less in vitro and clinical data exist concerning the susceptibility of *P. variotii*; however, this species is almost universally susceptible to amphotericin B and flucytosine (27) and is usually sensitive to miconazole, ketoconazole, and itraconazole (58). Although, fluconazole resistance is standard for this species, clinical success has been reported when fluconazole was used in combination with flucytosine in the treatment of *P. variotii* splenic abscess (67). However, in the former case a partial splenectomy was also attempted highlighting the role of appropriate surgical intervention in the management of *Paecilomyces* infection. The new triazoles are promising although in vitro data only exist for posaconazole with MICs ranging from 0.06 to 0.12 $\mu\text{g}/\text{mL}$ (68). *P. variotii* seems to be more susceptible to echinocandins than other species of *Paecilomyces* with MICs for caspofungin and anidulafungin <0.09 and <0.03 $\mu\text{g}/\text{mL}$, respectively (66,68).

PENICILLIUM SPECIES

Epidemiology and Mycology

The greater-than-200 blue-green molds that exist throughout nature make up the genus *Penicillium*. Most *Penicillium* species grow over a wide temperature range, but many are strongly or completely inhibited at 37°C and therefore, rarely cause infections in humans (3). The only true human pathogen in this genus is *Penicillium marneffeii*, although there are a few case reports of the following species causing human infection: *Penicillium brevicompactum*, *Penicillium chrysogenum*, *Penicillium decumbans*, *Penicillium citrinum*, *Penicillium commune*, *Penicillium crustaceum*, *Penicillium expansum*, *Penicillium glaucum*, and *Penicillium spinulosum* (69).

P. marneffeii is predominantly found in Southeastern and Far Eastern Asia and is a unique dimorphic species forming unicellular yeast-like organelles in tissue that can often be confused with *Histoplasma capsulatum* (3). Segretain initially isolated this organism in 1959 from the bamboo rat *Rhizomys sinensis* in Southeast Asia although the primary reservoir for this fungus remains unknown (70). The first reported case of disseminated infection occurred in an American missionary with Hodgkin's disease who had traveled extensively in Southeast Asia (71). By 1990, there were 30 reports of naturally occurring infection with *P. marneffeii*, but over the past decade, there has been a rapidly increasing incidence of invasive infections in AIDS patients as well as other immunosuppressed patients in the endemic areas.

P. marneffeii grows rapidly at 25°C as a mold on Sabouraud dextrose agar producing grayish, floccose colonies with brownish-red to wine-colored water-soluble pigment that diffuses throughout the agar (Fig. 5) (70). Microscopically, the conidiophores are divaricate with each penicillus consisting of four to five metulae on which four- to six-pointed phialides rest each with a chain of one-celled conidia (Fig. 6) (72). At 37°C, *P. marneffeii* converts to yeast-like spherical cells that multiply by fission (71). Differentiation between *P. marneffeii* and other species of *Penicillium* depends on a combination of microscopic evaluation and proof of dimorphism.

Clinical Presentation

P. marneffeii is endemic to China, Hong Kong, Indonesia, Thailand, and Vietnam and has been limited to populations who live in these areas or those who traveled to these endemic regions. Greater than 80% of those infected with *P. marneffeii* are immunocompromised (73); however, there have been several reports of *P. marneffeii* infection occurring in immunocompetent hosts (74–76). *P. marneffeii* can cause both focal infection and disseminated disease.

Disseminated disease has similar presentation in both immunocompromised and immunocompetent host. Hilmarsdottir et al. compared 46 HIV positive patients with 15 immunocompetent patients infected with *P. marneffeii* and found the most common manifestations to include fever, weight loss, adenopathy, hepatosplenomegaly, pulmonary symptoms, skin lesions, anemia, and leukocytosis or leukopenia (74). Symptoms may be present for weeks to years prior to diagnosis. In 75% of disseminated infections, umbilicated skin papules, easily confused with molluscum contagiosum, cutaneous cryptococcosis, or histoplasmosis, will be present on the face, trunk, and extremities (71,77). In addition, bone marrow infection, genital ulcers, osteomyelitis, arthritis, and retropharyngeal abscess have all been reported as



Figure 5 (See color insert) *Penicillium marneffeii*. Colonies flat mostly submerged with aerial mycelium that are white and submerged mycelium that are red. The colony characteristically exudes red pigment into the media.

complications of disseminated disease (73). The organism may be isolated from bone marrow, skin biopsy, or blood with a high rate of success in disseminated infections (71).

Focal infections with *P. marneffeii* may also occur. In fact, shortly after Segretain first identified *P. marneffeii*, he described the first focal infection after sustaining a needle stick injury to his finger while inoculating rodents (27). Other sites subject to focal infection have included hair, skin, nails, ear, sinuses, urinary tract, invasive pulmonary disease, brain abscess, and prosthetic-valve endocarditis (78). More recently, there have been several cases of peritoneal dialysis-associated peritonitis secondary to *P. marneffeii*. In all cases, the patients did well following removal of the peritoneal catheter and treatment with various antifungal regimens (69).



Figure 6 *Penicillium marneffeii*. Microscopy reveals four to five metulae on which four to six pointed phialides rest, each with a chain of one-celled conidia.

Treatment

P. marneffei is a curable infection if treatment is started early. Mortality rates without treatment are 90% in the non-HIV population and 100% in those infected with HIV (71). Most reports of antifungal susceptibility and treatment are in HIV patients since this population has the highest infection rate, but this information is applicable to other populations. Sekhon et al. performed in vitro antifungal susceptibility testing on a group of pathogenic isolates and discovered that itraconazole had the lowest MICs amongst all other antifungals tested (79). Amphotericin B, ketoconazole, miconazole, and flucytosine also had favorable MICs while fluconazole was not effective (79). More recently, in vitro testing with the new triazole voriconazole against 25 isolates of *P. marneffei* (MICs < 0.03 to 0.06 µg/mL) suggests that this new antifungal may be effective in the treatment of this organism (72). Currently, there is no in vitro or in vivo data for echinocandin efficacy against *P. marneffei* making the role of this new class of antifungals unknown.

Most clinical reports have treated *P. marneffei* infections successfully with amphotericin B alone or in combination with itraconazole, ketoconazole, or flucytosine. However, in one small study, itraconazole was given as monotherapy resulting in cure, but required two months for fungal cultures to sterilize (80). Another small, noncomparative study looked at the efficacy of voriconazole in 11 HIV-infected patients with disseminated *P. marneffei* infections. Six patients received 12 weeks of oral voriconazole, two received 2 weeks of intravenous followed by 10 weeks of oral voriconazole, one patient received 16 days of oral voriconazole, and the other two were discontinued secondary to abnormal baseline blood work. Eight of the nine evaluable patients were successfully treated and six patients seen at four-week follow-up remained disease free (81).

Since therapy with amphotericin B requires long-term intravenous access and is associated with multiple adverse effects, Sirisanthana et al. performed an open-label nonrandomized trial studying the efficacy of two weeks of amphotericin B followed by 10 weeks of oral outpatient itraconazole in HIV patients with disseminated *P. marneffei* infection (82). In this study, 72/74 (97.3%) of the patients responded to therapy (82), but in a previous study there was a 50% relapse rate within six months (80). A double-blinded randomized controlled trial studying the efficacy of secondary prophylaxis with itraconazole revealed that 57% of the placebo group relapsed within a year while none of those receiving itraconazole prophylaxis relapsed (83). The duration of secondary prophylaxis following highly active antiretroviral treatment-induced immune reconstitution remains unclear at this time. In conclusion, the standard therapy for disseminated *P. marneffei* infection is two weeks of amphotericin B followed by 10 weeks of oral itraconazole. In those with HIV, secondary prophylaxis with itraconazole should be initiated. The new triazoles, especially voriconazole, may offer another treatment option, but more studies are required (84).

SCOPULARIOPSIS SPECIES

Epidemiology and Mycology

Scopulariopsis is a large genus of saprophytic organisms found mostly in soil, but frequently isolated from food, paper, and other materials (85). Within the genus are members of both hyalohyphomycoses and phaeohyphomycoses. Of the hyaline species, *S. brevicaulis* is responsible for causing the majority of human infections while *S. candidum* and *S. acremonium* have also been reported to cause disease in humans (3).

Hyaline *Scopulariopsis* species grow on Sabouraud dextrose agar at both 28°C and 35°C forming powdery, white- to buff-colored colonies (Fig. 7) within three to five days (86,87). Microscopically, *Scopulariopsis* species (Greek word *scopula* means broom) form chains of rough-walled spherical conidia that sit on top of "broom-shaped" phialides (Fig. 8) (88).

Clinical Presentation

Scopulariopsis species are keratinophilic and are most commonly associated with onychomycosis; however, more recently there have been some reports of invasive infections. Of these, about 90% are associated with one of the following risk factors: AIDS, organ transplantation, corticosteroid therapy, peritoneal dialysis, surgery, cardiac disease, and trauma (85). Sites of these infections



Figure 7 *Scopulariopsis brevicaulis*. Expanding whitish, powdery colonies.

have included sinusitis (88–91), mastoiditis (89), keratitis (92), endophthalmitis (93), pneumonia (94), right middle lobe fungal ball (95), prosthetic-valve endocarditis (96,97), peritoneal dialysis-associated peritonitis (98), and recurrent subcutaneous infections (99,100).

Treatment

The ideal treatment of invasive infections due to *Scopulariopsis* remains unknown. This organism is resistant to most antifungal drugs currently available. In vitro antifungal susceptibility can vary greatly by isolate, but in general *Scopulariopsis* species are resistant to fluconazole and flucytosine, resistant or intermediately susceptible to amphotericin B and itraconazole, and frequently susceptible to ketoconazole and miconazole (85,101). One report identified 12 cases of *Scopulariopsis* infection in immunocompromised patients in whom prolonged therapy with conventional amphotericin B deoxycholate or lipid-based preparation, with or without additional surgery, was often ineffective (102).

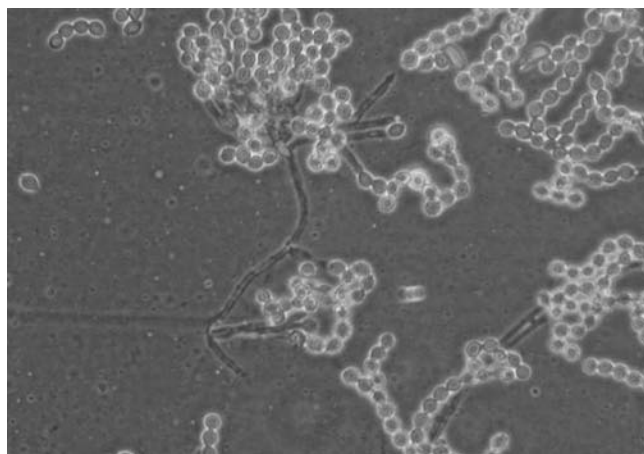


Figure 8 *Scopulariopsis brevicaulis*. Microscopy reveals chains of rough-walled conidia on well-developed annelidic conidiogenous cells.

Currently there is no data concerning efficacy of the new triazoles or echinocandins against *Scopulariopsis* species. In some large in vitro studies, voriconazole showed a superior activity to amphotericin B and itraconazole against *S. brevicaulis* (103,104).

Moreover, MICs of 8 mg/L and >8 mg/L were reported for caspofungin indicating limited activity of this echinocandin toward *Scopulariopsis* (102,105). Several case reports described combination therapies for the treatment of *Scopulariopsis* infection; however, there is no data to suggest their efficacy or which combination is the most promising. Steinbach et al. presented a patient in whom *Scopulariopsis* infection developed during therapy with lipid amphotericin B and who did not respond to voriconazole plus caspofungin therapy (102). Moreover, Wagner et al. described a patient who developed *Scopulariopsis* infection after stem cell transplantation despite being on liposomal amphotericin B plus caspofungin combination therapy (106). Both cases illustrate the limited clinical activity of combination therapy with echinocandins. Due to antifungal resistance of these organisms, early and aggressive surgical debridement/resection of infected areas should be considered in the treatment of invasive infections due to *Scopulariopsis* (89).

CONCLUSION

Human infections with these four hyaline molds present many difficult issues. These organisms are ubiquitous in nature with patients having constant exposure. Since more patients are immunosuppressed, and receiving indwelling medical devices and long-term antibiotic therapy than ever before, it is no surprise that the incidence of infection with these opportunistic fungi is on the rise. Management of these infections remains difficult due to intrinsic resistance to many of the antifungal drugs currently available. The most successful treatment strategies are based on removal of infected devices, surgical debridement of infected tissues, and resolution of the underlying immune system deficiencies. New antifungal agents, particularly the triazoles, offer some hope with enhanced in vitro efficacy against many of these opportunistic fungi; however, there is very limited clinical experience with these new antifungal drugs. Perhaps, in the near future, these new agents will help offset the rising incidence of human hyalohyphomycoses.

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21 | Management of Phaeohyphomycosis

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INTRODUCTION

It is important to emphasize that the term phaeohyphomycosis, which means condition of fungi with dark hyphae is not based on a single genus or species and actually, represents many genera and species. Another term that has been used to describe these fungi are dematiaceous fungi but this does not reflect their dark color primarily produced by melanins. However, a unifying theme for these fungi is their ability to produce melanin in their cell walls and form yeasts and/or hyphal-like structures in tissue. It is not surprising that this diversity of fungi is also reflected in their multiple clinical presentations and thus a single management style is not possible, but in this chapter, there will be an attempt to incorporate principles that will be helpful once these fungi are properly identified as causing disease.

Although the ability to produce melanin is not unique to this group of mycoses (i.e., *Cryptococcus* and some of the dimorphic fungi can produce melanin), the combination of their ability to produce melanin even during infection and the variety of morphologies of these fungi in tissue have allowed medical mycologists to lump this group of fungi together. In fact, with a broad definition over 100 dematiaceous molds have been reported to cause medical conditions from simple colonization to invasive disease in the human host and they represent an emerging group of mycoses in the last decade (1). From solid organ transplant recipients (2) to advanced AIDS patients (3) to cancer victims (4) multiple risk groups have appeared to be susceptible to phaeohyphomycoses.

CLINICAL PRESENTATIONS

This “wide net” spread to categorize this group of mycoses allows for a variety of clinical presentations. First, chromoblastomycosis is a chronic disease of the skin and subcutaneous tissue and is classically identified by the appearance of muriform fungal structures (sclerotic bodies) in the tissue. This indolent soft tissue disease is primarily diagnosed in tropical climates. Second, eumycetoma is a chronic deep tissue disease that primarily occurs in lower extremities with the fungi causing sinus tracts and mycotic grains within the subcutaneous and other soft tissues. Third, is the superficial ulceration of the skin and soft tissue or formation of subcutaneous cysts, and these presentations are primarily caused by direct trauma (5). For example, these lesions may be aided in their appearance by the thinning of the skin with chronic use of corticosteroids therapy. Fourth, foreign body or fomite introduction of these fungi into the body can occur by the contamination of instruments or fluids. Fifth, fungal keratitis is frequently caused by molds and along with *Aspergillus* spp, *Fusarium* spp, and *Paecilomyces* spp, the dematiaceous fungi are a major etiological group of fungi for this condition. Sixth, fungal sinusitis is a poorly defined entity but dematiaceous fungi have frequently been reported to produce allergic fungal sinusitis, fungus ball formation in sinuses, or even invasive disease in which the fungus breaks through sinus tissue planes and invades orbital tissue or the brain. Finally, there is systemic or disseminated phaeohyphomycosis in which several of this group of fungi may spread to multiple organs in severely immunosuppressed individuals or in some individuals (6,7) with brain abscess(s) since some of these fungi appear to have neurotropic features.

FUNGAL IDENTIFICATION

Along with a variety of clinical presentations, there are a series of different genera with substantial differences in their ability to produce human disease (Table 1) (8–10). For instance, there are several neurotropic dematiaceous molds such as the brain abscess-producing fungi (*Cladophialophora bantiana*, *Dactylaria gallopava*, *Fonsecaea pedrosoi*, and *Ramichloridium*

Table 1 Dematiaceous Fungi and Their Most Common Phaeohyphomycosis

Etiologic agent	Clinical presentation	References
<i>Alternaria</i> spp (<i>alternata</i>)	Osteomyelitis, cutaneous, sinusitis	(44–46)
<i>Aureobasidium</i> spp (<i>pullulans</i> , <i>mansonii</i>)	Peritonitis, cutaneous, spleen infection	(45–47)
<i>Bipolaris</i> spp (<i>australiensis</i> , <i>hawaiiensis</i> , <i>spicifera</i>)	Meningitis, sinusitis, keratitis, peritonitis, endocarditis, disseminated infection	(45,46,48–52)
<i>Chaetomium</i> spp (<i>atrobrunneum</i>)	Fungemia, cutaneous, brain abscess	(46)
<i>Achaetomium</i> spp (<i>strumarium</i>)		
<i>Cladophialophora</i> spp (<i>carrionii</i> , <i>bantiana</i>)	Chromoblastomycosis, brain abscess	(11,45,53–56)
<i>Cladosporium</i> spp	Colonizer, skin, keratitis	(45)
<i>Coniothyrium</i> spp (<i>fuckelii</i>)	Cutaneous, liver infection	(46)
<i>Curvularia</i> spp (<i>lunata</i>)	Sinusitis, keratitis, endocarditis, subcutaneous cyst, pneumonitis	(7,45,57)
<i>Dactylaria</i> spp (<i>gallopava</i>)	Brain abscess, disseminated infection, pneumonitis	(45,58,59)
<i>Exophiala</i> spp (<i>jeanselmei</i>)	Subcutaneous cyst, eumycetoma, keratitis, meningitis/brain abscess, disseminated infection, mycetoma, peritonitis	(17,45,60,61)
<i>Exserohilum</i> spp (<i>rostratum</i>)	Sinusitis, cutaneous, subcutaneous cyst, keratitis	(45,49)
<i>Fonsecaea</i> spp (<i>pedrosoi</i>)	Chromoblastomycosis, eumycetoma, pneumonitis	(45,62,63)
<i>Lasiodiplodia</i> spp (<i>theobromae</i>)	Keratitis	(64)
<i>Lecythophora</i> spp (<i>hoffmanii</i>)	Subcutaneous cyst, endocarditis, peritonitis	(64)
<i>Phaeoacremonium</i> spp (<i>parasiticum</i>)	Cutaneous, subcutaneous cyst	(45)
<i>Phaeoannellomyces</i> spp (<i>elegans</i> , <i>werneckii</i>)	Subcutaneous cyst	
<i>Phialemonium</i> spp (<i>curvatum</i> , <i>obovatum</i>)	Subcutaneous cyst, endocarditis, peritonitis	(64)
<i>Phialophora</i> spp (<i>verrucosa</i> , <i>richardsiae</i>)	Chromoblastomycosis, eumycetoma, keratitis, osteomyelitis, endocarditis	(63)
<i>Phoma</i> spp	Sinusitis, keratitis, subcutaneous cyst	(64)
<i>Ramichloridium</i> spp (<i>obovoideum</i> , <i>mackenziei</i>)	Brain abscess	(12)
<i>Rhinochadiella</i> spp (<i>aquaspersa</i> , <i>atrovirens</i>)	Colonizer; chromoblastomycosis, meningitis	(63–66)
<i>Scedosporium</i> spp (<i>apiospermum</i> , <i>prolificans</i>)	Eumycetoma, meningitis, pneumonitis, fungemia, disseminated infection	(64,67–72)
<i>Scytalidium</i> spp (<i>dimidiatum</i>)	Cutaneous, nail, subcutaneous cyst	(64)
<i>Wangiella</i> spp (<i>dermatitidis</i>)	Cutaneous, subcutaneous cyst, keratitis, brain abscess, arthritis, disseminated infection	(45,73,74)

Source: From Refs. 45, 75.

mackenziei) (11,12). In fact, these fungi have enough direct virulence potential that they can produce disease in both immunosuppressed and apparently immunocompetent hosts (13) and *Scedosporium apiospermum* can uniquely produce meningitis in “near-drowning” freshwater victims. On the other side of the virulence potential scheme are the fungal species that primarily colonize skin, airways, and rarely cause disease. These classic dematiaceous colonizing molds include *Cladosporium* spp, *Aureobasidium* spp, *Hormonema* spp, and *Rhinochadiella* spp. With these molds, it is necessary to actually show histopathological evidence or culture from a sterile body site to support their etiologic contribution to disease since they are rare causes of disease in most host risk group and frequently colonize skin and/or airways. In the middle of this virulence grouping of the dematiaceous molds are: *Alternaria*, *Bipolaris*, *Exserohilum*, *Exophiala*, *Phialophora*, and *Wangiella* species. These fungi have been shown to cause sinusitis, soft tissue disease,

keratitis, disseminated disease with or without meningitis, and even endocarditis (14). Since one can rank the potential virulence of these fungi and most reported disease with these molds occur in small case series, the initial steps in the management of this group of mold infections are to obtain correct identification of the mold to help predict outcome and plan a therapeutic strategy. Furthermore during this evaluation of identification, it may also be prudent to obtain *in vitro* susceptibility testing for the strain if it is causing disease and particularly do this testing in difficult cases in which there is a poor clinical track record. It is true that these dematiaceous molds do not have standard "break points" for *in vitro* susceptibility but there is a standard protocol for performing the MICs (15). In difficult cases there can be some *in vitro* impression of the antifungal drug activities against the specific dematiaceous mold producing the infection and in some cases MICs are so high that drug resistance can be predicted (16). In difficult-to-treat cases, this information may be helpful in selection of an antifungal regimen for medical treatment.

TREATMENT MODALITIES

The treatment of phaeohyphomycosis is primarily based on isolated human cases and several small case series (17). There are no robust randomized blinded studies for use of any antifungal agents for this group of infections to provide guidance. This deficiency in guidance is a combination of the variety of clinical syndromes produced by this group of fungi, the heterogenous risk groups with underlying diseases, and the number of genus strains that might have varying drug susceptibility. Despite the paucity of robust guidelines with accurate measured outcomes, there are actually several principles that can be followed that will generally provide a positive outcome for phaeohyphomycosis in most patients.

SURGERY

The first modality in the management of phaeohyphomycosis is the consideration of surgery. Primary treatment of surgically accessible lesions in selected soft tissue, brain, and sinus locations must be considered. For instance, subcutaneous cysts with dematiaceous fungi can and should be surgically removed as an encapsulated structure(s) since this modality can be curative. It is important that there is no spillage of cyst contents back into the wound during the surgical procedure and a simple aspiration of these cysts alone is probably not optimal. The best results with an ulcerative lesion or without a defined cyst will require very careful debridement and may be even approached with a Moh's like procedure to optimally define the borders of infection for surgical removal (18). An important principle in the local surgical management of these infections is that these fungi frequently grow with both hyphae and adventitial yeast-like forms in tissue (19) and thus it is not difficult to surmise that some of these fungal structures may be left or fall back in the wound during surgery to provide a nidus for relapse of infection. Therefore, it is probably wise to carefully lavage residual tissue in the wound after surgery with an antiseptic to help kill or remove these persistent forms. In fact, in many of these local cases, a systemic antifungal agent may also be used after surgery for an empiric period of time to potentially clear any residual fungi from the tissue.

For a single brain abscess, some form of surgical debridement is probably necessary for consistent cure. In this body site, complete surgical removal of a brain abscess that is ideal may not be possible without serious consequences, but even some careful debulking of the lesion may be helpful in reducing the burden of fungi prior to systemic antifungal therapy. Of course, it is possible that surgery might spread the infection to other tissue planes, but it is likely that debulking the mass of fungi far outweighs any concern about infection spread within the brain. Furthermore, all brain abscesses are accompanied by medical antifungal treatment no matter what the extent of surgery. Although occasionally medical therapy alone for brain abscesses has been successful, there are many failures without a combined surgical/medical approach for brain abscesses; therefore, the author encourages the combination approach.

Surgery for sinus disease is common. For instance, surgical removal of a fungus ball (eumycetoma) within the sinus may be curative. In some cases of eumycetoma such as Madura foot and chromoblastomycosis in which there is chronic scarring, poor lymphatic drainage, many fistulous tracts within the soft tissue and even underlying bone involvement, the inability to obtain free margins of disease with surgery, might create even more destruction of the

vascular supply, in these cases medical therapy alone may be considered the best treatment option. However, even in cases with these extensive soft tissue involvement, it is encouraged to have surgical evaluation and follow-up with any initial medical strategy that is planned.

ANTIFUNGAL DRUGS

The second component of management of these dematiaceous infections revolves around antifungal drugs. Although there are no robust definitive studies to guide the use of specific antifungal agents in phaeohyphomycosis, there is a rich history of *in vitro* susceptibility testing, animal model results, and reported cases of both failures and successes with antifungal therapy alone. It is impossible to match every phaeohyphomycosis (either genera or individual strains may vary) with a specific antifungal regimen, but it is reasonable to give some general principles that hold for many of these invading pathogens. Potassium iodide has been reported to be successful in cutaneous phaeohyphomycosis (20), but primarily standard antifungal agents have been used today in the management of phaeohyphomycosis. First, the broad-spectrum polyene class of antifungal agents do possess some *in vitro* activity against these fungi and have been used to treat disseminated phaeohyphomycosis cases successfully. However, for local cases of soft tissue infection, their toxicity profile, need for intravenous administration, occasional resistant strains, and the impreciseness for length of therapy generally do not make this class of agents very attractive for primary use. In most cases, the polyenes are used in cases of disseminated infection and even in these cases are probably most commonly used in combination with other agents rather than as a single agent. Furthermore, it is necessary to identify fungal species and possibly *in vitro* susceptibility before use. Second, flucytosine has excellent *in vitro* activity against many of the genera in this group. It has been used in animal models (21) and patients to successfully treat dematiaceous mold disease. There have always been concerns about the rapid development of direct drug resistance when flucytosine is used alone for antifungal treatment and this concern also includes the management of phaeohyphomycosis. Therefore, flucytosine is generally used in a combination regimen. It is a standard induction therapy regimen with amphotericin B for cryptococcal meningitis and is very reasonable to consider it as part of a combination regimen for the treatment of cerebral phaeohyphomycosis. Its different mechanism of action, excellent pharmacokinetics from oral administration to its high drug levels in the central nervous system make it an antifungal agent that should always be considered in serious, life-threatening phaeohyphomycosis. Third, the newest class of antifungal agents is the echinocandins (caspofungin, micafungin, and anidulafungin) that target the beta-glucan synthase enzyme used for cell wall formation. The echinocandins with their safety, little drug-drug interactions, and antifungal potency for many *Candida* species have become first line agents in invasive candidiasis and frequently are used in a secondary role for the management of aspergillosis. In fact, these echinocandins do possess *in vitro* activity against some of the genera in the dematiaceous groups (22–24). Therefore, they may have some role in certain cases of phaeohyphomycosis. Prior to their use in phaeohyphomycosis, it might be wise to determine the direct *in vitro* susceptibility of the strain to be treated in order to understand whether the echinocandin would have any potential to clinically inhibit the fungus in tissue. Furthermore, it is probable that echinocandins will be generally confined to combination therapy and in cerebral phaeohyphomycosis, which generally uses combination medical therapy. It is still uncertain how effective the echinocandins as a class are in CNS infections with their limited brain penetration. Fourth, terbinafine, the squalene epoxidase inhibitor, which attacks fungal cell membrane formation has been the pivotal drug in the management of dermatophyte infections. It has not been a linchpin drug for invasive mycoses and its experience in phaeohyphomycosis remains limited. However, it has excellent pharmacokinetics for skin and soft tissue infections; it does possess direct antifungal activity against some of the dematiaceous fungi (25), and has been successfully used in combination with other antifungal agents. Terbinafine's place in the management of phaeohyphomycosis is probably as a secondary agent that first needs the isolate to be shown to possess some *in vitro* antifungal susceptibility to it (26). It will primarily be used in combination with another agent such as an azole for refractory or relapsed infections.

Fifth, the azole drugs have become the most commonly used drugs in the medical management of phaeohyphomycosis. This development as a primary therapeutic choice for this group of fungi was first established by itraconazole and with the report of one series of

phaeohyphomycosis successfully treated in over 60% of cases with this antifungal agent (27). The extended-spectrum oral triazole became established as the drug of choice. Furthermore, the newer extended-spectrum triazoles such as posaconazole and voriconazole also have shown excellent *in vitro* activity against a wide range of dematiaceous fungi (23,28) and there have been several case series in which excellent therapeutic outcomes have been demonstrated with these newer azoles (29,30). Importantly, these new azoles have performed very well in CNS phaeohyphomycosis both in animal models (31) and human disease (32,33). Treatment success has been achieved both in meningitis and brain abscesses with these extended-spectrum azoles (34–36). There are several advantages of the extended-spectrum azoles (like voriconazole and posaconazole) in the treatment of phaeohyphomycosis. (i) They can be administered orally. (ii) The pharmacokinetics of these azoles is excellent both for skin/subcutaneous involvement and CNS disease. (iii) The precision of treatment length is still not known for phaeohyphomycosis. However, since these infections are primarily chronic, the length of treatment has also been extended for months of therapy. The azoles allow safe, long-term treatment schedules. (iv) Through *in vitro* susceptibility testing assessment, the potency and breadth of antifungal activity for these extended-spectrum azoles are broad and potent for many of the dematiaceous molds.

Since there are several classes of antifungal agents with direct antifungal activity against dematiaceous molds, the frequent clinical question in serious and refractory cases is: can combination therapy be of benefit? In fact, drug combinations from *in vitro* susceptibility studies generally show either additive or synergistic activity against many of the dematiaceous fungi (37). Therefore, there is some clinical support for the use of more than one drug for refractory or serious (life-threatening) disease but within specific isolates or disease locations, the value of more than one drug use remains arbitrary. For disseminated or intracranial disease in which there is limited surgical option, the use of combinations (polyene, flucytosine, terbinafine, echinocandins, and/or extended-spectrum azoles) is frequently considered. In fact, in most cases in which surgery is performed, antifungal drug treatment is used to insure elimination of any residual infection.

There are specific circumstances that will require creative solutions for treatment of these infections without robust clinical data for guidance.

1. Fungal keratitis (38,39). A portion of these infections are caused by dematiaceous molds. Treatment requires careful assessment by an ophthalmologist and generally with initial therapy, ophthalmic drops every one to two hours to control infection. At least one strategy is to alternate antifungal drops with different mechanisms of action such as amphotericin B, voriconazole, and an antiseptic like polyhexamethylene biguanide. Also in those cases, if there is extension deep into cornea or other eye structures in which topical agents may have reduced presence, use of systemic voriconazole may be effective, although topical voriconazole can penetrate through the cornea into the anterior chamber of the eye.
2. Allergic fungal sinusitis (40). There has been support for the use of corticosteroids to decrease immune stimulation or immunotherapy to induce tolerance to the fungal antigens in this condition (41). There are also proponents who would try to reduce antigen loads by prescribing antifungal agents to mimic the strategy that was successful for allergic bronchopulmonary aspergillosis. However, these fungi may be eliminated from sinus without specific antifungal therapy. Despite these several potential strategies for management there remain little evidence-based studies to confidently guide antifungal therapies in this condition and this area critically needs better studies. However, surgery will be required to remove a fungus ball in the sinus cavity (42).
3. Invasive or disseminated disease in the immunocompromised patient. It is essential that control of underlying disease is made and maintained. There needs to be an attempt to eliminate or reduce immunosuppressive drugs (i.e., corticosteroids) and correct immunosuppressive conditions such as neutropenia. Similar to most invasive fungal infections, the use of immune modulators [such as granulocyte-monocyte colony-stimulating factor (GM-CSF), gamma interferon] remain a possible option in select patients who are refractory to surgery and/or antifungal therapy but their specific use is poorly defined in phaeohyphomycoses. These infections are increasing in our cancer patients and may occur as

breakthrough infections during empiric or prophylactic antifungals. The mortality rate has reached 33% but some of these infections may be related to catheters/foreign bodies that need removal (4).

4. Chronic Skin Infections (chromoblastomycosis and eumycetoma) will likely require long-term antifungals (i.e., azoles) with careful surgical intervention when necessary and also control of bacterial superinfections in these chronic conditions that have sinus tracts and at times involvement of the underlying bone. The length of treatment for all the phaeohyphomycosis is not well defined and will need to be judged individually at the bedside or in the clinic. However, there are situations where these infections will relapse if therapy is stopped early so the majority of infections are treated for months.
5. Foreign body infections. The dematiaceous fungi can contaminate catheters, heart valves, and insertion of other foreign bodies (i.e., pacemakers) into sterile sites. For instance, intravenous catheters in cancer patients can act as a source for fungemia with *Aureobasidium* (4) or patient receiving chronic ambulatory peritoneal dialysis develops peritonitis with black molds (43). Similar to principles with other infections around foreign bodies, it is prudent to remove the foreign body if possible to guard against antifungal therapy failure related to biofilms and other factors that allow these organisms to persist despite treatment.

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22 | Pneumocystis

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HISTORY

The parasitic fungal genus *Pneumocystis* was discovered in 1909 by Chagas in the lungs of Trypanosome-experimentally infected Guinea pigs (1). He described the genus as *Schizotrypanum* and thought it to represent a life cycle variant of *Trypanosoma cruzi* (2). In 1910, Antonio Carinii also identified and mistook similar organisms in rat lungs as a novel trypanosome. The organism was correctly identified by the Delanoës in 1912 and renamed *Pneumocystis carinii* after Dr. Carinii (3).

In pre-World War II Europe, a novel type of pneumonitis, typically affecting premature infants and immunocompromised adults and clinically characterized by respiratory distress, cyanosis, and radiographically characteristic lung infiltrates was recognized (4). Mortality resulting from this pneumonitis was estimated to be 30% to 40% (5). Initial case series were published by Ammich (6) and Benecke (7), among others. In 1952, Vaněk and Jírovec isolated *P. carinii* from the alveolar lumina of immunocompromised adults who had succumbed to interstitial pneumonia (8).

Keen interest in *Pneumocystis* as a significant pathogen in immunocompromised hosts resumed in the 1980s as the incidence of pneumocystosis rose dramatically, coincident with the advent of human immunodeficiency virus (HIV).

In 1988, analysis of the *Pneumocystis* rRNA gene allowed resolution of the genus taxonomy from protozoa to fungi (9). In 2001 at the Seventh International Workshop on Opportunistic Protists, the name of the *Pneumocystis* organism causing exclusively human disease was changed to *P. jirovecii* in honor of the Czech parasitologist Otto Jírovec, one of the first researchers to describe *Pneumocystis* infection in humans (10). The inability of *Pneumocystis* to be propagated in culture continues to complicate the precise taxonomic classification of its various forms (12).

ORGANISM

Life Cycle and Genome

The genus *Pneumocystis* comprises a group of ascomycetous single-cell fungi belonging to the class Pneumocystidomycetes, order Pneumocystidales, and family Pneumocystidaceae. The life cycle of *Pneumocystis* has been described by means of microscopy. Three developmental stages of the organism are commonly seen in conjunction with additional intermediate forms. The small (1–4 μm) pleomorphic *trophozoite* commonly exists in clusters and can be identified on Giemsa stain by its reddish nucleus and blue cytoplasm. The trophozoite multiplies by binary fission during the asexual phase of the life cycle. In the sexual phase, the haploid trophic forms conjugate to form a diploid zygote that becomes a 4- to 6- μm *precyst* or *sporocyte*, which undergoes meiosis followed by mitosis resulting in a *cyst* or *spore case* (Fig. 1). Three intermediate cyst stages (early, intermediate, and late precysts) contain 2, 4, and 8 nuclei, respectively (12). The resulting cyst or spore case contains eight haploid *spores*. The cysts stain well with methenamine silver and toluidine blue O. The polymorphic spores are formed by compartmentalization of nuclei and cytoplasmic organelles and appear to be released through a perturbation of the cell wall. During infection, trophic forms (most of which are haploid) predominate over cyst forms (13).

The *P. carinii* genome comprises approximately eight million base pairs of DNA divided among 15 linear chromosomes that range in size from 300 to 700 kb (14). The genome of *P. jirovecii* is currently being delineated (13).

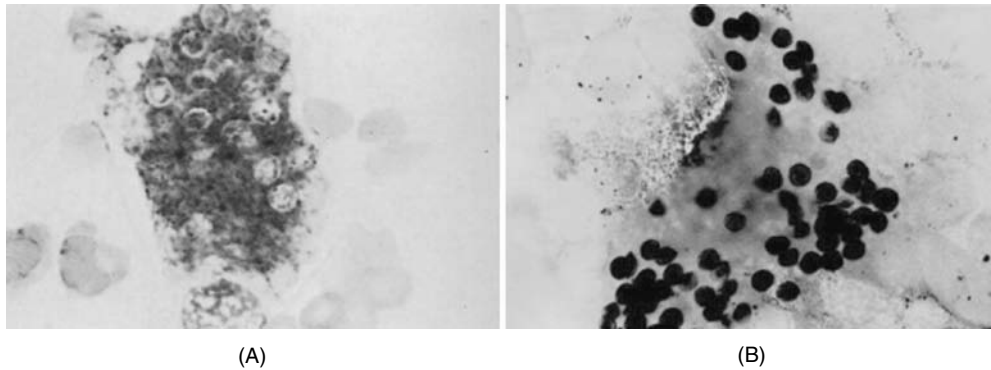


Figure 1 (A) *Pneumocystis* trophozoites and cysts (Diff-Quik, $\times 1000$), (B) *Pneumocystis* cysts (methenamine silver stain, $\times 1250$).

Cell Wall and Surface Antigens

The cell wall of *Pneumocystis* contains β -1,3-glucans and little or no chitin. The surface of *Pneumocystis* contains glucose/mannose, *N*-acetylglucosamine, and galactose/*N*-acetylgalactosamine residues (15). Cysts and trophic forms contain a number of immunogenic glycoproteins, such as mannose, glucose, galactose, and *N*-acetylglucosamine in their cell walls (16).

One distinguishing characteristic of *Pneumocystis* is the lack of ergosterol in the plasma membrane, a fact that renders it resistant to antifungal drugs that target ergosterol synthesis. Instead of ergosterol, the major sterol in *P. carinii* is cholesterol, which is postulated to be scavenged from the host lung (17). Other *P. carinii*-specific lipids include Δ^7 C-24-alkylated sterol and *cis*-9,10-epoxystearic acid, both potential drug targets. The major ubiquinone synthesized by *Pneumocystis*, coenzyme Q10, provides a drug target for ubiquinone analogs such as atovaquone (17).

Pneumocystis contains a family of proteins referred to collectively as the major surface glycoprotein (MSG), an immunogenic protein exhibiting shared and species-specific antigenic determinants (18–23). Rearrangement of the upstream conserved sequence (UCS) of MSG by recombination and gene conversion results in antigenic variation, which may allow *Pneumocystis* to evade the host immune response (18). MSG also facilitates interaction with host cell extracellular matrix proteins, such as fibronectin, vitronectin, possibly laminin, surfactant proteins A and D, and the mannose receptor (11,24–28).

EPIDEMIOLOGY

Pneumocystis is an opportunistic organism that infects patients with altered immune systems including those with acquired immune deficiency syndrome (AIDS) (29,30), transplant recipients, patients with malignancies, and immunocompromise due to medications such as corticosteroids and immunomodulators (11–31). In the infected immunocompromised host, *P. jirovecii* exists within the lung alveoli attached to alveolar epithelia and causes a distinct interstitial pneumonitis, commonly referred to as *P. carinii* pneumonia (PCP). Invasion of lung tissue or dissemination rarely occurs except in cases of overwhelming infection or severe immunosuppression (32).

Aside from the mammalian host lung, *Pneumocystis* occupies no known ecological niche. *Pneumocystis* is thought to colonize the human lung early in life. Vargas et al. showed 85% seroconversion at 20 months of age in a cohort of healthy Chilean infants (33). Current theory favors a model of airborne transmission of *Pneumocystis*. Based on the identification of organisms in healthy persons with intact immune systems, person-to-person spread is thought to occur (34–38).

There is strong data to suggest that pneumocystosis results from active acquisition from other persons, rather than latent reactivation within the immunocompromised host. For example, different *Pneumocystis* genotypes were found to cause recurrent episodes of pneumocystosis in AIDS patients (39). In another study, *Pneumocystis* genotypes recovered from patients in

different US cities reflected the place of diagnosis, rather than the place of birth of the patient (40). Lastly, Huang et al. demonstrated that a majority of sulfa-naïve patients presenting with AIDS-defining pneumocystosis harbored mutant dihydropteroate synthetase genes, suggesting acquisition of a mutant strain rather than in vivo mutation resulting from antibiotic selection (41).

Seasonal variation in the occurrence of pneumocystosis is unsubstantiated. Recent studies suggest that comparatively lower incidences of pneumocystosis among HIV patients living in developing countries may be due to under-diagnosis resulting from limited access to medical care (42).

PATHOLOGY AND PATHOGENESIS

Pathology

Formation of a foamy, eosinophilic exudate within the lung alveoli is the hallmark histological finding in pneumocystosis (Fig. 2). Microscopically, alveolar-capillary leakage and type I pneumocyte destruction have been noted (43,44). Physiologic changes in the lung occur similar to that seen in adult respiratory distress syndrome (ARDS) (45,46).

Pathogenesis

Once *Pneumocystis* is inhaled and deposited in the lung alveoli, the trophic form of *Pneumocystis* adheres to type I alveolar cells and initiates infection (43). As previously stated, adherence is facilitated by the *Pneumocystis* MSG (18). Through complementation studies in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, *Pneumocystis* mitogen-activated protein kinase (MAPK) signal transduction genes, cell wall genes, and *Cdc2*, *Cdc13*, and *Cdc25* have been implicated in organism replication (47–51). *Pneumocystis* may scavenge essential nutrients from the host tissue (43).

Pneumocystis may facilitate tissue injury and modulate the host inflammatory response through interference with host cyclin-dependent kinase regulatory pathways (52), alteration of

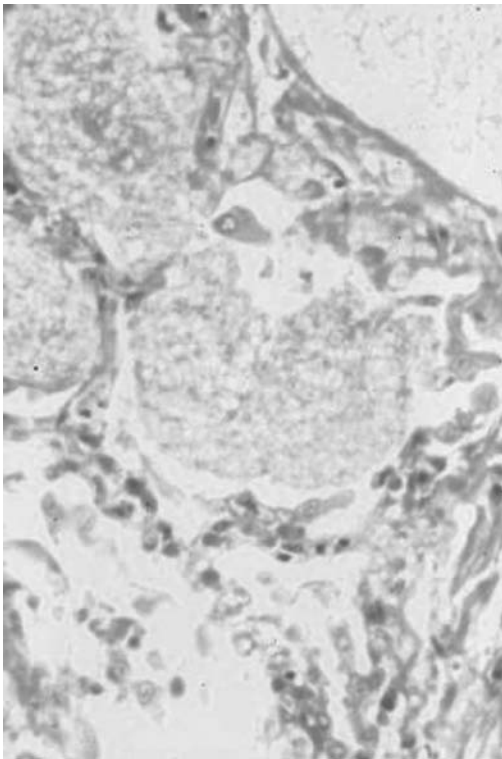


Figure 2 Eosinophilic infiltrate of *Pneumocystis* pneumonia within lung alveoli (H&E stain).

lung GTP-binding regulatory proteins, and induction of fibrinogen and intracellular adhesion molecule-1 (ICAM-1) expression (53).

Impaired host humoral immunity may play a role in the unregulated replication of the organism in pneumocystosis. *Pneumocystis* pneumonia occurs in humans and mice with B-cell immune defects (54,55). Monoclonal antibodies to MSG and other *Pneumocystis* antigens exert a therapeutic effect in experimental animal models of pneumocystosis. The role of antibodies in humans is unclear given the high prevalence of serum antibodies present in the population and lack of information regarding which antigen epitopes are protective (12,13).

Outbreaks of *Pneumocystis* pneumonia in severe combined immunodeficiency disease (SCID) mice suggest impaired cellular immunity contributes to the development of the disease (56). Depletion of CD4 cells, administration of corticosteroids, and immaturity of the immune system have all been shown to weaken host defenses against *Pneumocystis* infection (23,56–60).

In adult HIV patients, the risk of developing PCP greatly increases with CD4 levels less than 200/mm³ (61). The precise interplay among HIV, CD4 cells, and *Pneumocystis* remains unclear. Theus et al. showed that HIV patients infected with *Pneumocystis* retain CD4 memory, but fail to mount a proliferative response to whole *Pneumocystis* or MSG (62).

Other patient populations at risk for pneumocystosis include preterm infants, patients with primary immunodeficiencies such as SCID and hyper-IgM syndrome, patients with malignancy, solid organ and bone-marrow transplant recipients, and patients with collagen disorders (63–67).

Pneumocystis is cleared by the lung by alveolar macrophages through ingestion and intracellular killing of the organisms (68–70). Natural killer (NK) cells can be activated by *Pneumocystis* and may play a role in the host immune response (71,72).

CLINICAL FEATURES

The classic presenting symptoms of pneumocystosis are dyspnea, nonproductive cough, and fever. Occasionally, sputum production, hemoptysis, or chest pain is present (73). HIV patients with *Pneumocystis* pneumonia often have a more indolent presenting course; although the organism burden is higher, the lung damage is less severe (74,75). Acutely ill patients may manifest tachypnea, tachycardia, increased respiratory effort, or cyanosis. Lung auscultation is often underwhelming (15).

Classic radiographic findings include bilateral diffuse infiltrates extending from the perihilar region (76) (Fig. 3).

DIAGNOSIS

Given the expansive differential diagnosis of the immunocompromised host with abnormal respiratory findings, the diagnosis of pneumocystosis should be aggressively pursued by histopathologic demonstration of the organism. Patients with symptomatology, physical examination findings, and chest radiography suspicious for *Pneumocystis* pneumonia should have respiratory secretions collected for identification of the organism. Collection procedures include (in order of increasing sensitivity): induced sputum collection, bronchoalveolar lavage, and open-lung biopsy (15).

A number of stains can reliably identify *Pneumocystis* in respiratory secretions, including: methenamine silver, toluidine blue O, cresyl echt violet, Wright–Giemsa, Calcofluor white, and others (15). Immunological techniques such as immunofluorescence are widely used and may be more sensitive than histological stains (77,78). Other diagnostic modalities including DNA amplification by PCR are being tested with some encouraging results (79–81).

TREATMENT

Trimethoprim–Sulfamethoxazole (TMP–SMX)

TMP–SMX inhibits folic acid synthesis and is considered the first-line therapy for all forms of pneumocystosis (31). This drug has been effectively used with excellent success (82–85). TMP–SMX offers a low-cost, effective treatment regimen that can be given orally or parenterally at 15 to 20 mg/kg/day in divided doses for 14 to 21 days. Potential side effects of TMP–SMX



Figure 3 Typical chest radiograph of *Pneumocystis* pneumonia showing bilateral perihilar infiltrates.

include most commonly gastrointestinal symptoms; rash, including Stevens–Johnson syndrome; and anaphylaxis (15).

Trimethoprim with Dapsone

For mild-to-moderate pneumocystosis, TMP (15–20 mg/kg/day) with dapsone (100 mg/day) is as effective as TMP–SMX and less toxic (86). Dapsone can cause methemoglobinemia. Additionally, dapsone can cause hemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Clindamycin with Primaquine

Clindamycin (300–450 mg four times daily) with primaquine (15–30 mg daily) is comparable to TMP–SMX and TMP–dapsone with regard to efficacy and toxicity in the treatment of pneumocystosis (86,87). Primaquine may also cause hemolysis in patients with G6PD deficiency. The mechanism of action of clindamycin and primaquine against *Pneumocystis* is unknown.

Atovaquone

Atovaquone, a hydroxynaphthoquinone, acts on the electron transport chain of *Pneumocystis*. In the treatment of mild-to-moderate pneumocystosis in HIV patients, atovaquone was found to be less effective than TMP–SMX and equivalent to pentamidine (88,89).

Pentamidine Isethionate and Trimetrexate

The parenteral drugs pentamidine isethionate and trimetrexate are considered alternatives to TMP–SMX for the treatment of moderate-to-severe pneumocystosis in HIV and non-HIV patients (31). The mechanism of action of pentamidine against *Pneumocystis* is unknown. Pentamidine is typically given as a daily infusion of 4 mg/kg. Pentamidine is toxic with a side effect profile including cardiac arrhythmia, hypotension, and bronchospasm (15).

Trimetrexate

Trimetrexate inhibits *Pneumocystis* dihydrofolate reductase (DHFR). Trimetrexate was shown to be less effective than TMP–SMX for the treatment of moderate-to-severe pneumocystosis in AIDS patients (90). The drug is typically administered in a single daily dose of 45 mg/m².

Its main side effect is bone marrow suppression, which can be prevented by the concomitant administration of folic acid (15).

Adjunctive Corticosteroids

The administration of corticosteroids during the first 72 hours of treatment lessens the degree of hypoxemia and improves survival. Adjunctive corticosteroid treatment is recommended for all patients with moderate-to-severe disease (arterial oxygen pressure <70 mm;Hg or alveolar-arterial oxygen gradient for >35 mm;Hg) (91).

PREVENTION

Primary and secondary chemoprophylaxis directed against the development of *Pneumocystis* pneumonia should be considered in certain immunocompromised patient populations. Centers for Disease Control and Prevention guidelines recommend chemoprophylaxis for adult HIV patients with CD4 counts less than 200/mm³, and adolescent HIV patients oropharyngeal candidiasis or unexplained fever greater than 100°F or 37.7 °C for two weeks or longer, or who have recovered from a previous episode of pneumocystosis (92). Chemoprophylaxis is also indicated for infants born to HIV-infected mothers beginning at four to six weeks of age and continuing until the child's HIV status is determined. The rationale for initiating empiric prevention is based on the finding that roughly half of the cases of *Pneumocystis* pneumonia in infants with AIDS occur as the initial manifestation of AIDS, with high mortality (93). If the child is HIV-infected, prophylaxis should be continued throughout the first 12 months of life and thereafter determined by age-specific CD4 counts (15).

TMP-SMX is the drug of choice for *Pneumocystis* prophylaxis in HIV and non-HIV patients (15). Alternative regimens for those patients who cannot tolerate TMP-SMX include daily dapsone (alone, or in combination with pyrimethamine and leucovorin), daily atovaquone, and monthly inhaled pentamidine. The major side effect of aerosolized pentamidine is bronchospasm, and its administration requires a nebulizer and a negative pressure room.

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23 Management of Zygomycosis

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INTRODUCTION

The management of zygomycosis has always centered around a triad of strategies to cure this unusual, but not rare infection. These three therapeutic areas are (i) the control of the underlying disease, (ii) surgery, and (iii) antifungal therapy (1–3).

Control of Underlying Disease

The control of an underlying disease is extremely important to the final outcome of these infections (4). In the past, zygomycete infections such as rhinocerebral disease were frequently linked to poorly controlled diabetes and at times, specifically related to or linked to ketoacidosis. Diabetes is still a risk factor but the combination of better diabetes control and the use of statins with their antifungal activity may have combined to make this risk group less common. Despite a possible change in epidemiology, it is still critical to optimize diabetic control for successful management of this infection, and it is clear that diabetic renal dysfunction and the use of polyenes make this risk group very difficult to manage. Even more difficult to manage is the enlarging risk group of zygomycosis associated with hematological malignancies and bone marrow transplant recipients. This risk group provides a series of advantages for the fungus. For example, it is associated with voriconazole exposure, which may suggest that the patient had avoided an aspergillus infection only to survive long enough to contract an invasive zygomycosis with these ubiquitous, environmental molds. Furthermore, this patient population combines other advantageous conditions for the mold's survival in a weakened host such as neutropenia, graft-versus-host disease, high doses of corticosteroids, malnutrition, and relatively high iron states from multiple transfusions. These patients represent the "perfect storm" for the clinical growth of this mold. It is therefore essential to remember that at times this mold infection is an extreme marker for the end stage of a life-threatening disease and this fact needs to be factored into the treatment strategy and outcome.

Surgery

The second major strategic factor in the management of zygomycosis is the need to consider the value of surgical debridement. The well-known pathophysiology of zygomycosis describes the mold's angioinvasive nature in the mammalian host. It invades blood vessels causing ischemia/infarctions and eventually necrotic devitalized tissue. From rhinosinusitis to pneumonitis, it is important that dead necrotic material is surgically removed. Some of this devitalized tissue would not be effectively improved without debridement since antibiotics/antifungals would not be able to penetrate to the site of infection. Of course, all sites of infections and clinical conditions are not amenable to surgical debridement, and medical therapy alone will be necessary at times. Ideally, the infection site is surgically removed but on a practical basis it will be necessary to judge at the bedside the extent of the surgical debridement of this infection that is necessary and/or feasible. It can range from complete surgical removal of a cutaneous or subcutaneous infection to the very minimal ability to debride a zygomycete brain abscess. In the middle of these two polar extremes is the surgical debridement in the nasal and sinus areas connecting into the orbit or brain, which may require several surgical procedures to remove devitalized tissue. Therefore, although surgical intervention is a critical factor in the successful management of zygomycete infection, the precision of its use and the technical outcome are hard to develop robust guidelines and variable quality assurances, respectively.

Antifungal Therapy

The third major factor, which is the use of antifungal therapy, has recently engendered renewed enthusiasm for studies and attempts to optimize therapies. Historically, amphotericin B deoxycholate was the only active systemic antifungal agent for treatment of zygomycete infections. Although there were no randomized comparative studies, amphotericin B has always represented the most potent broad spectrum antifungal in vitro against the vast majority of zygomycete strains (genus and species). Clinical experience suggested that amphotericin B worked clinically. With a very large (929 published cases) retrospective review of the literature from 1940 to 2003, with a multivariate model of risk factors for mortality, both amphotericin B deoxycholate and lipid products of amphotericin B had a favorable outcome compared to no treatment (4). This large retrospective study continued to support the primary use of a polyene for zygomycosis. Furthermore, a recent retrospective review of 70 consecutive hematology patients with zygomycosis found that there was a twofold higher mortality with delayed amphotericin B administration for ≥ 6 days after diagnosis (5). Of course, many factors may have played a role into the delay of drug administration which added to the poorer prognosis, the principle is probably validated that the earlier administration of the polyene in the history of zygomycosis, the better the outcome and therefore, the clinician bears responsibility for earlier diagnosis and intervention to impact a potentially better outcome.

It is well known that the use of amphotericin B deoxycholate is accompanied by severe infusional reactions and renal toxicity. This is particularly common when doses of 0.7 to 1.0 mg/kg/day are used for zygomycosis in patients, who frequently already have compromised renal function. Therefore, the lipid formulations of amphotericin B, that is, liposomal amphotericin B, amphotericin B lipid complex, and amphotericin B colloidal dispersion, became very attractive therapeutic agents for the zygomycosis in the dosing range around 5 mg/kg/day. Although amphotericin B colloidal dispersion has been less frequently used secondary to early reports of increased infusional related toxicity, the other formulations have been used and particularly amphotericin B lipid complex has been reported in open trials to effectively treat zygomycosis (6). However, there have been no prospective "head-to-head" trials to compare efficacy of any of the polyene formulations with each other. Therefore, although most experts recommend a lipid formulation of amphotericin B as first line for induction therapy of zygomycosis because of amphotericin B deoxycholate toxicities, there is no firm agreement regarding which lipid product or how long to treat, and whether other agents or adjunctive therapies are beneficial.

In vitro antifungal activity against zygomycetes is always the initial platform to base therapy, and amphotericin B uniformly has direct activity on inhibiting most zygomycetes. In contrast, most azoles and echinocandins have very little antifungal activity in vitro against zygomycetes (7–9). However, one available azole does seem to separate itself in that posaconazole does have some inhibitory antifungal activity against several zygomycete genera. This in vitro antifungal activity allowed this agent to be used in treatment of zygomycosis and there are several reports of its efficacy as a salvage regimen (10,11). Furthermore, there has been a study of 30 different zygomycete strains that found the combination of posaconazole and amphotericin B to often demonstrate a synergistic effect on hyphae more commonly than conidia (12). Interestingly, although the echinocandins have poor antifungal activity against the growth of zygomycetes directly in vitro, there has been demonstrated an immunomodulatory activity of echinocandins against several molds such as *Rhizopus oryzae*. For example, the addition of caspofungin increased mold cell wall beta-glucan exposure and this impact allowed human neutrophil attacks against this remodeled cell wall (13).

Also, these in vitro mechanistic studies with antifungal agents have been supported and amplified by studies within animal models. Since disseminated zygomycosis can invade the central nervous system (CNS), there were questions about how the lipid preparations would compare. In a diabetic ketoacidosis murine model, it was found that liposomal amphotericin B at 15 mg/kg/day was superior for treatment compared to a lower dose of 7.5 mg/kg/day or amphotericin B 1 mg/kg/day (14). Therefore, there is some suggestion that dose may be important. In another study both formulation and dose were critical. In a comparison between liposomal amphotericin B and ABLC in a disseminated *R. oryzae* mouse model, there was generally better survival with the use of the liposomal amphotericin B preparation in both

the diabetic ketoacidosis and neutropenia models (15). When examining the CNS burden of infection, the liposomal product appeared to be more potent although ABLC at high doses showed a positive impact on CNS infection (15).

Posaconazole has had some animal model experience in zygomycosis and the results have been mixed and not as consistent as the polyene data. In one study of disseminated *R. oryzae* infection in both diabetic ketoacidosis and neutropenic models, posaconazole monotherapy had no benefit and there was also no benefit when combined with liposomal amphotericin B (16). These results were confirmed with another mouse model (12). However, a third study in a neutropenic model of disseminated *R. oryzae* using two strains did show that posaconazole monotherapy had antifungal activity but was not as potent as amphotericin B, but the combination did have a positive interaction including impact on CNS fungal burden (17). Thus, the limited animal models studied would suggest that the use of posaconazole for primary therapy has less assurance of activity compared to the polyene.

Since there are some conflicting results regarding echinocandins *in vitro* in which there is poor direct antifungal activity against zygomycete growth (18,19) but it may alter fungus to allow host cells to attack it, it was necessary to study this antifungal class in animal models of zygomycete infections. The echinocandins as monotherapy appear to have limited antifungal activity in disseminated zygomycete infections in mice (20). The combination of poor *in vitro* activity and a very meager response in animal models alone make it unlikely that the echinocandins will have much use as monotherapy in zygomycete infections. However, two reports of echinocandin–polyene synergy have been published for mouse survival (21,22). This positive interaction with lipid products of amphotericin B and all the commercially available echinocandins supports the continued investigation of combination therapy in difficult-to-manage cases of zygomycosis, and the echinocandins can be placed into combination consideration for this difficult-to-treat infection.

With the *in vitro* susceptibility testing, animal model efficacy studies, and clinical experiences from over 50 years, the optimal regimens for specific antifungal therapy for zygomycosis remain without support of robust comparative clinical trials. In truth, it is extremely difficult to marshal the members of medical centers needed to study this infection, and further complicating the studies is the variable impact of both surgery and the underlying disease on outcome. Therefore, the precision of optimal antifungal therapy is simply lacking. It is clear from the *in vitro* and animal studies that amphotericin B still ranks as the most potent antifungal agent available. It was also apparent that the high recommended doses of 0.7 mg/kg/day to 1.0 mg/kg/day had substantial morbidity in this patient population. Therefore, there has been a switch toward the use of lipid products of amphotericin B for the primary induction regimen. Treatment success in humans has been reported with all three lipid products of amphotericin B. However, no prospective clinical trials directly comparing the agents and/or dosing are available. Generally, there are retrospective studies with small numbers of patients in which the lipid products generally have some detectable clinical success in over half the patients (6,23–27). Although there is nothing conclusive about either the best preparation or the proper dose, the compilation of this experience supports the continued use of the lipid products of amphotericin B as primary induction therapy for disseminated zygomycosis. In no respects is this recommendation solidly evidence based, but a common induction regimen is 5 mg/kg/day of a lipid product of amphotericin B with specific emphasis on the liposomal product when there is CNS involvement for at least a four- to six-week induction period. The antifungal management will need to be carefully adjusted for each case both in dose and duration.

The lack of an intravenous formulation and the *in vitro*/animal data make the use of posaconazole as primary therapy for zygomycosis much less attractive unless there is non-life-threatening cutaneous disease that can be easily monitored. Despite this word of caution for posaconazole monotherapy for initial treatment, the clinical experience with this azole does suggest it can be used in the management of this infection. The bulk of support for posaconazole comes from retrospective, compassionate use studies in which cases were either refractory or intolerant to prior antifungal therapy. In approximately 100 patients and a difficult-to-treat population, approximately 60% to 70% of cases had a favorable treatment outcome with posaconazole (10,11). In a subset of very difficult patients, the use of a combination of amphotericin B plus posaconazole had a favorable response in approximately half the patients (28).

It is likely that posaconazole will continue to have some place in the management of invasive zygomycosis. Although not formally studied the ability to give this agent orally with limited toxicity, the use of sequential therapy for patients who have responded to an initial course of lipid formulation of amphotericin B has gained some favor by some experts. It allows the ability to lengthen therapy for zygomycosis and reduce long-term polyene exposure. If posaconazole is used there should be attention to drug exposure by drug level monitoring and/or attention to drug dosing. Patients tend to have better posaconazole exposure if given as 200 mg orally four times a day with food rather than 400 mg orally twice a day. The exact trough level is not certain for efficacy, but considering MIC values, it may be reasonable to have a serum level goal of 1 µg/mL at trough.

In regards to the use of adding an echinocandin to amphotericin B, there is only one retrospective review of 34 patients with rhino-orbital-cerebral zygomycosis (29). These patients were primarily diabetics and treatment with the polyene–echinocandin combination was successful in all six evaluable patients compared to approximately 40% success in the amphotericin B monotherapy group. There are great risks in interpreting these small, unrandomized, retrospective studies but with present information, there are no data that the combination causes harm and thus we will need a more robust study before formal recommendations can be made.

As with all infections with potentially poor outcome and an immunocompromised host, there have been studies to consider adjunctive therapies to provide an advantage for the host. Zygomycete infections are one of those mycoses with a rich history of adjunctive therapies. From cytokine therapies such as GM-CSF and interferon-gamma (30–32) to granulocyte transfusions (33), there have been attempts to bolster the host's immunity. In these few reports it is difficult to evaluate whether there was a positive clinical impact and these immune modulating strategies are generally used when heroic measures are needed and until better studies are performed. There are two other creative measures, which have been used in zygomycosis that are based around changing the local host environment to put the fungus at a disadvantage. First, it has been reported that hyperbaric oxygen was used to improve the oxygenation of devitalized tissue environment and directly kill or inhibit the fungus (34). It has appeared to cause no harm but on the other hand, there are no convincing data that it provides for a better outcome. It remains in the arena of "should we or can we do it" for specific refractory cases and there is no solid support that it is routinely necessary. Another adjunctive therapy has recently been considered and studied. Iron chelation with deferoxamine has been known as a risk factor for disseminated zygomycosis by its use as a siderophore to enhance iron uptake by the zygomycete and promote its growth (35). Newer iron-chelating agents, such as deferasirox, have the opposite effect and can steal iron from the zygomycete. Deferasirox has shown antizygomycete activity in vitro and in mouse models (35,36). These results were a platform to try it in human case reports with both success and failure (37,38). Therefore, to obtain some appreciation for the value of deferasirox, a randomized, double-blind, placebo-controlled pilot clinical trial is underway to more rigorously evaluate the utility of adding deferasirox to liposomal amphotericin B for the management of zygomycosis.

In summary, zygomycosis is generally treated with a three-part strategy: (i) surgical debridement, (ii) lipid amphotericin B, and (iii) control of the underlying disease. Despite its reputation as a difficult mold infection to treat and its significant difficulties to precisely study for robust recommendations, a careful plan of management can frequently be successful in zygomycosis if there is control of the underlying disease.

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24 Antifungal Management in Risk Groups: Transplant Recipients

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INTRODUCTION

Over the past decade, the epidemiology of invasive fungal infections (IFIs) in transplant patients has evolved. The use of new antifungal drugs and modifications in transplant practices, including changes in surgical techniques, conditioning regimens, and new immunosuppressive agents, has contributed to the shift in the numbers and types of IFIs encountered. To say the least, treatment of fungal disease in a complex transplant patient is challenging, and optimal outcome requires prompt diagnosis and consideration of several important factors including coinfection with immunomodulatory viruses, comorbid conditions, and drug interactions. The management of invasive mycoses within the complex milieu of the transplant recipient is discussed in this chapter.

TYPES AND TIMING OF INFECTIONS

For solid organ transplant (SOT) recipients, the timeline for infections following transplantation has traditionally been divided into three phases: the first month, months 2 through 6, and more than six months after the transplant procedure (Fig. 1). The risk for developing an invasive mycosis during each of these periods is linked to the patient's overall net state of immunosuppression and their exposure to fungal pathogens. During the first posttransplant month, technical and anatomical factors including those related to the surgical procedure and its aftermath, such as the presence of foreign bodies, ischemia with resulting devitalized tissue, remnant fluid collections, and prolonged intensive care support, contribute to the risk. *Candida* is the most common fungal pathogen encountered during this period. During the second through sixth posttransplant months, the SOT recipient has typically left the hospital, but the maximum impact of the induction immunosuppressive regimen begins to manifest in terms of infectious risks. In addition, patients are now routinely exposed to a broad array of fungal pathogens including *Aspergillus* and other molds, *Cryptococcus*, and *Pneumocystis jirovecii*. Latent infections from past exposures to the geographically restricted endemic fungi may also reactivate (1–3). Beyond the sixth month following transplantation, the risk for fungal disease for the majority of SOT recipients begins to decline, as most patients will have been placed on minimal maintenance immunosuppressive therapy. Infections after six months tend to occur either in the setting of continued chronic infection [e.g., cytomegalovirus (CMV)] or due to increased immunosuppression such as is required with recurrent or chronic rejection.

Rates of specific IFIs not only vary by time after transplant, but also by the type of solid organ transplanted. For example, invasive aspergillosis (IA) is rarely encountered in the pancreas transplant recipient, while lung transplant recipients are considered to be at relatively high risk for this infection. The incidence of IA during the first 12 months following lung transplant is 2.4%, compared with 0.8%, 0.3%, and 0.1% after heart, liver, and kidney transplant, respectively (4). Risk factors unique to each organ transplanted are likely to blame for these differences. In lung transplant recipients, factors contributing to the risk of IA include a vulnerable anastomotic site, continuous environmental exposure, *Aspergillus* colonization of the airways, CMV pneumonitis, acute rejection, and obliterative bronchiolitis (5). For liver transplant recipients, retransplantation, large volume intraoperative transfusion, pretransplant fulminant hepatic or renal failure, heavy *Candida* colonization peritransplant, choledochojejunostomy, and bleeding complications requiring reoperation have been identified as major factors for invasive candidiasis (IC) (6–9). New risk factors for IFIs are being continually identified and most recently,

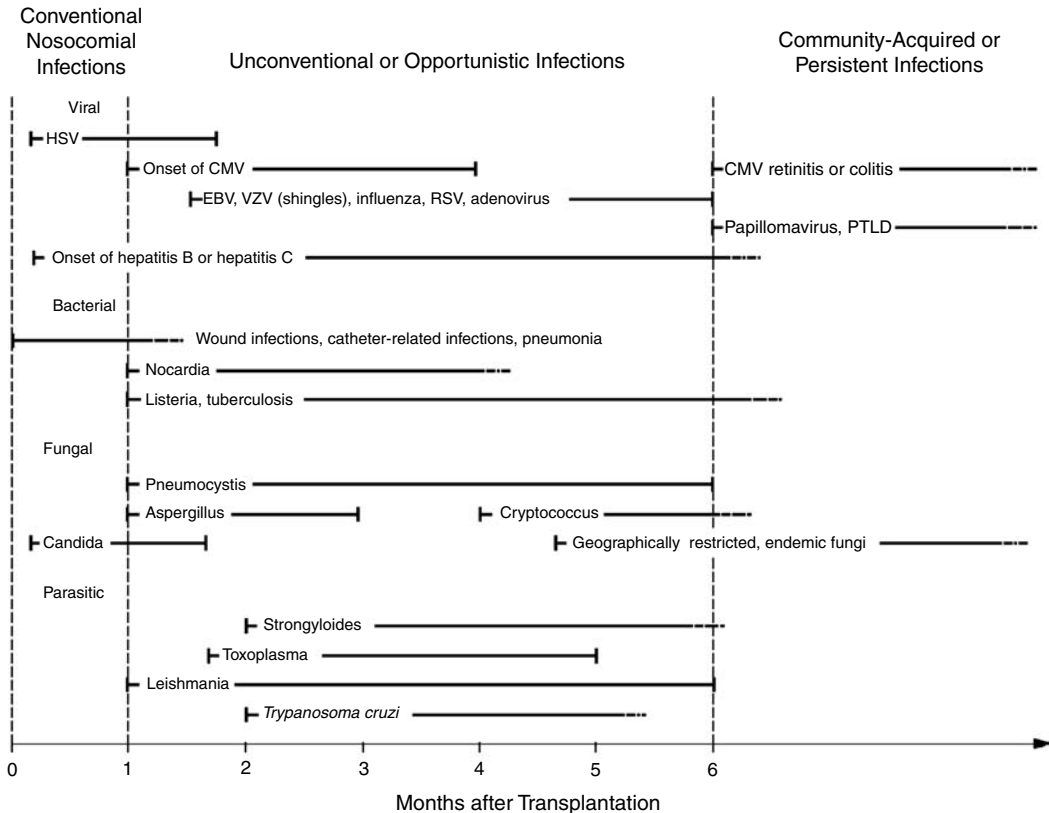


Figure 1 Periods of risk in solid organ transplantation. From Ref. 1.

renal failure, thrombocytopenia, human herpes virus-6, extracorporeal membrane oxygenation, delayed sternal closure, and ventricular assist devices have been implicated.

The most recent epidemiologic data for IFIs in transplant recipients comes from the CDC-sponsored Transplant Associated Infection Surveillance Network (TRANSNET), which prospectively monitored over 33,000 SOT and Hematopoietic Stem Cell Transplant (HSCT) recipients for IFIs between 2001 and 2006. Based on these data, for the SOT population combined as a group, *Candida* remains the most common fungal pathogen, causing 53% of IFIs, followed by *Aspergillus* (19%), *Cryptococcus* (8%), other molds (6%), endemic molds (5%), *Zygomycetes* (2%), other yeasts (2%), and *Pneumocystis* (1%). The median time to diagnosis for *Candida* and *Aspergillus*, the two most common pathogens, is 81 and 186 days, respectively (10).

For the HSCT recipient, host variables, transplant type, and complications posttransplant have a significant role in determining risk for IFI. For example, older age and type of underlying disease, source of stem cells and conditioning regimen, and coinfections and graft-versus-host disease (GVHD) are factors to consider. Though epidemiologic exposures are important, organisms of endogenous origin also play a significant role (11,12). Historically, two major periods of risk have been delineated for these patients (Fig. 2). The first is during the neutropenic window prior to engraftment (typically through posttransplant day 20–30) and the second, which occurs exclusively in recipients of allogeneic HSCT, is following engraftment and correlates with the diagnosis of acute GVHD (typically posttransplant days 30–100). Risk factors after engraftment are multiple and include: receipt of T-cell depleted or CD-34–selected stem cell products, multiple myeloma as underlying disease, delayed engraftment, grade II–IV GVHD, treatment with high dose steroids for GVHD, CMV disease, and respiratory viral infections (especially parainfluenza 3). In the past, *Candida* has been the most common fungal pathogen during the preengraftment phase. An endogenous site, usually the gastrointestinal tract, is thought to be

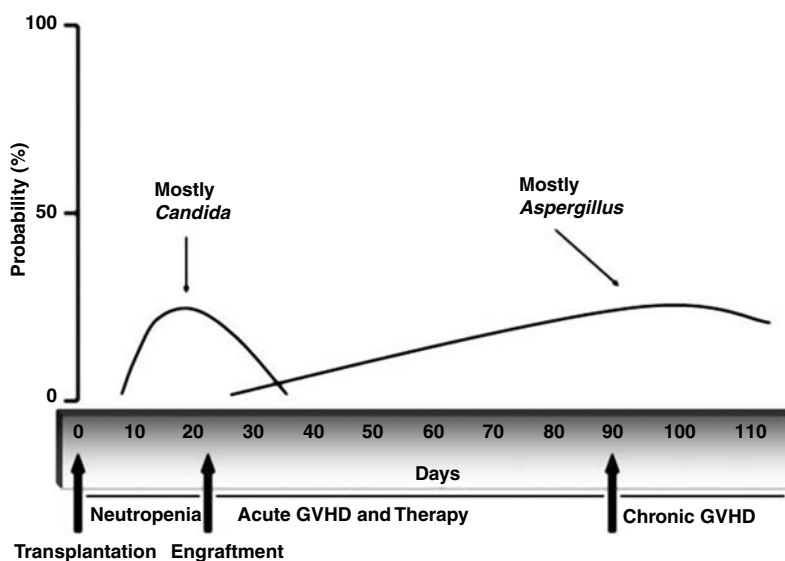


Figure 2 Periods of risk in hematopoietic stem cell transplantation. From Ref. 161.

the source of most *Candida* infections during this window, though *C. parapsilosis* may be more frequently acquired through an exogenous route via long-term central venous catheters (13–16). During the second risk period, infection with *Aspergillus* and other molds has predominated. Most recently, a third risk period for fungal disease has been described for HSCT recipients. This very late period, >6 months posttransplant, occurs in allogeneic HSCT recipients who require chronic immunosuppressive treatment for GVHD, and the risk factors for fungal disease during this window include neutropenia, clinically extensive chronic GVHD, CMV disease, and having received an unrelated or human leukocyte antigen (HLA)-mismatched peripheral blood stem cell transplant (12,17,18). In fact, the cumulative incidence of IA for HSCT recipients is higher at 12 months in patients with grafts from unrelated donors (3.9%), than grafts from allogeneic HLA-mismatched (3.2%), allogeneic HLA-matched (2.3%), and autologous (0.5%) donors. These rates were independent of the conditioning regimen employed.

A shift toward less IC and more IA in the HSCT population has been linked to the introduction of routine fluconazole prophylaxis and the use of preemptive therapy for CMV. In fact, the TRANSNET surveillance study has documented that *Aspergillus* has now surpassed *Candida* as the most common fungal pathogen following HSCT (12,17,19) and increasing numbers of non-*Aspergillus* molds, including the *Zygomycetes*, *Scedosporium*, and *Fusarium* are being diagnosed during the very late risk period. Overall in the HSCT population, *Aspergillus* is now responsible for 44%, *Candida* 28%, other molds 8%, *Zygomycetes* 7%, *Fusarium* 3%, and *Pneumocystis* 2% of IFIs. Several interesting caveats should be noted. First, infections with endemic fungi were rarely encountered in HSCT recipients. Second, *C. glabrata* (39%) has now replaced *C. albicans* (16%) as the most common infecting *Candida* species in the HSCT population (20,21).

PREVENTION

Preventing fungal infections in the transplant recipient is a worthy goal owing to high associated morbidity and mortality. In order to prevent fungal infections, attention must be given to the mode of transmission of the pathogen, the burden of the epidemiologic exposure, as well as the patient's overall net state of immunosuppression at different points in time. Preventive measures are often multifaceted and may include reducing environmental exposures and/or antifungal drugs, and will vary during unique periods of intense immunosuppression. For example, the majority of invasive mold infections are acquired by inhaling aerosolized fungal spores. Hence, as a first line of defense, heavily contaminated air, such as would occur at construction sites, bird coops, bat caves, and during activities such as soil tilling or mowing grass, should be avoided

at all times following any type of transplantation. In addition, for HSCT recipients, the degree of immunosuppression is so great and risk for acquiring a mold infection so high during the preengraftment window, that even having potted plants in the patient's room and consuming fresh fruits, vegetables, teas, or peppers, all which may contain fungal spores, should be avoided during this time. Some experts recommend that high-risk autologous and allogeneic HSCT patients should be housed in laminar airflow rooms outfitted with high-efficiency particulate air (HEPA) filters with a high rate of room air exchange, positive pressure relative to the corridor, and doors and windows that seal well. When traveling to other areas of the hospital, the patient should wear a highly effective mask and avoid areas of renovation or construction in the hospital (22–24). Damp mopping should be employed as standard housekeeping practice in order to avoid aerosolization of spores. Some authors also advocate avoiding water aerosols such as those created while bathing (showering), in addition to using water purification at the general or individual level, in order to protect from a waterborne source of molds. Finally, for *Candida*, potential acquisition from the hands of health care workers exists, so hand washing is strongly recommended (22–25).

Antifungal agents may be employed to prevent invasive disease with organisms such as *Candida*, which may be acquired from the gut during periods of neutropenia, and for mold pathogens, particularly in patients who remain at increased risk for disease but for whom a HEPA-protected environment is no longer practical. Such therapy is typically carefully targeted in both SOT and HSCT recipients, with timing and duration of antifungal therapy variable depending on the type of transplant and window of risk.

In lung transplant recipients, no well-controlled, randomized, multicenter study has been performed to establish a standard prophylactic approach; however, the majority of experts agree that the risk for invasive fungal disease is substantially high enough during the immediate posttransplant period to warrant antifungal prophylaxis. Thus, the majority of all lung transplant programs use some type of antifungal preventive therapy during the immediate post-transplant window, with the duration of prophylaxis varying between one and three months posttransplant (most are ≤ 6 months). Aerosolized amphotericin B-based regimens alone or in combination with a systemic azole such as fluconazole, or systemic therapy alone with a mold active azole such as itraconazole or voriconazole, are the regimens most often used. Small studies evaluating inhaled aerosolized amphotericin B deoxycholate (AmBd) and amphotericin B lipid complex (ABLC) have demonstrated these drugs to be safe in the lung transplant population. While aerosolized ABLC tends to be better tolerated than aerosolized AmBd, studies have documented minimal systemic absorption for both, and thus few systemic toxicities when amphotericin B-based drugs are administered via aerosolization. This, coupled with the ease of once weekly treatments amenable to long term and outpatient administration, are two of the attractive features of this particular approach to antifungal prophylaxis (26,27).

Centers have different approaches to preventive therapy once the lung transplant recipient has completed its initial prophylactic regimen. For example, if a surveillance bronchoscopy culture grows *Aspergillus*, approximately half of centers have a protocol to initiate antifungal therapy in order to prevent progression to invasive disease. Duration of therapy in this setting varies by center from <1 month to >6 months (28,29). Prophylaxis regimens are also not clearly defined when patients are undergoing treatment for rejection. Some centers will use mold active antifungal prophylaxis during treatment for rejection, particularly when alemtuzumab is used. Multicenter studies designed to assess the efficacy of antifungal prophylaxis regimens in the lung transplant population are needed.

Antifungal drugs that have been studied in randomized controlled trials as prophylaxis in the liver transplant population include fluconazole, itraconazole, and amphotericin B (30–35). Fluconazole is easily absorbed and has adequate tissue penetration while certain formulations of itraconazole have been associated with gastrointestinal intolerance and low serum concentrations. Amphotericin B was administered intravenously in this group, and despite having broad spectrum of activity, it may cause infusion-related adverse events or nephrotoxicity. One suggested and reasonable approach to prophylaxis in the liver transplant population, as well as pancreas and small bowel graft recipients, is to assess the risk for infection in each individual patient and initiate prophylaxis for patients with two or more high risk characteristics (Tables 1 and 2).

Table 1 Risk Factors for Invasive Candidiasis in Solid Organ Transplantation (7,99,135–143)

Organ	Risk factor
Small bowel	Anastomotic disruption
	Graft dysfunction
	Acute rejection
	Enhanced immunosuppression
	Multivisceral transplant
Pancreas	Enteric drainage
	Postperfusion pancreatitis
	Vascular graft thrombosis
	Wound infection with <i>Candida</i>
	Older donor age
Liver	Pancreatic after renal or simultaneous pancreatic renal transplant
	Posttransplant dialysis requirement
	Peritransplant colonization with <i>Candida</i>
	Antibiotic prophylaxis for spontaneous bacterial peritonitis
	Retransplantation
	Intraabdominal bleeding
	High intratransplant transfusion requirement
Hyperglycemia requiring insulin and exposure to more than three antibiotics	

Among HSCT populations, fluconazole prophylaxis has been studied and has become standard of care during the first 100 days posttransplant (36,37). Patients on fluconazole prophylaxis have less superficial and systemic infections, and candidemia is prevented. In addition, severe GVHD seems to occur less, and survival has been shown to be increased at eight years posttransplant in patients who received fluconazole prophylaxis. The main limitation to fluconazole prophylaxis has been its lack of activity against mold pathogens, which are a particular concern for allogeneic HSCT recipients who develop acute and/or chronic GVHD (11,36,38).

Table 2 Risk Factors for Invasive Aspergillosis in Solid Organ Transplantation (5,6,8,141,142,144–150)

Organ	Risk factor
Lung	Unhealed anastomotic site
	Airway ischemia
	Reperfusion injury
	Delayed sternal closure
	Decreased cough and delayed mucociliary clearance
	Receipt of single lung
	Presence of bronchial stents
	CMV pneumonitis
	Acute rejection
	Obliterative bronchiolitis
	Augmented immunosuppression for refractory rejection
	<i>Aspergillus</i> colonization of airways
	Pretransplant fulminant hepatic or renal failure
Liver	Primary allograft failure
	Retransplantation
	Large-volume intraoperative transfusions
	Bleeding requiring reoperation
	Cholechojejunostomy anastomosis
Heart	Requirement of renal replacement therapy
	Reoperation
	Cytomegalovirus disease
	Posttransplant dialysis
	Augmented immunosuppression for cardiac allograft vasculopathy
Episode of IA in heart transplant program 2 mo before or after transplantation date	

Most experts recommend that all HSCT patients with acute and/or chronic GVHD should receive mold active antifungal prophylaxis. Several antifungal agents have therefore been evaluated for this purpose. In a single center evaluation, aerosolized ABLC was prospectively evaluated as a prophylactic agent in allogeneic HSCT recipients and was shown to be safe and well tolerated as prophylaxis against invasive pulmonary IFIs (39). Itraconazole has a spectrum of activity that includes *Aspergillus* species, and in those able to tolerate the drug (gastrointestinal intolerance or hepatic toxicity) and in whom potential drug–drug interactions can be monitored, itraconazole appears to protect against IA compared with fluconazole (40–42). Newer azoles with mold activity (voriconazole and posaconazole) have also received attention regarding their potential for use as long-term prophylactic agents in the HSCT population. Posaconazole has been studied in allogeneic HSCT recipients with GVHD. Not only was posaconazole effective in protecting against IFIs, use of posaconazole also resulted in decreased mortality during the study period in neutropenic patients with hematologic malignancies (43,44). Voriconazole is currently under study in a large multicenter trial compared with fluconazole, the results of which are pending. Interestingly, there are currently reports of IFIs breaking through voriconazole treatment in the HSCT population. In particular, *Candida glabrata* and *Zygomycete* infections have been described (45–48). These studies suggest that prior azole exposure may select for infection with azole-resistant pathogens, and that exposure to one azole (fluconazole or voriconazole) may select for cross-resistance to other azoles (posaconazole). Further support of this concept is from in vitro data demonstrating that *A. fumigatus* previously exposed to fluconazole has altered susceptibility to itraconazole (49). These findings will likely impact the utility and choice of antifungal agents used for prophylaxis and highlight the negative impact of long-term prophylaxis.

Alternative strategies to the long-term use of antifungal agents as prophylaxis are needed. One such approach is to use new, minimally invasive diagnostic tests in a preemptive approach to preventing clinically symptomatic fungal disease. In such a strategy, patients would have the diagnostic test performed twice weekly during periods of highest risk for fungal disease. Antifungal therapy would be instituted only if the test is positive. This strategy would be effective only if the test is able to detect the infection very early in the course of disease. Two such tests that are currently FDA approved for the diagnosis of fungal disease, the galactomannan and glucan assays, have been shown to turn positive prior to the development of clinical symptoms and radiographic changes. The clinical utility of serial monitoring with each test has been studied in hematologic malignancy/HSCT populations for preemptive treatment strategies, and the results are promising enough to warrant larger multicenter trials in these populations (50,51). Unfortunately, the few studies of these tests performed to date in SOT populations have been disappointing (52–54).

Prophylaxis for PCP is generally recommended for transplant patients. In fact, prophylaxis has led to a substantial decrease in the rate of PCP infection in the SOT population, with the diagnosis decreasing from 33% (1992–1996) to 8% (1997–2003) in one center (55). The duration of PCP prophylaxis, as with other fungal pathogens, is variable depending on the type of transplant and prevalence of PCP in the area (Table 3).

ISSUES IN MANAGEMENT

Coinfections

The herpes viruses, cytomegalovirus (CMV), human herpes virus 6 (HHV-6), and human herpes virus 7 (HHV-7), have been described as having immunomodulatory properties that may facilitate infection with other opportunistic pathogens, including fungi (56–61). Perhaps most compelling are data supporting CMV disease as a risk for opportunistic fungal infections. In one center, 36% of liver transplant recipients with CMV disease developed an IFI over a one-year period compared to 8% without CMV disease (57). Preceding CMV disease, CMV pneumonitis, and CMV viremia were all associated with the development of an IFI, and being a CMV seronegative recipient of a CMV seropositive donor organ (CMV R–/D+) increased the risk by 19-fold. Further, in allogeneic HSCT recipients undergoing bronchoalveolar lavage, the

Table 3 Recommended Prophylaxis for *Pneumocystis* Pneumonia (151–160)

Prophylactic agents	
First line	Trimethoprim–sulfamethoxazole
Second line	Dapsone Dapsone–pyrimethamine Pentamidine Atovaquone
Type of transplant	Duration of prophylaxis
<i>Hematopoietic stem cell transplant</i>	
Allogeneic HSCT	Start 1–2 wk prior to transplant and continue at least 6 mo posttransplant Consider prophylaxis beyond 6 mo posttransplant
<ul style="list-style-type: none"> ● Allogeneic HSCT with: <ul style="list-style-type: none"> ○ Chronic GVHD ○ Increased systemic immunosuppression ○ Relapsed malignancy 	
Autologous HSCT	For most, no clear indication for prophylaxis
<ul style="list-style-type: none"> ● Autologous HSCT with <ul style="list-style-type: none"> ○ Underlying hematologic malignancy (lymphoma, leukemia) undergoing intense conditioning regimens or graft manipulation. ○ Recent receipt of fludarabine or 2-chlorodeoxyadenosine (2-CDA). 	Consider prophylaxis for 6 mo
<i>Solid organ transplant</i>	
Lung, heart/lung	Prophylaxis for 12 mo
<ul style="list-style-type: none"> ○ When the incidence in institution or region is >5% without prophylaxis. ○ If patient has a history of PCP or frequent opportunistic infections. ○ If patient is being treated for CMV or is considered at high risk for CMV infection. ○ If patient is receiving therapy for acute rejection or has neutropenia. 	Consider lifelong prophylaxis
Heart, liver, kidney, pancreas, small bowel	
<ul style="list-style-type: none"> ○ In patients with multiple rejection episodes and treatment for rejection. ○ In patients with graft dysfunction. ○ In patients with persistent viral infections such as CMV. 	Prophylaxis for 6–12 mo

concomitant isolation of CMV and *Aspergillus* predisposed to poor outcome (fatal in 9 of 10 patients) (62). Conversely, decreased rates of CMV disease have been associated with decreased IFIs. The use of prophylactic ganciclovir in heart transplant recipients reduces not only the rate of CMV disease but also of IFIs, with only 7% of IFIs reported in patients on ganciclovir prophylaxis compared to 17% who did not receive it ($p = 0.007$) (63).

General recommendations have been suggested for the management of CMV in the different transplant groups, and universal or preemptive therapy may be used (56). Universal prophylaxis is generally given to high-risk SOT patients (CMV R–/D+), with preemptive therapy reserved for SOT recipients considered to be at intermediate risk for CMV disease and for allogeneic HSCT recipients. For preemptive therapy, frequent monitoring for the development of CMV reactivation is required so that therapy can be given when viremia is first detectable, but the patient is still asymptomatic. It appears that both approaches decrease CMV disease compared to no prophylaxis or placebo (64,65). Whether universal and preemptive regimens are equally effective at also reducing associated fungal infections is still under investigation. In one study of SOT recipients, both regimens reduced the rate of acute allograft rejection;

however, only universal prophylaxis significantly decreased bacterial and fungal infections in the population under study (65). On the other hand, studies in liver transplant recipients receiving preemptive prophylaxis compared with those never requiring prophylaxis and high-risk patients receiving universal prophylaxis showed no difference in the rate of fungal infections, suggesting that CMV preemptive therapy is beneficial (64,66). The addition of CMV immune globulin alone or when added to ganciclovir appears to confer no additional protection against IFIs (67,68).

HHV-6 has also been implicated as facilitating infection with fungi (58,59,61). In liver transplant recipients, HHV-6 viremia has been associated with higher rates of IFI; IFI occurred in 32% of patients with HHV-6 viremia compared to 4% of those without viremia ($p = 0.009$). Further, HHV-6 was present either preceding the IFI or within one week of IFI in 80% of those who had both infections. HHV-6 was noted to be a strong predictor of IFI (OR 8.3, 95% CI = 1.2–58; $p = 0.0336$), thus corroborating previous studies, which showed a potential higher risk for IFI in patients with HHV-6 infection (61).

It is known that HHV-6 facilitates infection with CMV, and in turn, CMV is associated with IFI (59). Similarly, in a cohort of renal transplant recipients, patients with HHV-7 and coinfecting with CMV had a significant increase in CMV disease (60,69). Thus, both HHV-6 and HHV-7 may indirectly increase the risk for IFI through their effect on CMV. The use of ganciclovir has been shown to reduce the viral load of HHV-6, HHV-7, and CMV, and the incidence of CMV disease (70). Prophylactic ganciclovir has also been associated with reduced rates of IFI (63). It is therefore postulated that by treating this group of herpes viruses, one may manipulate their immunomodulatory effect and thereby reverse their permissive effect on susceptibility to IFIs.

Epstein-Barr virus (EBV), another herpes virus, may also be independently associated with IFI. In one series of pediatric liver transplant recipients, the rate of IFIs was reported to be 40% during the first year posttransplant. Of the posttransplant variables assessed, only bacterial infection, EBV infection, and tacrolimus administration were independently associated with development of IFI (71).

Comorbid conditions

Comorbid conditions present in transplant populations that have been associated with IFIs include GVHD, neutropenia, rejection, hypogammaglobulinemia, renal replacement therapy, and diabetes. An immune reconstitution syndrome has also recently been associated with the treatment of cryptococcosis in SOT recipients. It is important to recognize these comorbid conditions in order to ensure the optimal management for transplant recipients during periods of heightened risk for IFI and in order to promote a successful outcome for those patients who develop concomitant IFIs.

Neutropenia

The risk of IFI is increased in patients with neutropenia (12,21,72,73). The longer the duration of neutropenia, the higher the risk of developing an IFI (19). Colony-stimulating factors such as granulocyte colony stimulating factor (G-CSF) promote earlier recovery from neutropenia and have been used in the HSCT population (74–76). Peripheral blood stem cells (PBSC) generally have a more rapid engraftment of neutrophils (17 days vs. 24 days) and platelets (28 days vs. 47 days) than traditional bone marrow transplant. Unfortunately, recovery from neutropenia does not always translate into recovery of immune function (77). Recovery of normal lymphocyte counts precedes recovery of normal lymphocyte function. It can take up to one year for full functional capacity of all immune cell types.

Animal studies with a rabbit model of invasive pulmonary aspergillosis reveal a distinct pattern of response to IFI on histopathology depending on whether immunosuppression is secondary to neutropenia or to cyclosporine plus methylprednisolone (78). The pattern noted in the neutropenic rabbits was one of scarce mononuclear infiltrate with coagulative necrosis and intraalveolar hemorrhage, whereas in rabbits treated with cyclosporine plus methylprednisolone, the histology consisted of a neutrophilic and monocytic inflammatory necrosis and scant hemorrhage. Similarly, in humans, neutropenic patients including neutropenic HSCT recipients have angioinvasion by *Aspergillus* hyphae and intraalveolar hemorrhage with scant neutrophilic and monocytic infiltrate more frequently than nonneutropenic

patients with aspergillosis. In the neutropenic HSCT group, there is a trend toward coagulative necrosis (eosinophilic inflammatory infiltrate with loss of cell membrane but preservation of pulmonary architecture) while the nonneutropenic group shows inflammatory necrosis (neutrophilic and monocytic infiltrate with necrosis of pulmonary parenchyma) and granuloma formation on histology (79).

The histopathologic pattern found in patients with profound neutropenia and IA underscores the importance of neutrophils as a first line of defense as they prevent proliferation and angioinvasion of hyphae. In nonneutropenic patients, the host immune response is able to contain the hyphae and produces a necrotic inflammatory picture. Though the recovery of white blood cell counts is important in controlling the infection with IA, rapid recovery of the white blood cell count (with or without G-CSF) after profound neutropenia in hematologic malignancy after chemotherapy has led to complications (80,81). Pulmonary hemorrhage with hemoptysis, pneumothorax, and respiratory failure, characterized by hypoxia and worsening infiltrates, has been reported from a small study. The authors postulate that, along with the angioinvasiveness of *Aspergillus*, the necrotic inflammatory response, secondary to functional polymorphonuclear leukocytes interacting with mold and cellular debris, may stimulate cell metabolism and proteolytic enzymes to a degree which can injure the pulmonary vasculature.

GVHD

GVHD confers high risk for fungal disease, as it further inhibits immune recovery directly, through the deleterious effects of increased immunosuppressive cytokines, but also indirectly, due to the augmented iatrogenic immunosuppression required in this setting. The type of allogeneic HSCT transplant may influence the timing of GVHD. Though timing and rates of acute GVHD are similar for patients receiving PBSC (46%) compared with bone marrow cells (51%), PBSC recipients develop late-onset chronic GVHD after 180 days posttransplant. This is accompanied by increases in IFI and CMV disease during the same time period (82).

GVHD has also been reported in the SOT population, mostly in liver transplant recipients, though it appears that a relatively high incidence may occur in small bowel recipients as well. It is felt that small bowel and liver carry a significant immunologically competent lymphoid population that may increase the risk of GVHD, albeit without causing rejection. Biopsy of liver, gastrointestinal tract, and/or skin may show characteristic changes, such as damage to the interlobular bile ducts in the liver with occasional presence of apoptotic cells and canalicular cholestasis, denudation of the gastrointestinal mucosa with crypt destruction and apoptotic cells, and cutaneous findings of dyskeratotic cells and subepithelial vesicle formation, respectively. Infections including sepsis, CMV disease, and invasive candidiasis and aspergillosis, can complicate the period of intense immunosuppression required to treat GVHD, and mortality is fairly high (83–86). Most experts recommend antifungal prophylaxis while patients are receiving additional immunosuppression for treatment of acute as well as chronic GVHD.

Rejection

Rejection may occur at any time in the posttransplant period in the SOT population. Among the newer immunosuppressive agents, monoclonal antibodies such as alemtuzumab are being increasingly used to treat steroid-resistant rejection episodes. With the resulting intense and long-lasting immunosuppression secondary to lymphocyte depletion and other alterations of cellular immunity caused by alemtuzumab, patients are at risk for IFI. In a large study of 547 SOT recipients receiving alemtuzumab for either induction or rejection and followed for 12 months after the first alemtuzumab dose, opportunistic infections occurred in 10% of patients (87). The risk of developing an opportunistic infection was approximately five times higher when alemtuzumab was given for rejection versus induction (21.5% vs. 4.5%, respectively). Of the opportunistic infections, 32% were IFIs (18/62), 83% (15/18) of which occurred in patients who received alemtuzumab for rejection. The median time to infection was 58 days after alemtuzumab therapy and 245 days after transplant.

Importantly, lymphocyte depletion persisted even after 12 months following alemtuzumab administration. Median CD4 counts at baseline were 626 cells/mm³, and at 1, 3, 6, 9, and 12 months, median counts were 6, 15, 52, 92, and 127 cells/mm³, respectively. Patients who experienced rejection and received alemtuzumab had usually received other

immunosuppressive agents (methylprednisolone, another lymphocyte-depleting antibody such as rabbit antithymocyte globulin, or monoclonal CD3 antibody), and thus had a higher net state of immunosuppression, which increased the risk of IFI. Patients who received alemtuzumab for rejection had also experienced more transplant graft failure than those who received alemtuzumab for induction, and tended to be further out in their posttransplant period. As such, they had longer environmental exposure and generally were on different antimicrobial prophylaxis than those earlier in their posttransplant phase. In some centers, voriconazole and/or aerosolized amphotericin B–based prophylaxis are used when alemtuzumab is administered for steroid refractory rejection in lung transplant recipients (personal communication, B.D. Alexander).

Immune Reconstitution Syndrome

An immune reconstitution syndrome (IRS) has been reported in SOT patients infected with *C. neoformans* (88). In a review of 22 centers, 83 SOT patients with cryptococcosis were identified. An IRS-like syndrome was diagnosed in four patients based on worsening of a previously responding lesion not explained by failure of therapy or another infection. The four patients received standard treatment with a formulation of amphotericin B +/- flucytosine and were subsequently transitioned to fluconazole. However, the patients developed signs suggestive of worsening infection including increased inflammatory cells in CSF and enhancing CNS lesions. Cytology or histopathology revealed budding yeast cells consistent with *Cryptococcus* and even granuloma; however, all cultures remained negative. It was postulated that antifungal therapy elicited a vigorous proinflammatory Th-1 response in the host, leading to exacerbation of clinical signs and symptoms. The authors emphasized the importance of keeping this phenomenon in mind when faced with a seemingly worsening clinical picture following the initiation of treatment for cryptococcosis and to not merely ascribe it to failure of therapy (88). Whether or not the use of steroids would be beneficial in SOT patients with IRS requires study.

Hypogammaglobulinemia

Hypogammaglobulinemia in the posttransplant period has been associated with increased risk of infection in some studies (89–91). Increased rates of CMV infection as well as a trend in IFIs have been noted in SOT with hypogammaglobulinemia. Several of these patients developed hypogammaglobulinemia after receiving increased immunosuppression to treat rejection or bronchiolitis obliterans syndrome, and some had concomitant neutropenia. In a group of lung transplant recipients with severe hypogammaglobulinemia, IA accounted for 44% of infections (92). The authors suggest measures to manage these severely hypogammaglobulinemic patients including the reduction of immunosuppression when feasible, increasing prophylaxis for infection, and possible immunoglobulin replacement therapy. Though one study in liver transplant recipients did not find an increased risk of CMV disease, bacterial infection, or fungal infection with hypogammaglobulinemia in the posttransplant period, hypogammaglobulinemia was associated with increased mortality at one and five years posttransplant (93).

Metabolic Conditions

Other comorbid conditions have been associated with an increased risk of IFIs following transplantation. Liver transplant recipients have been considered at high risk for invasive mold infection if they required renal replacement therapy on the day of transplant or if hospital discharge was delayed beyond seven days posttransplant secondary to renal or hepatic insufficiency. Increased risk of IFI may be conferred by transplant for fulminant hepatic failure or retransplant. Both these groups may warrant antifungal prophylaxis (94). Posttransplantation dialysis is also a significant predictor of IC following liver transplant (7). Uremia and renal replacement therapy have been associated with impaired neutrophil function and increased neutrophil apoptosis (95,96).

Diabetes mellitus is frequently encountered following transplantation, often the consequence of steroid use. Poorly controlled diabetes is a well-described risk factor for the development of zygomycosis in both HSCT and SOT patients, and hyperglycemia requiring insulin therapy has been associated with candidemia in liver transplant recipients (97–99). Aggressive control of blood sugars is mandatory for these patients.

Antifungal Drug Interactions and Monitoring

The advent of newer azoles with activity against *Aspergillus* and other molds (voriconazole, posaconazole) as well as a new class of antifungals, the echinocandins (caspofungin, micafungin, anidulafungin) has increased the armamentarium of drugs available against IFIs. Drug interactions between antifungal agents and immunosuppressive medications exist and require dose modification, monitoring of serum levels, or occasionally, a change in medication regimen.

Voriconazole, a derivative of fluconazole, has a broad spectrum of activity. It is metabolized by the human hepatic cytochrome P450 enzymes, with highest affinity for CYP2C19. Inhibition of CYP3A4 by voriconazole is less than that of other azoles, such as ketoconazole and itraconazole. Voriconazole has significant drug–drug interaction with calcineurin inhibitors, and caution should be used when these agents are coadministered. Recommendations on dosage adjustment when starting voriconazole in patients already on cyclosporine or tacrolimus include reducing cyclosporine dose by 50% and tacrolimus by 66%. Calcineurin inhibitor levels should subsequently be monitored closely. Dosage adjustments also need to be made once voriconazole is discontinued so that therapeutic concentrations of these immunosuppressive drugs can be maintained. Coadministration of voriconazole and sirolimus is contraindicated by the manufacturer, but a few cases of SOT and HSCT patients treated with these agents concomitantly have been published. Doses of sirolimus were significantly reduced and serum levels tightly monitored to maintain concentrations in the therapeutic range.

In adults receiving voriconazole, variability exists between doses administered and trough levels, and low serum voriconazole levels have been associated with failure of therapy. If a patient is failing treatment with voriconazole, one should consider checking a voriconazole level to ensure absorption. A steady-state trough of at least 0.5 $\mu\text{g}/\text{mL}$ suggests adequate absorption. Other data suggest a higher success rate in patients with plasma voriconazole levels $>0.5 \mu\text{g}/\text{mL}$. In addition, an increase in the number of favorable responses was noted in transplant recipients with IA treated with voriconazole when random serum concentrations were above 2 $\mu\text{g}/\text{mL}$ (100–114).

Posaconazole is closely related to itraconazole, though with superior activity. It is currently only available as an oral formulation, which, if swallowing is impaired or severe oral mucositis is present, may limit its use. When used with calcineurin inhibitors, it has led to increased levels of cyclosporine with serious adverse events (nephrotoxicity, leukoencephalopathy, and death) when doses were not adjusted. When posaconazole therapy is initiated or discontinued, dose adjustment and more frequent clinical monitoring of cyclosporine and tacrolimus levels should be performed. The cyclosporine dose should be reduced to approximately three-fourths of the original dose, and the tacrolimus dose reduced to approximately one-third of the original dose when starting posaconazole.

Oral absorption of posaconazole is good, and the drug is generally well tolerated. However, patients with mucositis generally have poor food intake, which may require modification of posaconazole administration. In patients with mucositis who were neutropenic after chemotherapy and autologous stem cell transplant, though the mean posaconazole steady-state exposure and area under the curve (AUC) were not affected, posaconazole absorption was less in patients with mild-to-moderate mucositis. This may be overcome by administering the total dose in multiple divided doses (i.e., 800 mg total dose divided into 200 mg four times a day in lieu of 400 mg twice daily dosing). In addition, posaconazole should ideally be administered with a high-fat meal to facilitate absorption.

Itraconazole is also a potent inhibitor of the cytochrome P450 CYP3A4 enzyme, and when itraconazole is administered with cyclosporine or tacrolimus, increased plasma concentrations of these immunosuppressants result. The dose of cyclosporine should be decreased by at least 50%, if not more, after an intravenous itraconazole loading dose, and serum levels monitored. Concomitant administration of itraconazole and sirolimus also increase plasma concentrations of sirolimus, requiring dose adjustment or even discontinuation of the latter. Levels of all these immunosuppressants should be monitored frequently, and the dosages adjusted accordingly to achieve the desired serum concentrations and immunosuppressive effect.

Certain formulations of itraconazole, such as the capsules, are not reliably absorbed from the gastrointestinal tract, and levels of itraconazole should be monitored or itraconazole changed to a different formulation (oral solution, iv formulation) to achieve therapeutic levels. A loading

regimen may be used prior to standard or maintenance dosing to achieve the desired trough levels. Trough levels of itraconazole $>0.5 \mu\text{g}/\text{mL}$ have been suggested to achieve higher efficacy.

The polyene antifungals, amphotericin B deoxycholate, amphotericin B lipid complex, and liposomal amphotericin, are also used to manage IFIs in the transplant population. Infusion-related side effects (fever, chills, and rigors) and nephrotoxicity (elevated serum creatinine, hypokalemia) are the main adverse events with amphotericin B deoxycholate and, to a lesser degree, the lipid formulations. One should proceed with caution when administering these agents with calcineurin inhibitors and other nephrotoxic agents as altered kidney function may rapidly develop, and in transplant recipients, the lipid products of amphotericin B are favored over amphotericin B deoxycholate. Aerosolized amphotericin B, amphotericin B lipid complex, and liposomal amphotericin have been used mainly for prophylaxis in the SOT and HSCT population. They may precipitate bronchospasm and have been associated with nausea, vomiting, and taste disturbance, but were generally well tolerated and not systemically absorbed to a significant degree (27,39,101,115–122).

Of the echinocandins, caspofungin and anidulafungin are not metabolized by the human cytochrome P450 system. Micafungin demonstrates weak CYP3A4 inhibition. In general, these drugs are well tolerated and have few adverse effects. Transient elevation of liver transaminases has been reported in healthy volunteers and in HSCT and SOT patients concomitantly administered caspofungin and calcineurin inhibitors. The coadministration of caspofungin with cyclosporine led to an increase in the AUC of caspofungin by about 35% without increase in maximum concentration (C_{max}). The levels of cyclosporine were not increased. The manufacturer recommends caution when coadministering these agents, and they stress the importance of evaluating the risk–benefit ratio prior to use. Monitoring of liver enzymes during concomitant therapy with these agents is recommended. The administration of caspofungin with tacrolimus does not affect caspofungin pharmacokinetics but may reduce tacrolimus concentration by 25%. Careful monitoring of tacrolimus dosages and levels is required to maintain therapeutic concentrations.

Micafungin is well tolerated, and though nausea, vomiting, diarrhea, and hyperbilirubinemia were noted in HSCT recipients on micafungin prophylaxis, this was no different from those on fluconazole prophylaxis. No interactions were noted between micafungin and cyclosporine, tacrolimus, mycophenolate mofetil, fluconazole, or prednisolone. Micafungin has been administered to SOT and HSCT patients who were receiving calcineurin inhibitors, found to be well tolerated, and no dose modification of either medication was required. When micafungin is coadministered with sirolimus, trough levels of sirolimus may be increased and the AUC increased by 21%. Thus, sirolimus levels should be monitored and dosages adjusted to maintain concentrations in the therapeutic range.

Anidulafungin is also well tolerated though hypokalemia, nausea, and diarrhea were the most common adverse events in one noninferiority study. Coadministration with cyclosporine caused elevation of the steady-state AUC of anidulafungin by 22% without affecting the C_{max} , but the manufacturer does not recommend dose adjustment of either drug. Coadministration with tacrolimus did not result in alteration of either C_{max} or AUC of either drug, and no dose adjustments are recommended. Coadministration with voriconazole also does not affect these parameters nor is dosage modification recommended (101,123–134).

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Prophylaxis and Treatment of Invasive Fungal Infections in Neutropenic Cancer and Hematopoietic Stem Cell Transplant Patients

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INTRODUCTION

Invasive fungal infections (IFIs) are a major cause of morbidity and mortality among patients with hematologic malignancies, particularly in those affected by acute leukemia (1) and hematopoietic stem cell transplant (HSCT) (2–6). Advances in the HSCT area have led to a prominent increase of IFI incidence due to factors such as (i) the use of potent immunosuppressives as prophylaxis and treatment for graft-versus-host disease (GVHD), (ii) increasing reliance of alternative donors such as human leukocyte antigen (HLA) matched or mismatched unrelated donors, (iii) more umbilical cord blood unit transplantation procedures, (iv) performing multiple transplantation procedures, and (v) more frequent preventative measures against infections caused by highly virulent bacteria, *Candida* spp., or cytomegalovirus (5).

Pathogens Causing IFI in HSCT Recipients

Candida spp. were detected as the major cause of IFIs in early studies. Changes in clinical practice over time, however, involved a transition to peripheral blood stem cells rather than bone marrow as the hematopoietic graft source, frequent use of recombinant myeloid hematopoietic growth factors, and reduced-intensity conditioning regimens. These developments shortened the duration of posttransplant neutropenia and attenuated conditioning toxic effects such as mucositis. For these reasons, invasive mold infections now are a greater risk for the allogeneic HSCT recipient than invasive candidiasis (7). On the other hand, the increasing and extensive use of fluconazole and newer azoles with apparent reduced antimold activity prompted the emergence not only of resistant *Candida* spp. (i.e., *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*) (8,9) but also for serious IFIs caused by less commonly encountered fungi that frequently exhibit intrinsic resistance to many antifungal agents. Such organisms include *Zygomycetes* (*Mucor*) (8,10), *Fusarium* (8,11), or *Scedosporium* (8).

During the preengraftment period, *Candida* remains as an important cause of morbidity and mortality (12). Mortality rates may reach 40% in patients without tissue involvement and approach 90% in those with tissue invasion (13). Non-*albicans* species are an increasing cause of candidemia in 40% to 70% of patients with hematologic malignancies and HSCT recipients (14).

In contrast to *Candida*, *Aspergillus* IFI appears to predominate in the postengraftment, rather than in the preengraftment, neutropenic period. The principle affected groups include HSCT recipients receiving immunosuppressive therapy for GVHD as well as those given T-cell depleted grafts (9,15–16). The incidence of infection ranges from 10% to 20% in allogeneic HSCT recipients, whereas less than 2% of autologous HSCT recipients develop *Aspergillus* IFI (9). One large multicenter surveillance program reported that *Aspergillus* spp. associated with infections after HSCT included *A. fumigatus* (56%), *A. flavus* (19%), *A. terreus* (16%), *A. niger* (8%), and *A. versicolor* (1.3%) (4). Other less common and highly virulent mold IFI with mortality rates as high as 87% include *Fusarium* spp., *Scedosporium*, and *Zygomycetes* (5,17). *Scedosporium* infections

usually begin early in the first month after transplantation, while zygomycosis may develop as long as three months after transplantation, usually in association with GVHD or its treatment (5).

Incidence

Males outnumber females at a ratio of approximately 2:1 and the frequency of IFIs among traditional myeloablative allogeneic HSCT recipients at one year ranges from 10% to 25% (2,6,8,18–21). The cumulative incidence reported in a recent series for *Aspergillus* spp. infections was 23%, and for invasive candidiasis, it was 3% (22). Patients who develop extensive chronic GVHD, experience IFIs at even higher rates (20). Nonmyeloablative and reduced-intensity conditioning regimens, unfortunately, appear to have an incidence of IFIs similar to myeloablative conditioning regimens (6,21). Incidence rates are considerably lower (range 0–6%) in autologous HSCT recipients (3,5); onset in this population is usually during the neutropenic preengraftment period (23). The incidences of invasive aspergillosis and candidiasis in autologous HSCT are 8% and 2%, respectively (22). In a large series from the Italian SEFEM (Sorveglianza Epidemiologica Infezioni Fungine Nelle Emopatie Maligne) B-2004 study involving 1249 allogeneic and 1979 autologous HSCT recipients, the IFI incidence rates were 7.8% and 1.2% among allogeneic and autologous HSCT recipients, respectively (8). Morgan et al. reported a larger, prospective, multicenter surveillance trial in 4621 HSCT recipients collected from 19 USA centers (4). Cumulative incidence of *Aspergillus* IFI at 12 months was 0.5% after autologous HSCT, 2.3% after allogeneic HSCT from an HLA-matched related donor, 3.2% after transplantation from an HLA-mismatched related donor, and 3.9% after transplantation from an unrelated donor. The cumulative incidence at 12 months was similar after a myeloablative or nonmyeloablative conditioning regimen (3.1% vs. 3.3%) (4).

Risk Factors

Patient-, disease-, and treatment-related risk factors for IFI in adult HSCT recipients are shown in Table 1 (2,4,6,8,9,12,13,16,20,22,24). The likelihood for development as well as the infection severity is dependent upon the interplay of host and environmental factors. Therefore, features

Table 1 Risk Factors for the Development of IFI in Adult HSCT Recipients

Risk factor	Reference number
Recipient age >40 yr	2,4,6,8,9,12,13,16,20,22
Thrombocytopenia	2,4,6,8,9,12,13,16,20,22
Use of allogeneic HSCT	2,4,6,8,9,12,13,16,20,22
Infused CD34 ⁺ stem cell dose $\leq 2 \times 10^6$ /kg	2,4,6,8,9,12,13,16,20,22
Prior history of splenectomy, fungal infection, environmental exposure to fungus	2,4,6,8,9,12,13,16,20,22
Prolonged (> 10 days) neutropenia (ANC < 500 cells/ μ L)	2,4,6,8,9,12,13,16,20,22
Stem cell manipulation (e.g., CD34 ⁺ selection)	2,4,6,8,9,12,13,16,20,22
Presence of viral disease (e.g., CMV)	2,4,6,8,9,12,13,16,20,22
Severe mucositis	2,4,6,8,9,12,13,16,20,22
Development of \geq grade 2 acute GVHD	2,4,6,8,9,12,13,16,20,22
Mucosal fungal colonization	8,9,24
Use of systemic corticosteroids	8,9,24
Disrupted skin integrity	8,9,24
Lack of laminar air-flow in transplant room	8,9,24
Presence of a central venous catheter device	8,9,24
HLA-mismatched related donor	2,4,6,8,9,12,13,16,20,22
Matched unrelated donor	2,4,6,8,9,12,13,16,20,22
Use of UCB stem cells	2,4,6,8,9,12,13,16,20,22
Donor lymphocyte infusion	2,4,6,8,9,12,13,16,20,22
Multiple HSCT	2,4,6,8,9,12,13,16,20,22
Transplantations using T-cell depletion (e.g., ATG-, alemtuzumab-containing regimens)	2,4,6,8,9,12,13,16,20,22

Abbreviations: IFI, invasive fungal infection; HSCT, hematopoietic stem cell transplantation; ANC, absolute neutrophil count; CMV, cytomegalovirus; HLA, human leukocyte antigen; UCB, umbilical cord blood; ATG, antithymocyte globulin; GVHD, graft-versus-host disease.

such as the presence of compromised mucocutaneous barriers, environmental exposure to fungal pathogens, and the degree and duration of impaired immune competence may induce the development of severe IFIs in these immunocompromised patients.

Mortality Rates

Case-fatality rates for aspergillosis IFIs approach 90% in allogeneic HSCTs (15) and 70% in autologous HSCT (4). Mortality rates due to *Candida* infections correspondingly have varied from 20% to 50% in allogeneic HSCT recipients (25). More recently, IFIs have been managed more effectively as demonstrated in the SEIFEM B-2004 study; e.g., a 77% aspergillosis mortality rate for allogeneic and 14% for autologous HSCT (8). *Candida* IFI rates according to HSCT type were 57% and 44%, respectively (8). Another large surveillance trial of 4621 HSCT recipients revealed three months mortalities for aspergillosis of 54% for autologous HSCT to 85% in unrelated-donor HSCT (4). Nonmyeloablative conditioning regimens exhibited outcomes similar to ablative conditioning (6).

Time Intervals for Risk of IFIs

Because of the difficulty in diagnosis antemortem, many fungal infections unfortunately remain unidentified until autopsy (26). A recent autopsy survey involving 103 allogeneic HSCT recipients reported that 38 subjects died a median (range) of 125 (14–416) days after HSCT; 10 subjects had IFI at autopsy (18). The mean times after HSCT and death for aspergillosis, candidiasis, and zygomycosis were 4.4, 5.4, and 4.7 months, respectively. Only four patients were diagnosed antemortem as having IFIs (three aspergillosis and one candidiasis). This survey revealed that invasive aspergillosis had the highest prevalence for IFI (60%), followed by invasive candidiasis (20%), and zygomycosis (20%).

A bimodal distribution for time to IFI occurrence has been noted. *Candida* IFIs usually peak during neutropenia (prior to engraftment) while invasive aspergillosis IFIs begin and continue the second and third months after HSCT during the treatment of GVHD. Over the past decade, the "late period" has become increasingly significant as most mold infections occur in patients receiving GVHD therapy by six months or later (median 96 days after transplant) (7,23). A review of more than 5500 HSCT recipients at the Fred Hutchinson Cancer Research Center found that the annual incidence of invasive aspergillosis tripled between 1990 and 1993, reaching a plateau at 12% in the late 1990s (5). Another but relatively small study revealed that no autologous HSCT recipient developed invasive aspergillosis compared to a 15% rate in allogeneic recipients (27).

Use of a T-cell depleted allogeneic graft is associated with delayed immune reconstitution and increased incidence of viral and fungal infections. A recent study by Mihiu et al. (16) in patients who underwent T-cell depleted HSCT showed that late onset aspergillosis IFI (occurring >40 days post-HSCT) developed a median (range) of 164 (68–667) days after HSCT. The incidence of late onset aspergillosis IFI among unmodified, T-cell depleted, or reduced-intensity conditioning HSCT was 2.2%, 4%, and 6.8%, respectively.

IFIs caused by *Candida* and other yeasts are associated either with the transmigration of *Candida* organisms across mucosal surfaces damaged by cytotoxic chemotherapy or GVHD, or via direct introduction through indwelling venous catheters. By day 100 after HSCT, most recipients have reconstituted innate immunity that is essential for controlling *Candida* infections. In addition, at this time point many patients will have undergone removal of the indwelling vascular catheter. Prophylactic strategies to prevent invasive candidiasis should focus primarily on chemoprophylaxis during the period of highest risk.

On the other hand, invasive mold infections may result from environmental exposure to *Aspergillus*, *Fusarium*, and *Zygomycetes*. Effective preventive measures include high-efficiency particulate air (HEPA) filters and laminar airflow systems. Additionally, hospital water distribution systems have been implicated as reservoirs of opportunistic molds (28,29). Chemoprophylaxis and environmental approaches should be employed to prevent invasive mold infections.

MANAGEMENT OF IFIs

The strategies used when approaching a patient who is at-risk for developing an IFI or already has possible, probable, or definite evidence are detailed in Table 2. The treatment approaches, in addition to the obvious use of an antifungal antibiotic agent, may include surgery

Table 2 Treatment Strategies for Invasive Fungal Infections (IFIs)

Treatment strategy	Definitions	Goal
Prophylaxis	Administration of antifungal agent in febrile neutropenic patients or transplant recipients for prevention of IFI during the time period of high risk	Prevention
Empiric treatment	Administration of antifungal agent in patients with persistent fever despite broad-spectrum antibiotic therapy but no signs and symptoms of infection	Treatment
Preemptive therapy	Administration of antifungal agent in patients strongly suspected of having IFI due to fungal colonization, pulmonary/sinus symptoms, sepsis or radiologic findings compatible with IFIs, but no tissue documentation of IFI	Treatment
Definitive treatment of established IFI	Administration of appropriate antifungal therapy when an infectious organism has been documented and identified	Treatment

Table 3 Class and Properties of Current Antifungal Agents

Antifungal agent and class	Spectrum	Formulations	Major toxicity/side effects
Polyenes			
Amphotericin B deoxylate	<i>Histoplasma</i> , <i>Coccidioides</i> , <i>Candida</i> , <i>Aspergillus</i> , <i>Blastomyces</i> , <i>Rhodotorula</i> , <i>Cryptococcus</i> , <i>Sporothrix schenckii</i> , <i>Mucor</i> species	IV	Nephrotoxicity, hypotension, tachypnea, nausea, diarrhea, headache, hepatotoxicity
Amphotericin B lipid complex	<i>Aspergillus</i> , <i>Zygomycetes</i> , <i>Fusarium</i> , <i>Cryptococcus</i> , and many hard-to-treat <i>Candida</i> species	IV	Nephrotoxicity (less than amphotericin B deoxylate)
Liposomal amphotericin B	<i>Aspergillus</i> , <i>Candida</i> , <i>Cryptococcus</i> species	IV	Nephrotoxicity (less than amphotericin B deoxylate)
Amphotericin B colloidal dispersion	<i>Aspergillus</i> , <i>Candida</i> , <i>Mucor</i> , <i>Cryptococcus</i> species	IV	Nephrotoxicity (less than amphotericin B deoxylate)
Azoles			
Fluconazole	<i>Candida</i> , <i>Cryptococcus</i> species	Oral suspension, tablet, IV	Hepatotoxicity, leukopenia, hypokalemia, dose reduction required in renal impairment
Itraconazole	<i>Blastomyces</i> , <i>Histoplasma</i> , <i>Aspergillus</i> species	Capsule	Gastrointestinal (nausea, vomiting, diarrhea), rash
Second generation azoles			
Voriconazole	<i>Aspergillus</i> , <i>Candida</i> species, <i>Scedosporium apiospermum</i> , <i>Fusarium</i> species	Oral suspension, tablet, IV	Abnormal vision, hepatotoxicity, fever, rash, drug-drug interactions
Posaconazole	<i>Aspergillus</i> , <i>Candida</i> species, rare mold infections	Oral suspension	Fever, headache, anemia, neutropenia, thrombocytopenia, diarrhea, nausea, vomiting, hypokalemia
Echinocandins			
Caspofungin	<i>Candida</i> , <i>Aspergillus</i> species	IV	Diarrhea, nausea, pyrexia, hepatotoxicity, rash
Micafungin	<i>Candida</i> species	IV	Nausea, vomiting, hypokalemia, hypomagnesemia, hypotension
Anidulafungin	<i>Candida</i> species	IV	Rare (gastrointestinal)

(e.g., drainage, debridement) and modulation of the immune status (30). Several of these tools will be discussed later in this chapter. Current antifungal agents are detailed in Table 3. Selection of the appropriate drug treatment may be based on whether the strategy is to prevent or to treat an IFI (24).

Primary Chemoprophylaxis of IFIs

Two major issues to be considered include the assessment of risk factors for IFIs as well as recognizing changes in fungal epidemiology. Mold infection during preengraftment rarely has been reported; most infections occur in the late posttransplant period, usually in the context of grade 3–4 GVHD (7,9,15,27,31). In patients who have undergone nonmyeloablative or reduced-intensity conditioning, the duration of neutropenia generally is short and many recipients may not require antifungal prophylaxis. Patients with acute leukemia or those undergoing myeloablative conditioning regimens, however, are at considerable risk for developing prolonged neutropenia. In this patient population, because of the lack of specific symptoms and the difficulty in establishing an early diagnosis, empiric antifungal therapy has been the mainstay of the management of persistent neutropenic fever (lasting more than three to five days) despite appropriate antibacterial agents (32). One meta-analysis of randomized, controlled trials in severely neutropenic recipients found that prophylactic antifungal therapy was associated with a reduction in overall mortality and many centers follow this strategy (33).

Recommendations for antifungal prophylaxis in hematologic malignancy patients or HSCT recipients are detailed in Table 4. For many years, the older antifungal agent amphotericin B deoxycholate was the “gold standard” for treating mold and fluconazole-resistant yeast infections in immunocompromised hosts. Use of this product, however, was associated with significant toxicity including infusion-related reactions (hypotension and bronchospasm) as well as marrow suppression, nephrotoxicity, and hypokalemia (34,35). Lipid-based formulations, such as liposomal amphotericin B, amphotericin B colloid dispersion, and amphotericin B lipid complex, were developed to reduce such toxicities (36). Their efficacy was similar to “conventional” amphotericin B deoxycholate when given as empiric therapy in febrile neutropenic patients (35,37,38) and as prophylaxis in patients undergoing HSCT (39).

Available antifungal agents and recommended prophylaxis doses are detailed in Table 5. Recent antifungal randomized studies in those patients are shown in Table 6 (40–52).

Prophylaxis in the Preengraftment Period and Neutropenia for Patients Without a Previous History of IFIs

The 2000 CDC, Infectious Diseases Society of America (IDSA), and the American Society for Blood and Marrow Transplantation (ASBMT) issued guidelines recommending fluconazole therapy based on multiple randomized, placebo-controlled studies. The data demonstrate the

Table 4 Recommendations for Antifungal Prophylaxis in High-Risk Hematologic Malignancy Patients or HSCT Recipients

Clinical condition	Recommendations
AML: less-intensive induction or consolidation chemotherapy	Fluconazole 200 mg/day, from admission to resolution of neutropenia
AML: intensive induction or consolidation or high-dose chemotherapy	Posaconazole 200 mg TID or itraconazole 200 mg BID, from first day of chemotherapy until resolution of neutropenia
Autologous HSCT	Fluconazole 200–400 mg/day, from admission to resolution of neutropenia
Allogeneic HSCT: matched-related donor	Fluconazole 400 mg/day, from admission to day 75 after HSCT
Allogeneic HSCT: mismatched or unrelated donor or umbilical cord blood stem cell graft	Posaconazole 200 mg TID or itraconazole 200 mg BID or fluconazole 400 mg/day, from the last day of conditioning to resolution of neutropenia
Allogeneic HSCT: acute GVHD grade ≥ 2 or chronic extensive GVHD	Posaconazole 200 mg TID until resolution of GVHD or day 112 after HSCT with monitoring of cyclosporine or tacrolimus levels

Table 5 Available Antifungal Agents and Recommended Prophylaxis Doses

Antifungal agent class and generic name	Trade name	Recommended prophylaxis dose	Recommended treatment dose
Polyenes			
Amphotericin B deoxylate	Fungizone [®]	0.3 mg/kg/day IV	0.3–1 mg/kg IV
Amphotericin B lipid complex	Abelcet [®]	1 mg/kg/day IV	5 mg/kg IV
Liposomal amphotericin B	AmBisome [®]	1 mg/kg/day IV	3 mg/kg IV
Amphotericin B colloidal dispersion	Amphotec [™]	1 mg/kg/day IV	3–4 mg/kg IV
Azoles			
Fluconazole	Diflucan [®]	100–400 mg/day IV/po	400 mg/day IV
Itraconazole	Sporanox [®]	200 mg/day IV	200 mg/day IV q12 h
Second generation azoles			
Voriconazole	Vfend [®]	4 mg/kg IV q12 h	6 mg/kg IV q12 h for 1st 24 hr, then 4 mg/kg IV q12 h
Posaconazole	Noxafil [®]	200 mg po q8 h	400 mg po q12 h
Ravuconazole (Phase II study)	NA	NA	NA
Echinocandins			
Caspofungin	Cancidas [®]	50 mg/day IV	70 mg/day IV loading dose, then 50 mg/day IV
Micafungin	Mycamine [®]	50 mg/day IV	100–150 mg/day
Anidulafungin	Eraxis [®]	NA	100–200 mg/day IV, then 50–100 mg/day IV

Abbreviations: NA, not available.

benefit of fluconazole therapy as prophylaxis against *Candida* infections in HSCT recipients during the preengraftment period (53) as well as for patients with febrile neutropenia (54), for the prevention of IFIs and as prophylaxis to decrease colonization, superficial fungal infections, and IFIs (55). Other studies support fluconazole as protective against IFI by reducing overall mortality when given 75 days post bone marrow transplant (56) when compared with discontinuing prophylaxis at time of engraftment (57). The beneficial effect on mortality persisted in allogeneic HSCT recipients after eight years of follow-up (58).

Voriconazole, on the other hand, did not show a survival advantage over fluconazole (41). Moreover, more recent additions to the IFI armamentarium possess many significant drug–drug interactions, especially with cyclophosphamide-based conditioning regimens and calcineurin inhibitors (59). Drug–drug interactions of new generation antifungal agents are detailed in Table 7.

Although voriconazole is one of the primary drugs used for the treatment of invasive aspergillosis, the data regarding its utility as a prophylactic agent are limited. One small, randomized study evaluated the efficacy of voriconazole as prophylaxis in the prevention of lung infiltrates due to mold infections during induction chemotherapy for acute myelogenous leukemia patients (60). The incidence of lung infiltrates by day 21 after therapy was borderline significantly lower in the voriconazole arm compared to placebo ($P = 0.06$). A retrospective study in 315 allogeneic HSCT recipients showed that voriconazole-treated patients had a significantly lower incidence of invasive aspergillosis than those who did not receive the drug ($P < 0.0005$) (61). Voriconazole prophylaxis showed efficacy comparable to fluconazole prophylaxis in a multicenter trial conducted in allogeneic HSCT recipients (41); the incidence of IFI at 6 or 12 months after HSCT was similar ($P = 0.11$ and $P = 0.50$). Breakthrough *Zygomycetes* IFIs during voriconazole treatment, however, have been reported by several groups and represent a major concern for using this agent as prophylaxis (62–65).

Table 6 Recent Randomized Studies Involving Antifungal Prophylaxis in HSCT Recipients and Neutropenic Patients with Hematologic Malignancies

References	No. and type of patients	Comparison of antifungal agents	Efficacy	Statistical significance
(47)	557 Neutropenic	ITRA 2.5 mg/kg BID po vs. Ampho B 500 mg qid po	ITRA>Ampho B	$P = 0.004$
(48)	317 Neutropenic	FLU 400 mg/day IV vs. Ampho B 0.5 mg/kg/day IV	NS	68% vs. 67%, $P > 0.05$
(49)	355 Allo and auto HSCT	FLU 400 mg/day po or IV vs. Ampho B 0.2 mg/kg/day IV	NS	$P > 0.05$
(50)	277 Neutropenic	ITRA 100 mg bid po vs. Ampho B 500 mg tds + nystatin 2 MU qd	NS	65% vs. 53%, $P > 0.05$
(46)	837 Neutropenic (155 allo and 261 auto HSCT)	VOR 6 mg/kg IV q12 h, then 3 mg/kg IV q12 h vs. Lipo Ampho B 3 mg/kg/day IV	VORI>Lipo	$P = 0.02$
(44)	140 Allo HSCT	ITRA 200 mg IV q12 h × 2d, then 200 mg IV q24 h or 200 mg po q12h vs. FLU 400 mg/day IV/po	ITRA>FLU	91% vs. 75%, $P = 0.01$
(45)	304 Allo HSCT	ITRA 2.5 mg/kg tid po or 200 mg/day IV vs. FLU 400 mg/day IV/po	ITRA>FLU	93% vs. 85%, $P = 0.03$
(51)	494 Auto HSCT	ITRA 2.5 mg/kg bid po vs. FLU 400 mg/day po	NS	99% vs. 98.8, $P > 0.05$
(40)	195 Neutropenic (107 auto and 37 allo HSCT)	ITRA 200 mg bid po or 200 mg/day IV vs. FLU 400 mg/day po or 400 mg/day IV	NS	89% vs. 88%, $P > 0.05$
(41)	600 Allo HSCT	VOR 200 mg bid po vs. FLU 400 mg/day po	NS	
(52)	600 Allo HSCT	POS 200 mg tid po vs. FLU 400 mg/day po	POS>FLU	1.0% vs. 5.9% IA, $P = 0.001$
(42)	660 Neutropenic	POS 200 mg tid po vs. either FLU 400 mg/day po or ITRA 200 mg bid po	POS>FLU or ITRA	1% vs. 7% IA, $P < 0.001$
(43)	882 (405 Auto and allo HSCT)	MICA 50 mg/day IV vs. FLU 400 mg/day IV	MICA>FLU	80% vs. 73.5%, $P = 0.03$

Abbreviations: allo, allogeneic; auto, autologous; HSCT, hematopoietic stem cell transplantation; Ampho B, amphotericin B deoxycholate; ITRA, itraconazole; FLU, fluconazole; VOR, voriconazole; POS, posaconazole; MICA, micafungin; Lipo, lipid amphotericin B; IA, invasive aspergillosis; NR, not reported; NS, not significant.

Posaconazole, an orally administered agent, has shown efficacy superior to standard azole agents as prophylaxis in patients at high risk for IFIs (42,52). In a randomized trial conducted in 600 allogeneic HSCT recipients receiving treatment for GVHD, posaconazole therapy was associated with fewer cases of invasive aspergillosis (2% vs. 7%, $P = 0.006$) and a lower incidence of IFI-related mortality (1% vs. 4%, $P = 0.046$) when compared to fluconazole (52). Furthermore, posaconazole treatment was associated with a significantly lower occurrence of breakthrough IFIs (2% vs. 8%, $P = 0.004$). In a parallel randomized study conducted during the same time period, 602 AML and myelodysplastic syndrome (MDS) neutropenic patients had a lower incidence of IFIs when given prophylaxis with posaconazole compared to either fluconazole or itraconazole (2% vs. 8%, $P < 0.001$ and 1% vs. 7%, $P < 0.001$). This effect extended throughout the entire study period (5% vs. 11%, $P = 0.003$) and was associated with lower overall ($P = 0.048$) and IFI-related ($P = 0.01$) incidences of mortality (42).

Regarding other agents, one multicenter, randomized trial showed micafungin (50 mg/day) superiority over fluconazole (400 mg/day) (80% vs. 73.5%) as prophylaxis during the preengraftment neutropenic period in patients undergoing HSCT. "Treatment success" was significantly higher (80% vs. 74%, $P = 0.03$) in the micafungin arm, i.e., lower likelihood of

Table 7 Drug–Drug Interactions of New Generation Antifungal Agents and Precaution Measures

Antifungal agent	Recommendations		
	Requires monitoring of antifungal drug blood concentrations	Requires switching to alternative drug when used with	Contraindicated
Fluconazole	Cyclosporine, tacrolimus, phenytoin, warfarin, rifampin	Simvastatin, lovastatin	
Itraconazole	Cyclosporine, tacrolimus, digoxin, warfarin, carbamazepine, rifabutin, ritonavir, amprenavir, indinavir	Simvastatin, lovastatin, alprazolam, diazepam, triazolam, midazolam	Cyclophosphamide, vinca alkaloids, quinidine, ergot alkaloids, cisapride
Voriconazole	Cyclosporine, omeprazole, tacrolimus, warfarin, sulfonyleureas, simvastatin, lovastatin, vinca alkaloids, calcium channel blockers, benzodiazepines, phenytoin		Cyclophosphamide, cisapride, ergot alkaloids, quinidine, sirolimus, terfenadine, carbamazepine, barbiturates, rifampin, rifabutin
Caspofungin	Rifampin, carbamazepine, dexamethasone, phenitoin, tacrolimus, cyclosporine		
Micafungin	Sirolimus, nifedipine		

being switched to empiric antifungal therapy and fewer episodes of breakthrough aspergillosis (1.6% vs. 2.4%) (43). Mattiuzzi et al. (66) compared caspofungin to itraconazole in 192 hematologic malignancy patients undergoing induction chemotherapy. Caspofungin prophylaxis was comparable in efficacy to itraconazole without significant differences in IFIs, adverse events or mortality rates. Finally, anidulafungin, another echinocandin that has not yet been evaluated in the empiric setting, was found effective and safe in the prophylaxis of neutropenic children at high risk for IFIs (67).

Antifungal Prophylaxis in the Preengraftment Period for Patients with a Previous History of IFI

Mold-active agents recently have been recommended as prophylaxis in this patient subset (68–70). While there are no randomized studies addressing antifungal prophylaxis therapy in subjects with a previous history of IFI, a recent case series by Hill et al. addressed outcome of 16 hematologic malignancy patients who underwent allogeneic HSCT despite a prior IFI (70). Voriconazole or caspofungin prophylaxis was administered to all patients; four subjects experienced recurrent fungal infection resulting in one death. The 45% two-year survival, however, was similar to that for other high-risk hematologic malignancy patients undergoing HSCT.

Antifungal Prophylaxis in the Postengraftment Period

Therapy using low-dose amphotericin B deoxycholate administered prophylactically in the posttransplant setting has been a disappointment due to infusion-related side effects and drug-related toxicity. The lipid formulations of amphotericin B are less toxic but are more expensive and still may be associated with renal toxicity in a substantial percentage of patients (60,71,72). In two older studies, fluconazole therapy has been an effective antifungal prophylaxis after either autologous or allogeneic HSCT (56,57). In addition to reducing the rate of IFIs, overall survival improvements have been noted by three months after HSCT when prophylaxis was continued through day 75 (56). More recently, Marr et al. compared fluconazole versus placebo prophylaxis and had eight years of follow-up; fluconazole treatment provided persistent protection against *Candida*-related death (58). The obvious downside of prophylactic fluconazole

use is breakthrough infections by fluconazole-resistant species such as *Aspergillus*, *C. glabrata*, and *C. krusei* (73,74).

Studies comparing use of itraconazole and fluconazole through 180 days after HSCT found no differences in overall survival; however, the rate of IFIs was lower in the itraconazole group than in the fluconazole cohort (5–9% vs. 12–25%, $P < 0.05$). In contrast, patients receiving itraconazole experienced significantly more hepatotoxicity and nephrotoxicity that necessitated drug discontinuation (36% vs. 16%, $P < 0.01$) (44,45). Hepatic injury appeared more common in patients who received cyclophosphamide as part of conditioning regimen (59). Similarly, concomitant itraconazole plus vincristine therapy led to increased neurotoxicity (75) (Table 7). Importantly, the prospective, randomized, phase III study recently completed by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) in myeloablative allogeneic transplant recipients that compares voriconazole versus fluconazole has not yet been fully analyzed.

Ullmann et al. (52) prospectively compared posaconazole versus fluconazole for infection prophylaxis at onset of GVHD. This international, randomized, double-blind study involved 600 hematologic disease patients. At the end of the fixed 112-day treatment period, posaconazole was as effective as fluconazole in preventing all IFIs (5.3% vs. 9%, $P = 0.07$) but was superior to fluconazole in preventing proven or probable invasive aspergillosis (2.3% vs. 7%, $P = 0.006$). There were fewer breakthrough IFIs (2.4% vs. 7.6%, $P = 0.004$) in the posaconazole-treated cohort, particularly invasive aspergillosis (1% vs. 5.9%, $P = 0.001$). Overall mortality was similar but deaths attributed due to IFIs were reduced in the posaconazole group (1% vs. 4%, $P = 0.046$). The incidence of treatment-related as well as serious adverse events was similar in the two groups (36% vs. 38% and 13% vs. 10%, respectively). For patients intolerant of triazoles, the echinocandins (caspofungin, micafungin, and anidulafungin) represent potential effective alternatives for prophylaxis use in the postengraftment period.

Antifungal Prophylaxis for Neutropenia in High-Risk Hematologic Malignancy

Three different groups studied prophylaxis using lipid amphotericin B. Penack et al. (76) compared liposomal amphotericin B (L-AMB) 50 mg on alternate days versus no prophylaxis. In 219 neutropenic episodes involving 132 hematologic malignancy (predominantly AML) patients, prophylaxis reduced the incidence of IFI including invasive *Aspergillus* infections. Mattiuzzi et al. (77) reported a retrospective comparison of amphotericin B lipid complex (ABLC) 2.5 mg/kg thrice weekly in newly diagnosed AML or high-risk myelodysplasia patients undergoing induction chemotherapy versus a historic control group that had received prophylactic L-AMB 3 mg/kg thrice weekly. The IFI rates did not differ between the two regimens. A third study examined the safety of high-dose L-AMB prophylaxis (7.5–10 mg/kg weekly) in neutropenic acute leukemia patients or allogeneic HSCT recipients (78). High dose L-AMB therapy was not well tolerated by the allogeneic HSCT patients due to infusion-related toxicity.

Posaconazole was compared in randomized fashion to fluconazole or itraconazole in a multicenter study in AML and MDS patients with anticipated prolonged neutropenia (42). Prophylaxis was given with each cycle of chemotherapy until recovery from neutropenia or IFI, for up to 12 weeks. Proven or probable IFIs were lower in the posaconazole-treated patients (2% vs. 8%, $P < 0.001$). In addition, fewer invasive aspergillosis infections were observed in the posaconazole group (1% vs. 7%, $P < 0.001$) and survival was significantly superior as well ($P = 0.04$). Serious adverse events, however (predominantly affecting the gastrointestinal tract), were higher in the posaconazole group (6% vs. 2%, $P = 0.01$). Despite a lack of evidence, IV voriconazole or caspofungin often are used as alternatives in this setting.

Duration of Antifungal Prophylaxis

Most studies have demonstrated that for AML or for other hematologic malignancy patients undergoing chemotherapy or autologous transplant, antifungal prophylaxis should continue until the neutrophil count has recovered ($>500/\mu\text{L}$). For allogeneic HSCT recipients, patients receiving daily corticosteroid doses equivalent to prednisolone >1 mg/kg for 0 to 13 days and 0.25 to 1 mg/kg for 14 to 27 days are at increased risk of invasive aspergillosis (7). Therefore, allogeneic HSCT recipients should continue fluconazole prophylaxis until day 75 after HSCT or the commencement of posaconazole for prophylaxis during GVHD treatment. The duration of individual patient therapy should reflect the grade of GVHD and the extent of

immunosuppressive therapy required. In patients receiving alemtuzumab, the duration of prophylaxis is not well defined; IFI risk may persist for up to 12 months after its cessation (79).

Secondary Prophylaxis After IFI

If an IFI was diagnosed during a previous course of chemotherapy, the usual routine is to administer “secondary prophylaxis” to patients presenting for further cycles of chemotherapy or for HSCT. Unfortunately, there are no large published studies that have been performed in this setting; i.e., supportive evidence is limited only to case series and no agent can be recommended over another.

Risk of recurrent IFI appears related to the number of risk factors in effect (80). For patients with 0–1 risk factors, the rate of another IFI is 6%, increases to 27% for 2–3 risk factors and is extremely high at 72% if >3 risk factors are present. Secondary prophylaxis has been reported to be well tolerated and effective in two series, e.g., patients with AML (81) and in allogeneic HSCT recipients (82). Reports of low reactivation IFI rates have been published for therapy with conventional amphotericin B (83), lipid formulations of amphotericin B (68), itraconazole (84), voriconazole (85), and caspofungin (82) when compared with untreated controls. Posaconazole also was used successfully as initial treatment for zygomycosis in an AML patient and then again as secondary prophylaxis during allogeneic HSCT (86). Therapy clearly is indicated in this setting as shown in a review of 197 cases of proven/probable aspergillosis in patients undergoing treatment for hematologic malignancy or autologous HSCT. A 62% progression rate was reported in patients who did not receive antifungal therapy compared to 16% in treated patients (84). In order to minimize the chance of recurrence of IFI, clinicians should consider timing of the transplant, duration of prior antifungal therapy, hematopoietic stem cell source, feasibility of surgical resection, and possibility for use of donor granulocyte transfusions during the period of profound neutropenia.

Treatment of IFIs

Current evidence-based data on prophylactic and empiric use of antifungal agents are detailed in Table 8. Many agents possess significant clinical antifungal activity for the treatment of an IFI. Voriconazole was compared to liposomal amphotericin B as empiric antifungal therapy in febrile, neutropenic cancer patients, half of whom underwent HSCT (46). No significant differences were observed between treatments in overall response rates, but voriconazole was

Table 8 Current Data on Prophylactic and Empiric Antifungal Therapy

Antifungal agent	Prophylactic therapy	Empiric therapy
Amphotericin B deoxycholate	Formally standard of care but no longer due to toxic effects	Marked toxicity with long-term use
Lipid-based amphotericin	Preferable over amphotericin B deoxycholate due to superior tolerability	Toxicity and high cost are important issues when used long term
Fluconazole	Disadvantage is lack of activity against invasive molds; previous prophylaxis with azole derivatives may limit activity against <i>Candida</i>	Strong evidence-based activity during pre-engraftment. Resistant species and pathogens limit use in postengraftment period
Itraconazole	Effective antifungal, but previous prophylaxis with azole derivatives may limit activity against <i>Candida</i>	Effective antifungal, but tolerability may be limiting factor
Voriconazole	Moderate evidence-based efficacy	Moderate evidence-based efficacy
Posaconazole	Evidence-based efficacy	Good evidence-based efficacy
Ravuconazole	Insufficient data	Insufficient data
Caspofungin	Good evidence-based efficacy	Some evidence for efficacy in patients with hematologic malignancy
Micafungin	Insufficient data	Good evidence in HSCT recipients during preengraftment
Anidulafungin	Some evidence-based efficacy	Insufficient data

associated with significantly fewer breakthrough IFIs than liposomal amphotericin B ($P = 0.003$). Infusion-related adverse events, azotemia and hypokalemia, occurred more frequently with liposomal amphotericin B while visual hallucinations were more common with voriconazole.

Posaconazole was found to be an effective antifungal therapy in the empiric treatment of IFIs in 66 febrile, neutropenic patients (that included HSCT recipients) (87). In a small nonrandomized study in febrile, neutropenic leukemia patients, 78% of micafungin-treated patients attained "success"; i.e., this therapy was not discontinued nor did any IFI-associated deaths took place (88). Walsh et al. (89) published a prospective, randomized trial in febrile, neutropenic cancer patients comparing caspofungin to liposomal amphotericin B as empiric therapy of IFIs. Rates of overall response, breakthrough IFI, and resolution of fever during neutropenia were similar between the treatment groups. In contrast, successful resolution of baseline infection and survival through seven days of follow-up significantly favored the caspofungin group, primarily as a result of treatment discontinuation from increased toxicity due to liposomal amphotericin B that included infusion-related events and nephrotoxicity.

High-Dose or Combination Antifungal Therapy

Invasive mold infections are associated with high mortality rates and some investigators have advocated the routine use of antifungal agents in combination, in part based upon success in treating patients with cryptococcal meningitis and invasive candidiasis. Combination therapy, however, may be more toxic, certainly is more costly from a financial perspective and is based on very few prospective study data. Some investigators have suggested that this combination therapy strategy be reserved for salvage therapy of immunosuppressed, seriously ill patients rather than for use during prophylactic, preemptive, or empiric antifungal therapy. Other issues in the combination agent debate include: improved response rates and a survival advantage reported in recent monotherapy studies; response rates in most published combination therapy studies do not suggest large gains over monotherapy; the lack of sustained survival advantage to combination therapy studies; and finally, the consideration of host defenses in treatment-responsive patients (90,91).

Recently, single-agent posaconazole showed greater safety and efficacy than high-dose lipid amphotericin B alone or in combination with caspofungin as salvage therapy for invasive aspergillosis in hematologic malignancy patients (92). The overall response rate was 40% for posaconazole, 8% for high-dose lipid amphotericin B ($P \leq 0.001$), and 11% for combination therapy ($P < 0.002$). Aspergillosis contributed to death in 40% of the posaconazole group, 65% of the lipid amphotericin B cohort, and 68% of the combination group ($P \leq 0.008$). By multivariate analysis, posaconazole therapy independently improved response ($P < 0.001$). High-dose amphotericin B alone or in combination was associated with a significantly higher rate of nephrotoxicity ($P \leq 0.02$) and hepatotoxicity ($P < 0.03$). Use of single-agents in higher dose also may be a means to enhance response and avoid antifungal drug combinations. Safdar et al. (93) compared high-dose to standard-dose caspofungin in hematologic malignancy patients undergoing HSCT. Response rates (CR or PR) were higher but not significant (44% vs. 29%, $P = 0.1$) yet overall outcome was statistically superior (OR 3.066, 95% CI = 1.092–8.61; $P = 0.033$). The authors speculated, however, that the better results may have reflected the use of granulocyte-macrophage colony-stimulating factor (41% vs. 14% in standard group; $P = 0.04$), interferon gamma (26% vs. 5% in standard group; $P = 0.003$), or the combination employed for immune enhancement.

Recent reports demonstrate the success of combination antifungal therapy regimens that utilize new generation antifungals (94,95). Based on available data to date, however, combination therapy is not warranted at the initial diagnosis of invasive aspergillosis. Although randomized, controlled trials with rigorous study design are needed to resolve this issue, the number of agents to consider, the permutations and combinations possible, as well as the significant expense likely preclude solving this dilemma in this fashion (90). Perhaps use of information from observational databases may help resolve this clinical problem.

Treatment of IFIs by Resistant Fungi in Patients on Antifungal Prophylaxis

Prolonged prophylaxis with fluconazole or voriconazole has been reported to result in emergence of resistant fungal species, such as *Zygomycetes* (96). Echinocandin (caspofungin or

micafungin) treated patients also appear at increased risk for secondary fungal infections caused by *Zygomycetes* (43,97). On the other hand, breakthrough disseminated *Aspergillus ustus* infection, an uncommon fungal organism affecting HSCT recipients, as well as breakthrough *Candida glabrata* fungemia, were reported in allogeneic HSCT recipients receiving voriconazole or caspofungin prophylaxis (98,99). Kontoyiannis et al. (96), in a case-control study analyzing risk factors for zygomycosis, showed that voriconazole use was an independent variable. This study also revealed an increasing incidence of zygomycosis, while there was a trend for fewer episodes of invasive aspergillosis during the voriconazole treatment period.

Breakthrough fungal infections after allogeneic HSCT in patients receiving antifungal prophylaxis have been increasing in the past few years. Trifilio et al. recently noted that higher numbers of patients were developing secondary fungal infections rarely seen in transplant recipients (e.g., *C. glabrata*, *C. kruzei*, *Cunninghamella*, *Rhizopus*, *Mucor*) (100). The duration of voriconazole therapy was long and ranged from 6 to 956 (median: 133) days. Importantly, fungal infections occurred in patients with lower plasma voriconazole ($\leq 2 \mu\text{g/mL}$) concentrations. This group investigated the role of monitoring plasma voriconazole concentrations in HSCT recipients and administered voriconazole at doses 200 to 800 mg per day (101). Median (range) plasma voriconazole concentrations remained low at 1.2 (0–12.5 $\mu\text{g/mL}$) and they found only a weak correlation between dose and blood concentration ($r = 0.14$, $P = 0.045$).

Voriconazole (102) and liposomal amphotericin B (103) are listed as evidence-based options for primary antifungal therapy in patients with invasive aspergillosis and in patients not responding to or who are intolerant of first-line antifungals. Caspofungin (104) and posaconazole (105) also have been shown to achieve 40% to 50% CR or PR as second-line antifungal therapy. Zygomycosis IFI, on the other hand, remains a challenge due to the high morbidity and mortality in HSCT population. Accurate diagnosis and appropriate medical treatment with posaconazole (with or without surgery) can enhance survival (106).

Immune Enhancement Strategies

A variety of clinical and investigatory immune enhancement strategies of the host immune system are being developed. Restoration of host immune function is critical for the effective treatment of IFIs in this group of patients. Many such treatment options are detailed in Table 9 (107–114).

CONCLUSIONS

While the response to treatment of established IFIs has improved with the use of new antifungal agents, further improvements likely require better prophylaxis strategies. An in-depth risk-benefit assessment should be undertaken that takes into account many factors including patient-, disease-, and treatment-related risk factors, fungal epidemiology of the institution, available diagnostic tools, risk of breakthrough IFI, drug toxicity, and drug–drug interactions. During the

Table 9 Immune Enhancement Strategies in Hematologic Malignancy Patients and HSCT Recipients with IFIs

Strategy	References	Study groups	Response rate (%)	Survival rate (%)
Clinical		Hematologic malignancies		
Donor granulocyte transfusions	(107)	HSCT recipients	35	NS
	(108)	HSCT recipients	NR	NS
	(109)	HSCT recipients	57	28
Recombinant				
IFN- γ -1	(110)	HSCT recipients	45	55
rh-M-CSF	(111)	HSCT recipients	NR	27
Adoptive T cells	(112)	HSCT recipients	90	NR
Preclinical Model				
Pentraxin	(113)	Mice		
DC vaccine	(114)	Mice		

Abbreviations: IFN, interferon; NS, not significant; NR, not reported; DC, dendritic cell; rh-M-CSF, recombinant macrophage-colony stimulating factor.

preengraftment period of neutropenia, echinocandins, voriconazole, and lipid amphotericin B compounds are all acceptable choice for prophylaxis. Fluconazole has been recommended for all HSCT recipients until posttransplant day 75 and micafungin is an acceptable prophylaxis alternative prior to engraftment. Combinations with more mold-active prophylaxis can be reserved for patients at highest risk. Voriconazole currently is the optimal choice for high-risk patients in the postengraftment period. In patients unable to tolerate voriconazole, echinocandins or lipid amphotericin B compounds may be effective alternatives. Posaconazole has potent activity against *Zygomycetes*, which has gradually increased in incidence after prolonged use of potent immunosuppressive regimens and antifungal prophylaxis. As the mold-active agents also may suppress early markers of fungal infection (e.g., galactomannan antigen), more aggressive diagnostic methods are needed. Finally, the approach to prophylactic antifungal therapy should be balanced against the financial costs.

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Advances in medical therapy have allowed for increased survival of the most premature infants. These critically ill infants are immunocompromised and are often supported with invasive devices placing them at risk for invasive candidiasis. This chapter describes the therapeutic options for prophylaxis, empiric therapy, and treatment of neonatal candidiasis.

EPIDEMIOLOGY

Candida species are the third most common pathogen in nosocomial blood stream infections among premature infants (1–3). In a large multicenter cohort, the cumulative incidence of candidiasis in infants ≥ 2500 g birth weight admitted to the Neonatal Intensive Care Unit (NICU) was 0.3% (3/1139), 3.1% among VLBW (very low birth weight, <1500 g birth weight) infants, and 7.0% among ELBW (extremely low birth weight, <1000 g birth weight) infants (4). Mortality from all *Candida* species in premature infants has been consistently reported at 20% (2,3,5–7). In addition to a high mortality rate, candidiasis in infants is associated with significant morbidities in survivors including poor neurodevelopmental outcome, periventricular leukomalacia, chronic lung disease, and severe retinopathy of prematurity (8–11).

Candida albicans is the most commonly isolated species in infants (12–14). However, the epidemiology of candidiasis is changing, and the percentage of cases caused by *C. albicans* has recently declined from 80% to as low as 29% in some centers (12,15). In one center, 5% (1/22) of cases of neonatal candidiasis from 1981 to 1990 were due to *C. parapsilosis* (16). This percentage increased to 60% (53/89) from 1991 to 1995. *C. albicans* continues to be associated with increased rates of end organ damage and a higher attributable mortality relative to the other *Candida* species (6,17). *C. glabrata*, *C. krusei*, *C. lusitanae*, and *C. tropicalis* are less commonly isolated in infants (15,18).

RISK FACTORS

Specific risk factors for invasive disease tend to fall in one of two categories—those that increase colonization of mucosal surfaces or those that disrupt or impair immune function.

Increased Colonization

Infants are initially colonized vertically at birth or horizontally in the nursery by parents or caregivers (19,20). On admission to the NICU, 5% to 10% of all infants were found to be colonized with *Candida* (21). By seven days of life, colonization rates increased to 50% and rose to 64% by one month of age (20,22,23).

Third-generation cephalosporins and other broad-spectrum antibiotics enhance fungal colonization by destroying competing bacterial flora (13). In a cohort of over 6000 infants, multivariable modeling revealed that third-generation cephalosporin or carbapenem use in the past seven days was associated with candidiasis (24). Gastric acidity is considered to be protective against *Candida* infection by suppressing fungal growth in the upper gastrointestinal tract. The use of H₂ blockers has been linked to an increased risk of candidemia in infants (4).

Impaired Immune System

Prematurity is the strongest risk factor for candidiasis in infants. The immune system of the preterm infant has defects in chemotaxis, cytokine and antibody production, and phagocytosis (25,26). Invasive medical devices such as central venous catheters and endotracheal tubes compromise an already underdeveloped layer of skin and place the preterm infant at an especially high risk for candidiasis. The use of steroids such as hydrocortisone and dexamethasone has been associated with increased risk of candidiasis in infants (27–30).

CLINICAL MANIFESTATIONS

Candidemia

Signs and symptoms of candidemia are often nonspecific and subtle in infants and may include temperature instability, lethargy, apnea, hypotension, respiratory distress, abdominal distension, hyperglycemia, and feeding intolerance (31,32). Although longer periods of candidemia are associated with increased risk of dissemination, dissemination can occur in the setting of only a single positive blood culture (25,31).

Meningoencephalitis

Meningitis complicates approximately 15% of cases of candidemia in infants; a rate that has been falling over the last 30 years, but is still much higher than the rate of meningitis observed in candidemic adult patients. This fact has important implications for diagnostic workup and therapeutic approaches, as clinicians cannot assume that the central nervous system (CNS) is not involved in otherwise seemingly straightforward candidemia.

Candidiasis of the CNS often results in granulomas, parenchymal abscesses, and vasculitis, and unremarkable CSF parameters in the presence of CNS infection are common (11,13,33). Only 25% of infants in one series of infants with *Candida* meningitis had abnormalities noted on CSF examination (8).

Urinary Tract Infections

Renal disease may present with rising creatinine, hypertension, flank mass, or acute urinary obstruction from fungal mycetoma (34,35). Patients with candiduria should have a renal ultrasound to detect the two types of renal involvement: nonshadowing echogenic foci and parenchymal infiltration (34). Often, radiographic findings persist despite proper therapy, negative cultures, and clinical recovery (36).

Optic Complications

Optic infections may result in chorioretinal infections or, more rarely, lens abscesses (37,38). As with meningitis, rates of chorioretinal lesions complicating candidemia are falling (39,40). A recent review of 123 candidemic VLBW infants found only one patient with optic involvement (39). However, infants with candidemia in the absence of direct optic infections have been shown to be at increased risk for the development of severe retinopathy of prematurity (9–11,37,41).

Other Organs

Endocarditis is documented in <5% of candidemic infants. Candidemia may also result in infections of the bones and joints. Assessment of end-organ damage in the presence of neonatal candidemia should include an ultrasound of the head and abdomen, lumbar puncture, ophthalmologic exam, and an echocardiogram.

PROPHYLAXIS

A survey of 219 U. S. neonatologists revealed that 34% used antifungal prophylaxis in their practice (42). Agents used for prophylaxis included fluconazole, oral nystatin, and amphotericin B deoxycholate. However, there are no formal studies describing the use of amphotericin B deoxycholate for *Candida* prophylaxis in infants.

Nonabsorbable Agents

Use of nonabsorbable oral agents for prevention of candidemia in infants is controversial (43). Nystatin is the most commonly used nonabsorbable agent in infants (42). A small randomized, controlled trial of infants <1250 g birth weight given nystatin (100,000 units every eight hours) or placebo demonstrated a significant decrease in both colonization rates and invasive *Candida* infections, 6% (2/33) in the nystatin group versus 32% (11/34) in the placebo group (44). A recent larger study of nystatin prophylaxis evaluated 3991 infants randomly assigned to nystatin or nystatin only if orally colonized with *Candida* (45). In this study, the rate of candidiasis for VLBW infants not receiving prophylaxis was 45% (104/232). This rate of candidiasis is an order of magnitude higher than rates observed for VLBW infants in previous multicenter cohort

Table 1 Fluconazole Prophylaxis Trials

Study	Population	Type	Results
89	<1500 g	RCT	Nystatin: 44% (4/9) Fluconazole: 0% (0/8), $P < 0.05$
23	<1500 g	RCT	Placebo: 4% (2/50) Fluconazole: 4% (2/53), $P = \text{NS}$
47	<1000 g	RCT	Placebo: 20% (10/50) Fluconazole: 0% (0/50), $P < 0.01$
48	<1000 g	RCT	Previous dosing: 5% (2/41) Twice weekly: 2% (1/40), $P = 0.83$
51	<1000 g	Retrospective cohort	No prophylaxis: 7% (15/206) Fluconazole: 2% (5/240), $P = 0.01$
50	<1500 g	Retrospective cohort	No prophylaxis: 8% (9/119) Fluconazole: 0% (0/136), $P < 0.01$
49	<1000 g	Retrospective cohort	No prophylaxis: 6% (13/206) Fluconazole: 1% (2/178), $P < 0.01$
52	<1500 g	Retrospective cohort	No prophylaxis: 17% (40/240), Fluconazole: 4% (10/225), $P < 0.01$
53	<1500 g	RCT	Placebo: 13% (14/106), Fluconazole: 3% (7/216), $P < 0.01$

Abbreviation: RCT, randomized controlled trial.

studies (5,7). Although nystatin administration decreased rates of candidiasis, the significance of this decrease is difficult to interpret in the presence of such a high rate of candidiasis in the control group. There is also concern that nystatin may be inactivated prior to reaching the intestines, the target region for the drug (46). Nystatin is also ineffective in protecting against invasion of other anatomic sites: skin, respiratory tract, and catheter sites.

Fluconazole

Several randomized placebo-controlled trials have been completed for fluconazole prophylaxis in preterm infants (Table 1). A study of 103 VLBW infants demonstrated lower rates of colonization in the fluconazole group but no difference in invasive disease (23). A second study of 100 ELBW infants found a significant decrease in both *Candida* colonization and the incidence of candidiasis (47). The placebo arm in this single center study had one of the highest rates (13/50; 26%) of invasive candidiasis reported in ELBW infants. A subsequent study of fluconazole prophylaxis at the same institution evaluated 3 mg/kg twice weekly dosing (12 doses total) versus the previous regimen (26 doses) in ELBW infants (48). Rates of candidiasis were similar between the twice weekly dosing group 3% (1/40) and the older regimen 5% (2/41) ($P = 0.68$). Twice weekly dosing might decrease drug toxicity and cost versus the more frequent dosing schedule. Several cohort studies have demonstrated decreased incidence of candidiasis using fluconazole prophylaxis (49–52).

Finally, a large eight-center study evaluated fluconazole prophylaxis in 322 VLBW infants (53). The infants were randomized 1:1:1 to receive placebo, fluconazole prophylaxis at 3 mg/kg/dose, or fluconazole prophylaxis at 6 mg/kg/dose. The rate of candidiasis in the two prophylaxis groups was 3% (7/127) and the rate in the placebo group was 14% (14/106). The rate of candidiasis in the placebo group ELBW infants, 14% (6/43), was twice that observed in previous large multicenter studies (5,7). Rates of candidiasis in the infants 1000 to 1499 g birth weight were over 10 times as high as previous observational studies, 13% (8/63) versus 1% in the 16 center NICHD Neonatal Research Network. Resistance to fluconazole was not observed during the courses of these randomized trials and side effects of fluconazole were minimal.

Although, fluconazole prophylaxis of older immunocompromised patients is effective in reducing candidiasis, fluconazole prophylaxis trials in infants to date have taken place in units with high baseline rates of candidiasis and have not evaluated the long-term neurodevelopmental consequences of prophylaxis; therefore, most NICUs do not routinely use antifungal prophylaxis.

EMPIRIC THERAPY

Antifungal therapy initiated three days earlier relative to the first positive culture in infected ELBW infants has been associated with improved mortality and morbidity (11). The decision to initiate empiric therapy in adult patients is largely based on a predictive model of risk in the treatment of neutropenic patients with prolonged febrile episodes but no such model exists for infants (54). In an effort to develop a similar model, a retrospective multicenter cohort study examined risk factors and subsequent candidiasis from blood cultures of 6172 premature infants (24). The study found that thrombocytopenia, gestational age 25 to 27 weeks, gestational age <25 weeks, and cephalosporin/carbapenem use seven days prior to blood culture were independently associated with candidiasis when controlling for the other variables in the analysis. Scores were assigned to these predictors as follows: gestational age <25 weeks = 2, gestational age 25–27 weeks = 1, platelet count <150,000 = 2, third-generation cephalosporin or carbapenem within seven days of positive blood culture = 1. A combined score of 2 or greater was found to be 85% sensitive and 47% specific in predicting candidemia. However, these data should be interpreted with caution. They provide early pilot data for subsequent prospective multicenter evaluations but are not sufficient for the development of routine guidelines for empiric therapy in premature infants. Although the case for empirical therapy is strengthened by the fact fluconazole and amphotericin B are well tolerated in infants and early experience with the echinocandins are also positive, empiric therapy warrants further investigation prior to its widespread use (55).

TREATMENT

Removal of the central venous catheter within 24 hours of a positive blood culture is a critical component of treatment (56). Delayed removal of central venous catheters in candidemic infants has been associated with increased mortality and morbidity, including worse neurodevelopmental outcomes (6,56,57).

Amphotericin B Deoxycholate

Amphotericin B deoxycholate is the most often used agent for invasive candidiasis in infants (58). However, there are limited pharmacokinetic data in infants. Two studies involving a total of 17 infants demonstrated a longer half-life of the drug in infants compared with adults (59,60). Test doses are not recommended as the drug is much better tolerated in infants than in adults (59,61). CSF penetration is poor in adult patients but was found to reach 40% to 90% of serum levels in preterm infants (59). Side effects observed in infants include electrolyte abnormalities and nephrotoxicity (62). Amphotericin B deoxycholate is effective against most *Candida* species causing disease in infants with the exception of *C. lusitaniae* (63). A prospective observational study of 59 infants with invasive candidiasis found that all isolates were sensitive to amphotericin B deoxycholate (14).

There is only one published randomized controlled trial for treatment of candidemia in infants (Table 2). This 23-patient study compared amphotericin B deoxycholate (1 mg/kg/day) versus fluconazole (10 mg/kg loading dose followed by 5 mg/kg/day) (64). Although the study was not sufficiently powered for noninferiority, there was no observed difference in survival between the two groups. Five (45%) of the infants in the amphotericin B group suffered thrombophlebitis leading to abscess formation compared with only 1/12 (8%) of the infants receiving fluconazole. One infant in the amphotericin B group suffered acute renal toxicity and two additional infants demonstrated an increase in liver enzymes. Only one infant receiving fluconazole experienced an increase in liver enzymes.

Lipid Preparations of Amphotericin B

Published experience with lipid preparations of amphotericin B in infants is limited (63). Serum levels on day 1 and day 28 of therapy were reported for 17 infants receiving 1 mg/kg/day of liposomal amphotericin B (L-amB) (65). Levels were lower than in adults and children receiving similar doses. Population pharmacokinetics of amphotericin B lipid complex (ABLC) was evaluated in 28 infants with a median birth weight of 910 g (66). Serum levels in infants receiving both 2.5 mg/kg/day and 5 mg/kg/day were similar to levels reported in adults. There was no accumulation of drug noted after multiple doses. Although therapeutic levels of

Table 2 Prospective Trials of Antifungals

Study	Type of study	Drug	Survival
90	Prospective cohort	Fluconazole	97% (30/31)
64	RCT	Amphotericin B	54.5% (6/11)
		Fluconazole	67% (8/12)
77	Prospective cohort	Fluconazole	70% (14/20)
91	Prospective cohort	Fluconazole	80% (32/40)
92	Prospective cohort	ABLC	81.8% (9/11)
93	Prospective cohort	L-amB	72.7% (32/44)
61	Prospective cohort	L-amB	94.6% (35/37)
67	Prospective cohort	Amphotericin B	85.3% (29/34)
		L-amB	83.3% (5/6)
		ABCD	85.7% (12/14)
69	Prospective cohort	Amphotericin B	100% (4/4)
		L-amB	64.2% (52/81)
		ABLC	75.9% (22/29)
82	Prospective cohort	Caspofungin	90% (9/10)

Abbreviation: RCT, randomized controlled trial.

amphotericin B were detected in urine samples, little to no amphotericin B was detected in the CSF.

Amphotericin B colloidal dispersion (ABCD) and L-amB were compared with amphotericin B deoxycholate in 56 candidemic infants (67). Infants with a serum creatinine <1.2 mg/dL were given 1 mg/kg/day of amphotericin B deoxycholate ($n = 34$) while the remaining infants were given either 5 mg/kg/day of ABCD ($n = 16$) or L-amB ($n = 6$). Potassium supplementation was required in 16 (47%) of the infants receiving amphotericin B deoxycholate and none of the 22 infants receiving the lipid preparations. Renal function improved during the course of treatment of all three groups. In a separate cohort of 44 infants treated with 1 to 5 mg/kg/day of L-amB, the only side effect noted was hypokalemia occurring in 16 (36%) of the infants (68). A total of 32 (73%) of the infants responded to therapy, including 5/6 (83%) of the infants with meningitis. Another report observed 118 infants given L-amB ($n = 81$) or ABLC ($n = 29$) (69). Both antifungals were started at 1 mg/kg/day and increased as tolerated to a maximum dose of 5 mg/kg/day. No difference in mortality was observed between the two groups and efficacy was 94% and 86% with L-amB and ABLC, respectively. L-amB was used in a series of 41 infants with candidiasis with a success rate of 95% (39/41) (61). The authors noted that eradication of infection occurred earlier when the target dose of 5 to 7 mg/kg/day was reached faster and concluded that clinicians should initiate therapy with this higher dose.

There is some concern about the ability of the lipid preparations to clear renal and CSF *Candida* infections (66,70). However, successful treatment of infants with *Candida* meningitis has been reported (68,71).

Flucytosine

Flucytosine (5-FC) is not an option for candidiasis as monotherapy because resistance develops rapidly. Use of 5-FC for candidiasis is further limited by lack of a parenteral formulation in the United States. However, 5-FC is occasionally administered in combination with other antifungals for *Candida* meningoencephalitis (72). In a cohort of 17 cases of candidal meningitis (including 11 infants), improvement was noted in 15 patients on combination therapy of amphotericin B deoxycholate and 5-FC (73). Conversely, an analysis of 27 ELBW infants with *Candida* meningitis revealed that time to clear infection was longer in infants given combination flucytosine and amphotericin B than those treated with amphotericin B alone (6). Bone marrow suppression is the predominant toxicity.

The Azoles

Until recently, pharmacokinetic data of fluconazole in infants is extremely limited and nonexistent for the smallest infants (<750 g birth weight) (74–76). Pharmacokinetic data obtained in

12 infants with a mean birth weight of 912 g demonstrated decreasing drug half-lives over the first few weeks of life (74). The infants received 6 mg/kg every 72 hours. A recent population pharmacokinetic analysis of 55 infants (23–40 weeks gestation) suggests that 12 mg/kg/day of fluconazole provides for therapeutic levels in most infants (77).

A group of 19 preterm infants with a mean birth weight of 1725 g treated with fluconazole experienced clearance of infection in 18 cases (95%) (78). The one failure was caused by *C. glabrata*. The infants were given a 10 mg/kg loading dose followed by 5 mg/kg/day. Only one infant, with syphilitic hepatitis, required a dosing change because of elevation in liver function tests. A prospective report of 40 infants given 6 mg/kg/day of fluconazole reported an overall mortality of 20% (8/40). Elevated liver function tests were observed in two (5%) infants.

Voriconazole, a second-generation triazole, has been found to be active *in vitro* against both *C. glabrata* and *C. krusei* as well as some isolates that have developed resistance to fluconazole. However, voriconazole should be used with caution in instances where fluconazole resistance is likely (79). To emphasize this point, the authors observed an infant at their institution that developed resistance to both azoles while on treatment doses of fluconazole (80). Voriconazole is not recommended in patients with renal insufficiency as its cyclodextrin carrier is renally cleared (81). The drug is metabolized by the liver, and good CSF penetration has been observed. Torsades de pointes has been rarely seen during therapy in adult patients. Other side effects include allergic reactions, elevated transaminases, and visual disturbances.

A 10-week-old infant with hemophagocytic lymphohistiocytosis with a cutaneous *Trichosporon beigeli* infection was given 6 mg/kg every 12 hours of voriconazole (82). Serum levels obtained were subtherapeutic for invasive fungal infections. The authors recommended a dose of 8 mg/kg every eight hours based on their findings. Voriconazole safety has not been studied in infants.

The Echinocandins

Caspofungin was not tested in infants prior to market approval, and there remains very little experience with caspofungin in infants. Odio et al. reported on a series of 10 infants receiving caspofungin as salvage therapy after initial therapy with amphotericin B deoxycholate (83). In this report 9/10 (90%) infants survived, and no adverse drug events were observed. The infants were given a dose of 1 mg/kg/day for two days before increasing the dose to 2 mg/kg/day for the duration of treatment. Another report of the successful use of 1.6 mg/kg/day of caspofungin in two infants also noted no adverse drug events. The authors reported hypercalcemia in an infant given a loading dose of 100 mg/m² followed by 70 mg/m²/day (80). No further electrolyte abnormalities were noted once the dose of caspofungin was decreased to 35 mg/m²/day.

Although no pharmacokinetic data were obtained in these studies, increased clearance of the drug has been observed in pediatric patients relative to adults. In a series of patients (ages 2–17 years), weight-based dosing using 1 mg/kg/day yielded suboptimal levels (84). The data suggested that a body surface area approach using daily maintenance doses of 50 mg/m²/day was necessary to obtain appropriate drug levels. However, caspofungin pharmacokinetics has yet to be studied in children less than two years old.

Micafungin was evaluated in premature infants in a single dose (0.75–3.0 mg/kg) pharmacokinetic study (85). The half-life in infants weighing >1000 g was 8 hours and 5.5 hours in infants 500 to 1000 g. The area under the curve (AUC) in infants >1000 g was approximately 50% less than that observed in older children, and the AUC was approximately 50% less in infants 500 to 1000 g compared to infants >1000 g. The substantially reduced AUC observed in infants is extremely important because the echinocandins (as a class) have shown a dose–response relationship.

We recently enrolled 12 preterm infants in a multidose pharmacokinetic study of micafungin (86). Infants received 15 mg/kg of micafungin every 24 hours for five days. The mean gestational age at birth of the infants was 27 weeks (range, 23–29 weeks), and the mean age at enrollment was 16 days of life. Analysis of the trial data suggested that 15 mg/kg dosing in preterm infants corresponds to approximately 4 to 5 mg/kg dosing in adults. We observed no statistical differences between infants <1000 g and those ≥1000 g. No adverse events were felt to be related to micafungin therapy.

Anidulafungin, which requires reconstitution with ethanol, has been studied in children age 2 to 17 but not in infants (87). The acceptable alcohol exposure during gestation is 0 (88); therefore, anidulafungin should not be used in this patient group in its current formulation. The experience with the variable kinetics of micafungin and caspofungin in children and infants suggests that caution should be employed, as infants may need higher than expected dosages.

CONCLUSION

Candida infections are of increasing importance as physicians care for a growing number of immunocompromised, premature infants. *Candida* speciation patterns continue to change with the introduction of newer antifungals for both prophylaxis and empirical therapy. Treatment options are greater now than ever before, but physicians are hampered by a lack of randomized controlled trials in this special population. Amphotericin B deoxycholate (1 mg/kg/day) or fluconazole (12 mg/kg/day) should continue to be used as first line agents for candidiasis. Micafungin 10 mg/kg/day, L-amB (5 mg/kg/day), and ABLC (5 mg/kg/day) are acceptable alternatives.

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27 Newer Antifungal Agents in Pediatrics

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INTRODUCTION

In recent years, the antifungal landscape has been changing rapidly, with several newer agents approved and clinicians striving to determine the optimal niches for each new agent in the overall therapeutic armamentarium. Unfortunately, studies with these agents in pediatric patients are slow and often clinical development and investigation stop after only initial pharmacokinetic exploration. Even the limited data we have on pediatric antifungals points to interesting differences between both adult and pediatric pharmacokinetics for the same agent, as well as differences among pediatric pharmacokinetics within the individual drug classes. For example, voriconazole displays nonlinear pharmacokinetics in adults but linear pharmacokinetics in children, demanding higher doses in smaller patients and possibly explaining treatment failures due to underdosing using the approved adult dosing regimen. Likewise, caspofungin requires higher doses in pediatric compared to adults, and dosing is best accomplished by using a body surface area regimen rather than a body weight scheme. Micafungin displays a clear trend toward lower plasma drug levels obtained in neonatal patients, emphasizing the importance of the neonatal period as a separate age group among pediatric patients.

These unique attributes to pediatric dosing underscore the necessity to more fully evaluate antifungals in children and not merely extrapolate data from adult studies to our pediatric patients, as well as the overall difficulty for pediatricians managing sick children to effectively utilize antifungals. This chapter provides an overview of the current state of systemic antifungal therapy in pediatric patients and focuses only on newer antifungal agents recently approved and on the available data in pediatric and neonatal patients. The emphasis here includes the newer triazoles, voriconazole and posaconazole, and the echinocandins, caspofungin, micafungin, and anidulafungin.

TRIAZOLES

The triazole antifungals are heterocyclic synthetic compounds that inhibit the cytochrome P450 dependent 14α -lanosterol demethylation, a vital step in fungal cell membrane ergosterol synthesis (1,2). Triazoles can be subdivided into first (fluconazole and itraconazole) and second generation (voriconazole and posaconazole) agents.

Voriconazole

Pediatric Pharmacokinetics

Voriconazole (VFend®; Pfizer Inc., New York, NY), a synthetic derivative of fluconazole, was the first available second-generation triazole approved in 2002 by the U.S. Food and Drug Administration (FDA) (1). It has potent activity against a broad spectrum of clinically relevant fungal pathogens, including *Aspergillus*, *Candida*, *Cryptococcus*, and some less common molds (3). Voriconazole is available in intravenous, oral tablet, and oral suspension formulations, and when administered orally its bioavailability is >90% in adults (4). To achieve steady-state concentrations within one day, an intravenous or oral loading dose is recommended before changing to a maintenance dose.

An open, multicenter study investigated the safety, tolerability, and pharmacokinetics of intravenous voriconazole in immunocompromised pediatric patients (5). This study included a single-dose study of 11 patients ages 2 to 11 years (mean age 5.9 years) receiving 3 or 4 mg/kg of body weight and a multiple-dose study of 24 patients ages 2 to 11 years (mean age 6.4 years) receiving a loading dose of 6 mg/kg twice daily (BID) on day 1, followed by 3 mg/kg BID on day 2 to day 4, and 4 mg/kg BID on day 4 to day 8. In contrast to the previously established nonlinear elimination of voriconazole in healthy adult volunteers (6) and adults (4), this study found linear pharmacokinetics of voriconazole in children over a dosage range of 3 and 4 mg/kg BID. Children require higher dosages of voriconazole than adults to achieve comparable serum levels, and a maintenance dose of 4 mg/kg BID in children approximates only 3 mg/kg BID in adults (5). Extrapolation of area under the curve (AUC) and maximum serum pharmacokinetic levels in children revealed an estimated 11 mg/kg/dose would be required to achieve adult values obtained with 4 mg/kg/dose if linear pharmacokinetics was maintained throughout the full dosage interval.

A subsequent pharmacokinetic study evaluated higher voriconazole doses and studied nine patients in each of two age groups (2–6 years, 6–12 years) (7). Each child received either 4 mg/kg/dose followed by 6 mg/kg/dose or 6 mg/kg/dose followed by 8 mg/kg/dose. Each child also received at least two different doses in escalating order and then was switched to oral voriconazole. The intravenous doses had similar pharmacokinetic parameters within each age cohort, except a greater AUC level in the 8 mg/kg/dose range in the older age cohort. After oral dosing, the older age group had higher AUC and maximum serum levels compared to the younger children. However, even at the 8 mg/kg/dose, the AUC (34,681 ng × hr/mL) in children was still less than that observed with adults (42,000 ng × hr/mL) at the 4 mg/kg/dose level. Interestingly, the bioavailability of oral voriconazole in children was lower (65%) than seen in adults (96%) (7). This study also demonstrated a high interpatient pharmacokinetic variability, as has been reported for studies concerning voriconazole drug level monitoring, but no significant differences were found in the 2–6 year versus 6–12 year age groups except at the 8 mg/kg/dose.

The European Medicines Agency (EMA)-approved dosing for voriconazole in children is to load with 7 mg/kg/dose for one day and then continue with the same 7 mg/kg/dose as a maintenance dose. This dosing recommendation is a clear step forward in the correct use of voriconazole in children compared to earlier uses when adult dosages were followed and infected children were probably underdosed. However, this second pharmacokinetic study raised several important points that remain inconclusively investigated. The higher dose of 8 mg/kg/dose still yielded an AUC level lower than in adults who received a standard dosing and does suggest that the optimal voriconazole dose in children is likely even higher. With linear pharmacokinetics in children, clinicians have a wider therapeutic window with which to safely increase the voriconazole dose. These dosing concerns are especially problematic with the known differences in metabolism across patient subpopulations and the concerns of a lack of standard effective voriconazole level goals for children.

For children, voriconazole is also available as an orange-flavored suspension (40 mg/mL). A common practice in pediatrics is to crush tablets and administer them to pediatric patients through a gastric or duodenal tube. A recent study examined the bioavailability of crushed or whole voriconazole tablets in an open-label, randomized, two-way crossover study in 20 healthy volunteers (8). While there was a slightly faster time to maximum serum concentration with the crushed tablets (0.5 hours vs. 1.5 hours), the bioavailability was equivalent in crushed and whole tablets.

Voriconazole is generally well tolerated. The most common drug-related adverse events in children are transient and reversible and include visual disturbances (blurred vision and photophobia), elevated hepatic enzymes, and skin reactions (photosensitivity and pruritus) (5,7,9).

Voriconazole is known to be mostly metabolized by the hepatic enzyme CYP2C19, a subfamily of the cytochrome P450 (CYP) (10). Due to a point mutation in the gene encoding CYP2C19, some individuals are poor metabolizers with fourfold higher levels of voriconazole compared to extensive metabolizers. Additionally, patients can be either homozygous or heterozygous for this metabolization phenotype. Poor metabolizers compose about 3% to 5% of

the Caucasian and African population, but up to 15% to 20% of the Asian population (5). This suggests that optimal dosing of voriconazole may be related to pharmacogenomics, which could loosely be assisted by using ethnicity as an indirect marker. Therefore, the genotype of CYP2C19 plays a crucial role in the pharmacokinetics of voriconazole. Moreover, voriconazole inhibits the metabolism of tacrolimus resulting in a significant increase in the trough concentration of tacrolimus in the blood (11). Close monitoring of blood levels of tacrolimus in children with coadministration of voriconazole and tacrolimus is warranted.

Clinical Experience and Pediatric Data

A multicenter, randomized, open trial compared the outcome of patients receiving either intravenous voriconazole or amphotericin B deoxycholate for primary therapy of invasive aspergillosis (12). In the voriconazole arm a total of 144 patients (13–79 years, mean 48.5 years) received a loading dose of 6 mg/kg IV BID on day 1, followed by 4 mg/kg IV BID for at least seven days and 200 mg BID orally for a total of 12 weeks. In the amphotericin B treatment group, 133 patients (12–75 years, mean 50.5 years) were administered 1.0 to 1.5 mg/kg IV once daily (QD). The overall response rate at 12 weeks showed statistically significantly more complete and partial responses in patients treated with voriconazole (52.8%) compared to patients treated with amphotericin B (31.6%). Voriconazole-treated patients also had a higher survival rate than amphotericin B-treated patients (70.8% vs. 57.9%; $p = 0.02$) and showed significantly fewer drug-related severe adverse side effects. While this study revolutionized the landscape of invasive aspergillosis treatment, the study did not include children younger than 12 years and enrolled very few under 18 years of age. Therefore, no firm conclusions can be made on the superiority of voriconazole over amphotericin B in this age group.

A minority of 52/144 (36%) patients in the voriconazole group and a majority of 107/133 (80%) patients in the amphotericin B group received another licensed antifungal therapy besides their primary study drug, resulting in a smaller final group of patients receiving only voriconazole or amphotericin B. Patients receiving voriconazole as initial therapy and then switching to another antifungal drug still had a higher rate of complete and partial response than patients who obtained amphotericin B as initial drug followed by another antifungal agent (48% vs. 38%) (13), suggesting that the initial therapy with the triazole was critical.

An additional open study confirmed these promising data by administering voriconazole as primary (52%) or salvage therapy (48%) to 116 patients with invasive aspergillosis (14). Up to 48% of the patients demonstrated a complete or partial response, whereas in 21% the disease was stable and in 31% failed. The results of these clinical studies have led most experts to recommend voriconazole as the preferred antifungal against invasive aspergillosis.

Voriconazole is also effective in the treatment of *Candida* infections. In a randomized, double-blind multicenter trial including 391 immunocompromised patients with esophageal candidiasis, oral voriconazole (200 mg BID) showed a primary success rate of 98.3% compared to 95.1% in patients treated with oral fluconazole (200 mg QD) (15). In another multicenter study of 422 patients with candidemia, voriconazole was as efficacious as amphotericin B followed by fluconazole (16). Moreover, voriconazole also demonstrates similar success rates and less breakthrough fungal infections compared to liposomal amphotericin B in patients with neutropenia and persistent fever (17).

The largest pediatric study with voriconazole reported on 58 children (ages 9 months to 15 years, mean 8.2 years) with invasive fungal infections refractory to or intolerant of conventional antifungal therapy (18). Voriconazole was administered as a loading dose of 6 mg/kg IV BID on the first day of therapy, followed by 4 mg/kg IV BID. If feasible, the IV dose was then later switched to an oral dose of 100 or 200 mg BID for children weighing <40 kg or ≥ 40 kg, respectively. Responses to the therapy with voriconazole were complete or partial in 26 (45%), stable in 4 (7%) and failed in 25 (43%) patients. *Aspergillus* spp. was the most common infecting organism (72%) and 43% of those patients responded to the therapy. In a subsequent case series, voriconazole was utilized as salvage therapy in seven children (age range 2–13 years, median 5 years) with invasive aspergillosis (19). Complete and partial response was observed in each of two patients, stable response in one patient, and failure of the voriconazole treatment in two patients. These are the largest published reports of voriconazole use in children, and while they demonstrated that voriconazole is generally as effective in children as adults, the incorrect

dosages utilized in these small studies don't answer the fundamental question of true efficacy in children.

At present, there are no studies available in neonates. There are ongoing concerns about the effect of voriconazole on the developing retina in neonates due to the well-documented visual disturbances in pediatric and adults.

Posaconazole

Pharmacokinetics

Posaconazole (Noxafil[®]; Schering-Plough Research Institute, Kenilworth, NJ) is a second-generation extended-spectrum triazole and derivative of itraconazole that was approved by the FDA in September 2006. Posaconazole has excellent activity against both yeast and mold infections, specifically including activity against zygomycosis where voriconazole has no antifungal efficacy (20). Posaconazole is currently only available as an oral suspension, with an intravenous formulation being formulated but not yet ready for clinical use. Posaconazole has a large volume of distribution and is highly protein bound (>95%) (20). Dose adjustments are not necessary in patients with renal (21) or hepatic impairment (22).

The bioavailability of posaconazole increases significantly when administered in divided doses. One study showed the highest bioavailability of posaconazole in fasting healthy volunteers receiving 800 mg/day divided in four doses compared to 800 mg/day divided in either one or two doses (23). When administered in the fed state, 800 mg/day divided in two doses gave similar serum levels to dosing four times daily and is more practical for compliance. Another study reported on posaconazole in 98 adults with febrile neutropenia or refractory fungal infections (24). Posaconazole was administered in three regimens: 400 mg BID, 600 mg BID, or 800 mg QD. A daily dose of 800 mg/day given as 400 mg BID provided the greatest posaconazole exposure.

There are currently very limited pediatric pharmacokinetic data for posaconazole. Serum samples obtained on 12 pediatric (ages 8–17 years) and 194 adult (ages 18 to 64 years) patients from a multicenter, phase 3, open-label study of patients with invasive fungal infections refractory to standard antifungal therapies were analyzed as preliminary comparisons of adult versus child pharmacokinetics (25). All patients received a maintenance dose of 800 mg/day in divided doses except for one pediatric patient who took 400 mg/day as a divided dose on the day of sample collection. The mean plasma concentrations of posaconazole in children and adults were 776 ng/mL (median 579 ng/mL; range 85.3–2891 ng/mL) and 817 ng/mL (median 626 ng/mL; range 0–3710 ng/mL), respectively, suggesting similar plasma concentrations in pediatric and adults. One limitation with this study is that while the age range of the 12 pediatric patients was 8 to 17 years, it consisted of a single eight-year-old patient, a single 10-year-old patient, and all other patients were ≥ 12 years old. This skew toward teenagers could explain the results similar to adult dosage findings. Pediatric experience with posaconazole therapy is very limited in general, with only approximately 150 patients who have received the drug on a compassionate use basis (Schering-Plough, data on file) and it is unclear if the limit on absorption seen in adults extends to pediatric patients. A dedicated pediatric posaconazole pharmacokinetic study is ongoing.

Posaconazole is overall well tolerated. The most common drug-related adverse events are gastrointestinal (nausea, vomiting, abdominal pain) and hepatic (elevated hepatic enzymes) (26,27).

Clinical Experience and Pediatric Data

In adults with severe graft-versus-host disease, posaconazole was as effective as fluconazole in antifungal prophylaxis, but more effectively prevented invasive aspergillosis as well as breakthrough invasive fungal infections and reduced deaths due to invasive fungal infections (28). Moreover, in adults with neutropenia, posaconazole was superior to either fluconazole or itraconazole in preventing invasive fungal infections (2% vs. 8%) (29).

Segal et al. reported on seven pediatric (ages 9–18 years) and one adult patient (age 36 years) with chronic granulomatous disease and invasive filamentous fungal infections, who received posaconazole as salvage therapy (26). Except one patient who received posaconazole

as a dosage of 200 mg thrice daily, the remaining seven patients received 400 mg BID. Treatment with posaconazole led to a complete response in 7/8 adults including 6/7 pediatric patients. Importantly, in that study the prior antifungal drug was voriconazole in 7/8 patients which was discontinued due to failure ($n = 6$) or intolerance ($n = 1$). Two open-label, multicenter, compassionate trials investigated the outcome of 24 patients including three pediatric patients (ages 7, 17, and 18 years) with active zygomycosis treated with posaconazole oral suspension (200 mg four times daily or 400 mg BID (30). Overall, 79% of patients had a complete or partial response while all three pediatric patients were treated successfully (one complete response and two partial responses).

Raad et al. reported on the outcome of posaconazole (800 mg/day in two or four divided doses) as salvage therapy in patients with underlying hematologic malignancy and invasive fusariosis (31). A subgroup of this study consisted of 16 patients with leukemia including three pediatric patients (ages 10, 10, and 14 years). Successful outcome occurred in 50% of the subgroup population; however, only 1/3 pediatric patients (33%) survived the study.

There have been recent studies which confirmed the promise of posaconazole as salvage therapy in adults with invasive aspergillosis and zygomycosis (27,31,32). Nevertheless, devoted pediatric efficacy studies with invasive fungal infections have not yet been performed.

ECHINOCANDINS

The echinocandins are a novel class of antifungal agents which noncompetitively inhibit the synthesis of β -(1,3)-D-glucan, an essential component of the fungal cell wall that provides structural integrity and is essential for fungal cell growth and division (1,33). As β -(1,3)-D-glucan is not shared by mammalian cells, these are highly selective for fungi (34). Of significance, the cell wall of *Cryptococcus neoformans* and *Zygomycetes* lack critical amounts of β -(1,3)-D-glucan and therefore the echinocandins are not effective against these fungal pathogens (35). Interestingly, the echinocandins are fungicidal against *Candida* and fungistatic against *Aspergillus* (36). Because of their large molecular weight, echinocandins are not well absorbed orally and must be administered intravenously (1).

Caspofungin

Pharmacokinetics

Caspofungin (Cancidas[®]; Merck Co., Whitehouse, NJ) was the first echinocandin to receive approval from the FDA in 2001 and is a semisynthetic derivative of the natural product pneumocandin B₀ (1,37). Caspofungin is primarily excreted by the liver and not metabolized by the cytochrome P450 enzymes (38,39). It shows linear pharmacokinetics after single dosing but modest nonlinearity after multiple dosing (39). In adults, a loading dose of 70 mg on the first day, followed by a maintenance dose of 50 mg daily is recommended (39). Dose reduction is not necessary in patients with renal impairment or mild hepatic insufficiency (40,41). However, in patients with moderate hepatic insufficiency (Child-Pugh scores 7 to 9, on a scale from 5 to 15, with higher scores indicating worse liver function) reduction of the maintenance dose to 35 mg/day after the initial 70 mg loading dose is recommended (41). A slight reduction of tacrolimus exposure by approximately 20% was seen with coadministration of caspofungin, and therefore close monitoring of tacrolimus concentrations is recommended (42). Moreover, caspofungin interacts with cyclosporine A resulting in an increased plasma concentration of caspofungin by about 35% without an alteration of the cyclosporine A plasma levels (40).

Walsh et al. reported on 39 children (2–11 years) and adolescents (12–17 years) (mean 7.7 years) with a new onset of fever and neutropenia in whom caspofungin was administered either on a basis of weight (1 mg/kg/day) or body surface area (BSA) (50 or 70 mg/m²/day) (43). Pediatric pharmacokinetic data were compared to those in adults receiving caspofungin 50 or 70 mg/day for mucosal candidiasis. The weight-based regimen in seven children (1 mg/kg/day) yielded a significantly smaller area under the concentration–time curve over 24 hours (AUC_{0–24}) compared to adults (50 mg/day) and was therefore discontinued. However, in children ($n = 10$) and adolescents ($n = 8$) receiving 50 mg/m²/day using the BSA regimen the AUC_{0–24} following multiple doses was similar to that of adults receiving the FDA-approved 50-mg daily regimen. Moreover, children ($n = 12$) taking 70 mg/m²/day showed comparable

AUC₀₋₂₄ values after multiple doses to adults (70 mg/day). The β -phase half-life of caspofungin was approximately one-third (37%; $p = 0.001$) less in children than in adults. Even BSA dosing revealed that adolescent and pediatric concentrations descend more rapidly than in adults, with statistically significant decreases in end-of-infusion concentrations of caspofungin with increasing age. Therefore, a BSA dosing regimen is now recommended for caspofungin therapy in pediatric patients. Only five patients (12.8%) experienced drug-related clinical adverse events (fever, diarrhea, phlebitis, proteinuria, and skin rash) and two patients (5.1%) laboratory adverse events (hypokalemia and increased serum aspartate transaminase).

To investigate efficacy and safety of caspofungin at 50 mg/m²/day also in a younger age group, a pharmacokinetic study was conducted in eight immunosuppressed children ages 3 to 24 months with fever and neutropenia (44). In young children clearance ($t_{1/2} = 8.04$ hours) was reduced ~38% relative to adults at 50 mg/day ($t_{1/2} = 13.0$ hours) and no confirmed invasive fungal infection during study course was observed. The authors concluded that caspofungin administered at 50 mg/m²/day in young children 3 to 24 months of age reaches comparable plasma exposure to that of adults receiving 50 mg/day.

Another study investigated retrospectively the safety of caspofungin in 25 pediatric immunocompromised patients (ages 0.3 to 26.2 years, median 9.8 years), who obtained caspofungin for documented ($n = 13$) or suspected ($n = 8$) fungal infections or for prophylaxis ($n = 4$) (45). Caspofungin doses ranged from 0.8 to 1.6 mg/kg/day for patients weighing <50 kg and from 50 to 75 mg/day for patients weighing ≥ 50 kg. Caspofungin was generally well tolerated with side effects occurring only in 3/25 (12%) patients (hypokalemia and elevated total serum bilirubin).

Clinical Experience and Pediatric Data

A multicenter, retrospective, noncomparative survey in Germany reported on 64 immunocompromised pediatric patients ages 0.4 to 17.9 years (median 11.5 years) who received caspofungin for proven (26.6%), probable (21.8%), and possible (26.6%) invasive fungal infections or empirical treatment (25%) (46). The mean daily maintenance dose was 1.07 mg/kg (0.40–2.92 mg/kg) or 34.3 mg/m² (16–57 mg/m²) for a median treatment duration of 37.0 days (3–218 days). An overall successful outcome was achieved in 67.7% of patients and the overall survival rate was 75% at the end of treatment. However, the majority of patients (68.7%) received caspofungin in combination with another antifungal treatment regimen, resulting in only one-third of patients receiving caspofungin as monotherapy. Moreover, the BSA regimen (50 mg/m²), which has been shown to result in a more optimal caspofungin exposure to pediatric patients compared to the weight-based regimen, was utilized in only 5/64 patients (43).

Two smaller studies investigated the efficacy of caspofungin in children with invasive fungal infections. One preliminary report from an ongoing prospective, multicenter trial study showed good overall response to caspofungin in 28 children with documented fungal infections (loading dose 70 mg/m² on day 1, followed by a maintenance dose of 50 mg/m²) (47). Success rates were 88% and 50% in patients with invasive candidiasis and invasive aspergillosis, respectively. Another study reported on an overall 50% survival rate and 65% successful response rate in 20 pediatric patients with invasive fungal infections who received caspofungin for salvage or first-line therapy (48). Caspofungin is also effective in the empirical treatment of children with febrile neutropenia. Khayat et al. showed in 26 pediatric patients that caspofungin is as least as effective as liposomal amphotericin B and demonstrates better tolerability (49).

To date the use of caspofungin in neonates with invasive candidiasis has been limited. Odio et al. reported on the use of caspofungin for salvage therapy in 10 neonates (nine premature and one term) with invasive candidiasis refractory to the treatment with amphotericin B deoxycholate (50). In this patient population, invasive candidiasis was caused by *Candida albicans* ($n = 4$), *C. parapsilosis* ($n = 3$), *C. tropicalis* ($n = 2$), and *C. glabrata* ($n = 1$). Despite initial therapy with amphotericin B deoxycholate, blood cultures remained positive in all patients for 13 to 49 days. Caspofungin was initiated with a dose of 1 mg/kg/day for two days followed by 2 mg/kg/day for 15 to 21 days in nine patients or with a dose of 0.5 mg/kg/day for three days followed by 1 mg/kg/day for 28 days in one patient. Between three and seven days, all positive blood cultures cleared. A total of 8/10 neonates resolved on caspofungin therapy, one patient responded after relapsing and a second administration of caspofungin was needed, and

one patient died of an overwhelming bacterial septicemia. There were no clinical or laboratory adverse events attributable to caspofungin administration.

In a subsequent study, six premature neonates with invasive candidiasis refractory to amphotericin B deoxycholate received caspofungin as monotherapy at an initial dose of 0.5 to 1 mg/kg/day (days 1–3) and a maintenance dose of 1 to 3 mg/kg/day (51). All six neonates were successfully treated (complete response) and tolerated caspofungin therapy very well. A linear scaling of the observed pharmacokinetic data was performed and compared to historical pharmacokinetic data in pediatric patients (either children 2–11 years or adolescents 12–17 years) receiving 50 mg/m²/day and adults receiving the standard dose of 50 mg/day. Caspofungin plasma concentrations at doses of 2 mg/kg/day or 25 mg/m²/day in premature neonates corresponded to 50 mg/day given in adults and therefore this dose has been recommended for further evaluation in neonates.

The largest retrospective neonatal report showed that caspofungin (1 mg/kg IV QD) added to amphotericin B, fluconazole, or flucytosine treatment in 13 neonates (12 premature and one term) with persistent candidemia sterilized blood cultures in 11/13 patients within three days (range 1–21 days) (52). Adverse events included elevation of liver enzymes ($n = 4$), hypokalemia ($n = 2$), and severe thrombophlebitis ($n = 1$).

Micafungin

Pharmacokinetics

Micafungin (Mycamine[®], Astellas PharmaUS, Inc., Deerfield, IL) is the second available echinocandin that was approved by the FDA in 2005 for esophageal candidiasis and for prophylaxis in patients undergoing hematopoietic stem cell transplantation (53). It is a water-soluble lipopeptide synthesized after cleavage of the hexapeptide FR901370, a natural product of the fungus *Coleoptima empedri* (53). Micafungin is metabolized in the liver and excreted into bile and urine and there is no metabolism by the cytochrome P450 enzymes. It exhibits a linear dose-dependent relationship in both adult and pediatric patients (54,55) as well as premature infants (56). Dose reduction is not necessary in patients with moderate hepatic or renal dysfunction (57). There does not appear to be a drug interaction with micafungin and tacrolimus (58) but a mild inhibition of cyclosporin A metabolism by micafungin (59).

There are several pharmacokinetic studies on micafungin in pediatric patients that have been completed. A multicenter, phase I, open-label dose escalation study assessed the pharmacokinetics and safety of micafungin in neutropenic pediatric patients and included 77 children stratified by age (2–12 years and 13–17 years) (54). They received an initial micafungin dose of 0.5 mg/kg/day, which was then escalated to higher dose levels of 1.0, 1.5, 2.0, 3.0, and 4.0 mg/kg/day. Plasma half-life, clearance, and volume of distribution did not differ over time or across dosage cohorts and were similar to adult pharmacokinetics as also reported by Townsend et al. (60). Interestingly, there was an inverse relation between increased clearance and decreased age, suggesting higher doses of micafungin might be needed in younger age groups.

Arrieta et al. performed a meta-analysis of the use and safety of micafungin in 244 pediatric patients (<16 years of age) from five clinical trials (61). Pediatric patients were treated with up to 8.6 mg/kg/day for up to 681 days. The safety profile was similar to that of adults and in only 25% of patients ≥ 1 adverse event possibly related to micafungin occurred. In a subpopulation of 77 neutropenic febrile pediatric patients who received micafungin for prophylaxis at doses between 0.5 and 4 mg/kg/day, there was an inverse relation for age and clearance again noted as described earlier (54,61). The average clearance in this patient subpopulation was found to be 1.5 to 2 times greater in patients eight years and younger compared to patients older than eight years (61). Another study in Japan involving 19 pediatric patients with deep fungal infections caused by either *Aspergillus* or *Candida* species reported linear pharmacokinetics for micafungin (1–6 mg/kg/day) as well as similar dose-proportionality when compared with adult data (55). In contrast to the two previous studies, no age-dependent pharmacokinetic profile was noticed among the three different age groups (<2 years, 2–5 years and 6–15 years) regarding plasma half-life (55). Furthermore, Hope et al. reported a linear relationship of clearance and weight in 72 children (62).

In pediatric patients, drug-related adverse events occurred in approximately 12% of patients and included diarrhea, vomiting, and headache (54). No attributable nephrotoxicity and infusion-related toxicity was noted, but minimal hepatotoxicity was observed.

In addition to the pediatric data, there are two pharmacokinetic studies in neonates available. Heresi et al. evaluated micafungin in 18 premature infants weighing >1000 g using three dosages (0.75 mg/kg/day, 1.5 mg/kg/day, 3.0 mg/kg/day) and in five premature infants weighing 500 to 1000 g using 0.75 mg/kg/day (56). There was a shorter half-life and a more rapid clearance per body weight in the smaller weight group compared with the >1000 g group. Moreover, clearance for micafungin in neonates weighing <1000 g was 1.7-fold and 2.6-fold greater than the clearance of 2 to 8 years and 9 to 17 years old children, respectively, as reported previously by Seibel et al. (54). A shorter plasma half-life and a higher clearance of micafungin were also seen in neonates after a single dose compared to children ages 2 to 8 years (61). Overall, micafungin was well tolerated in neonates and no serious drug-related adverse events were reported (56).

Clinical Experience and Pediatric Data

A pediatric multicenter, randomized, double-blind trial compared the efficacy of micafungin versus liposomal amphotericin B (L-AmB) as first-line therapy in 98 pediatric patients with proven invasive candidiasis (63). The study included 19 premature infants, 57 patients younger than 2 years, and 41 patients ages 2 to 15 years. Micafungin and L-AmB were administered at a daily dose of 2 mg/kg and 3 mg/kg, respectively, to premature infants ($n = 19$), children younger than 2 years ($n = 57$), and children ages 2 to 15 years ($n = 41$). Treatment success, as determined by both clinical and microbiologic response, and survival during treatment and the 12-week follow-up were similar in both the micafungin and L-AmB treatment group (72.9% vs. 76.0% and 75% vs. 76%, respectively). Notably, micafungin compared to L-AmB was slightly more effective in patients with neutropenia (85.3% vs. 76.9%) but less effective in patients 2 to 15 years old (63.6% vs. 73.7%).

Another multicenter, randomized, double-blind phase III study compared the efficacy and safety of micafungin with that of fluconazole for prophylaxis against invasive fungal infections during neutropenia (64). A total of 882 patients were enrolled including 84 pediatric patients (<16 years). Success rate, defined as the absence of suspected, proven, or probable fungal infection at the end of treatment and at follow-up after four weeks, was better in patients receiving micafungin than in patients receiving fluconazole (80.0% vs. 73.5%, $p = 0.03$). Micafungin was also superior to fluconazole in pediatric patients with success rates of 69.2% vs. 53.3%. Despite the improved efficacy over fluconazole in pediatric patients, the treatment success rates of micafungin in children (69.2%) was lower than adults aged 16 to 64 years (81.1%) and adults >64 years (97.0%). It is unclear if this lowered success rate in children is due to an ineffectively low pediatric dose of micafungin and that this success rate could be increased with optimal dosing. This lowered success rate in pediatric patients was also seen in an open-label noncomparative study of candidemia that enrolled 126 patients, including 20 (15.9%) children (<16 years of age) (65). In patients receiving at least five doses of micafungin 84.9% of adult ($n = 106$), but only 75% (15 of 20) of pediatric patients responded to micafungin therapy, possibly because of inadequate dosing in children.

There are several noncomparative studies investigating the efficacy of micafungin in patients with invasive aspergillosis and candidiasis. Denning et al. investigated the outcome of 225 patients with proven or probable invasive aspergillosis including 58 children (<16 years of age) (66). A total of 26/58 (44.8%) pediatric patients responded to micafungin therapy including 12/27 (44.4%) children younger than 10 years compared to a 35.6% overall response rate. However, only 34/225 patients received micafungin as monotherapy with a 50% response rate when administered as primary therapy but only a 40.9% response rate when given as salvage therapy. In a further analysis of those 58 pediatric patients (<16 years) with probable or proven invasive aspergillosis, micafungin was administered in two children as monotherapy and in 56 children in combination with other antifungal agents (67). The overall response rate was 45%, complete and partial responses were seen in 16% and 29%, respectively. In pediatric ($n = 16$) and adult ($n = 69$) bone marrow transplant recipients who had refractory invasive aspergillosis,

micafungin in combination with their existing antifungal regimen showed an overall complete and partial response of 39% in adults and 38% in pediatric patients (68).

Another study found that micafungin is more effective in *Candida* than in *Aspergillus* infections (61). Fifty-three children with invasive candidiasis and 58 children with invasive aspergillosis received micafungin at a dose of 1 to 2 mg/kg/day and showed complete and partial responses in 72% and 49%, respectively (61).

At present there are no reports on efficacy of micafungin in neonates, but a large phase III trial comparing micafungin versus amphotericin B deoxycholate for neonatal candidiasis is currently underway.

Anidulafungin

Pharmacokinetics

Anidulafungin (Eraxis[®], Pfizer, New York, NY) was approved by the FDA in February 2006 for esophageal candidiasis, candidemia, peritonitis, and intraabdominal abscesses (69). Antifungal dosing in adults for anidulafungin was studied at 50 and 100 mg/day for esophageal candidiasis and invasive candidiasis, respectively (1). Anidulafungin has linear pharmacokinetics and the longest β -half-life compared to caspofungin and micafungin. The volume of distribution and clearance is greater in anidulafungin than in micafungin (33).

A multicenter, phase I/II dose escalation study investigated the pharmacokinetic profile and safety of anidulafungin in neutropenic pediatric patients at high risk for invasive fungal infections (70). A total of 25 children, equally distributed in the 2- to 11-year-old group and in the 12- to 17-year-old group, received anidulafungin at either 0.75 mg/kg/day ($n = 13$) or 1.5 mg/kg/day ($n = 12$). Plasma drug concentrations and drug exposures were similar for patients between age groups and a weight-adjusted clearance was consistent across age. In children receiving anidulafungin at 0.75 mg/kg/day or 1.5 mg/kg/day, pharmacokinetic data were similar compared to adults administered doses of 50 or 100 mg/day. This important feature distinguishes anidulafungin from caspofungin where a higher dosing based on body-surface area is recommended. No drug-related serious adverse events occurred. Anidulafungin was well tolerated and its dosing can be based on body weight.

Clinical Experience and Pediatric Data

To date there are no reports available on the efficacy of anidulafungin in pediatric patients and neonates.

CONCLUSION

The recent explosion of newer antifungal agents has greatly augmented the clinical spectrum of drugs available against invasive fungal infections (Table 1). However, the importance of devoted

Table 1 Spectrum of Activity of Selected Antifungal Agents

Antifungal	Important clinical uses
Amphotericin B	<i>Blastomyces dermatitidis</i> , <i>Coccidioides immitis</i> , <i>Cryptococcus neoformans</i> , <i>Histoplasma capsulatum</i> , <i>Paracoccidioides brasiliensis</i> , <i>Sporothrix schenckii</i> , most <i>Candida</i> species, <i>Aspergillus</i> , <i>Zygomycetes</i> (Not: <i>C. lusitanae</i> ; Less effective: <i>Scedosporium</i> , <i>Fusarium</i> , <i>Trichosporon</i>)
Fluconazole	Most <i>Candida</i> , <i>C. neoformans</i> , <i>B. dermatitidis</i> , <i>H. capsulatum</i> , <i>C. immitis</i> , <i>P. brasiliensis</i> (Not: <i>C. krusei</i> , <i>Aspergillus</i> ; Less effective: <i>C. glabrata</i>)
Itraconazole	<i>Candida</i> , <i>Aspergillus</i> , <i>B. dermatitidis</i> , <i>H. capsulatum</i> , <i>C. immitis</i> , <i>P. brasiliensis</i>
Voriconazole	<i>Candida</i> , <i>Aspergillus</i> , <i>Fusarium</i> , <i>B. dermatitidis</i> , <i>H. capsulatum</i> , <i>C. immitis</i> , <i>Malassezia</i> species, <i>Scedosporium</i> , dematiaceous molds (Not: <i>Zygomycetes</i> ; Less effective: <i>C. glabrata</i>)
Posaconazole	Same as voriconazole, except has activity against <i>Zygomycetes</i> and <i>C. glabrata</i>
Caspofungin	<i>Candida</i> , <i>Aspergillus</i>
Micafungin	(Not: <i>C. neoformans</i> , <i>Fusarium</i> , <i>Zygomycetes</i>)
Anidulafungin	

Table 2 Preferred Pediatric Dosing of Approved Systemic Antifungal Agents

Drug class	Antifungal drug	Preferred adult dosing	Preferred pediatric dosing
Polyene	Amphotericin B deoxycholate	1–1.5 mg/kg/day	1–1.5 mg/kg/day
	Amphotericin B Lipid complex	5 mg/kg/day	5 mg/kg/day
	Amphotericin B colloidal dispersion	5 mg/kg/day	5 mg/kg/day
	Liposomal amphotericin B	5 mg/kg/day	5 mg/kg/day
Triazole	Fluconazole	100–800 mg/day; 3–6 mg/kg/day	6–12 mg/kg/day
	Voriconazole	Load: 6 mg/kg/dose BID × 1 day Maintenance: 4 mg/kg/dose BID	Load: 7 mg/kg/dose BID × 1 day Maintenance: 7 mg/kg/dose BID
Echinocandin	Posaconazole	400 mg BID	Unknown
	Caspofungin	Load: 70 mg QD × 1 day Maintenance: 50 mg QD	Load: 70 mg/m ² QD × 1 day Maintenance: 50 mg/m ² QD
	Micafungin	50–100 mg QD	4–12 mg/kg QD
	Anidulafungin	50–100 mg QD	0.75–1.5 mg/kg QD

Abbreviations: QD, once daily; BID, twice daily.

pediatric data has been largely underestimated. There have been no large phase III antifungal clinical trials dedicated to children; therefore, most of the information has been extrapolated from adult to pediatric patients (Table 2). Only a few antifungal studies include pediatric patients at all. In these studies, pharmacokinetics appears more complex in children than in adults, and at times the pharmacokinetics is directly opposite those seen in adults (Table 3). While limited pharmacokinetic studies have begun to correct pediatric antifungal dosing, there has been only a small body of work studying the efficacy of antifungal agents in pediatric patients. This situation seems paradoxical, as the known dosing differences would clearly lead to efficacy differences when adult doses were studied in children. It will only be through the performance of larger pediatric trials that pediatric dosing and indications will be improved and benefit children.

Table 3 Pharmacokinetic Differences in Pediatric Antifungal Dosing

Antifungal drug	Unique pediatric trait
Amphotericin B products	No known dosing differences, but children can generally tolerate higher doses than adults
Fluconazole	Dose higher in children due to shorter half-life
Voriconazole	Linear pharmacokinetics in children as compared to nonlinear pharmacokinetics in adults Optimal pediatric dose still not yet determined, and it is possible to increase dose further if needed
Posaconazole	Likely 6–9 mg/kg/dose BID, but pharmacokinetic studies are ongoing
Caspofungin	Need to use body-surface area dosing scheme and not weight-based dosing in children due to rapid clearance
Micafungin	Dosing depends on age, with smaller children requiring high doses and neonates requiring even higher doses
Anidulafungin	Appears to be no difference in dosing between children and adults

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28 | Fungal Infections in Burn Patients

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INTRODUCTION

In North America, burns are one of the primary causes of injury deaths, especially in children younger than 14 years of age (1,2). Furthermore, the United States also has one of the highest per capita burn death rates of any industrialized country. Fortunately, the survival rates from burns have improved substantially in the past decade due to advances in modern medical care in specialized burn centers. The advances in medical care along with longer burn unit stays and the survival of thermal injury patients that previously would have died early have brought about an important consequence, the increase in nosocomial infections. Although most infections in burn patients are due to bacterial infections, in several retrospective and prospective epidemiologic surveys of burn units, the incidence of invasive fungal infections, especially in the severely burned patient was estimated to be approximately 10% to 21% (3).

EPIDEMIOLOGY

In patients with severe burns, more than 75% of all mortalities are due to sepsis from either infected burn wounds or other infectious complications (4). In a prospective epidemiologic study by Allen and colleagues, the investigators reported an overall mortality due to invasive candidiasis of 21% and were able to correlate the incidence of *Candida* colonization and invasive candidiasis with the degree of total body surface area (TBSA) of burns (3). In burn patients with a TBSA burn between 40% and 49% were found have a 40% incidence of invasive candidiasis, while those patients with TBSA burns of 60% to 90% had an incidence of candidemia or candidiasis as high as 70% (4).

In intensive care units (ICU) across North America, including burn units, sepsis due to fungal infections has increased approximately 200% over the past 20 years (5). This increase has been described in the National Nosocomial Infections Surveillance System (NNIS) study that reported the rate of fungal infections rose from 2.0 to 3.8/1000 discharges (6). This increase was detected primarily in certain ICUs within the hospital, such as the burn and trauma units, the neonatal ICU, the cardiothoracic ICU, the surgical and medical ICU, and the hematology/oncology units (6). The highest number of nosocomial fungal infections was reported in the burn/trauma units at 16.1/1000 discharges compared to an incidence of 9.6 in the surgical ICU (6). In a separate study evaluating fungal infections in ICUs, the National Epidemiology of Mycoses Survey (NEMIS) reported that the nosocomial bloodstream infection rate due to *Candida* spp. was 9.8/1000 admissions to the ICUs (7).

Of all of the fungal pathogens capable of producing disease in humans, *Candida* spp. accounts for the majority of infections in immunocompromised patients, especially the burn patient (6,8,9). *Candida* spp. are currently the fourth most commonly isolated organism recovered from the bloodstream of hospitalized individuals and ranks first as the most common cause of fungal infections found in burn patients (10–12).

The management of patients who have suffered extensive burns has greatly improved over the past 10 to 20 years. Because of this, survival rates have improved dramatically (12). However, because of the aggressive management of these patients and improved survival rates, infection is currently the main cause of morbidity and mortality, especially late mortality in this patient population (12). Nonbacterial causes of invasive burn wound infections have been increasingly recognized over the past decade as important causes of morbidity and mortality in severely burned individuals. The most common nonbacterial cause of infections in thermal injury remains fungi. Most fungal infections tend to occur after the second or third week of their thermal injury. The overall incidence of invasive fungal infections is estimated to be between

Table 1 Etiology of Fungal Infections in Burn Patients

-
- *Candida* species
 - *C. albicans*
 - *C. parapsilosis*
 - *C. glabrata*
 - *C. tropicalis*
 - *C. rugosa*
 - *C. guilliermondii*
 - *C. krusei*
 - *Rhodotorula rubra*
 - *Trichosporon beigelli*
 - *Aspergillus* species
 - *A. fumigatus*
 - *A. flavus*
 - *Zygomycetes*
 - *Rhizopus*
 - *Mucor* spp.
 - Hyalohyphomycetes
 - *Fusarium* spp.
 - *Scedosporium apiospermum*
 - Phaeohyphomycetes
 - *Alternaria*
 - *Curvularia*
-

8% and 15% of burn injury patients depending on the extent of TBSA burn and length of stay in the burn unit (11–15).

The most commonly encountered opportunistic fungal pathogens in burn patients include *Candida* spp., *Aspergillus fumigatus*, *A. flavus*, *A. terreus*, *Zygomycetes*, *Fusarium* spp., *Trichosporon asahii*, *Alternaria*, and *Curvularia* (12–17) (Table 1). Of these, the *Candida* spp. are the most commonly encountered fungal pathogens in most burn units. Unfortunately, there are few recent epidemiologic studies evaluating the changing fungal flora of burn units and burn unit patients.

In the NEMIS study evaluating the incidence of nosocomial infections in the ICUs, burn units had the highest rates of nosocomial candidiasis at approximately 16.1/1000 admissions to the burn unit compared to an incidence of 9.6/1000 admissions to the SICU (7).

Although the current incidence of invasive burn wound infections due to *Candida* and systemic candidiasis is unknown, during the 1990s the incidence was estimated to be approximately 20% to 30% in patients with burns greater than 30% total surface area (3,18). In the study by Allen et al., the investigators found that in patients with a TBSA burn between 40% and 49%, 60% of these patients were eventually colonized by *Candida* spp. In patients with extensive TBSA burns of 60% to 90%, almost 100% of these patients were colonized by *Candida* spp. by the 10th day of burn unit stay (3). In a prospective, multicenter study evaluating the fungal epidemiology in different burn units, the investigators found that patients who had occlusive dressing over their burns were more likely to be colonized by *Candida* spp. when compared to those patients that had open dressings and had not been exposed to occlusive dressings (15). The true incidence of candidemia and invasive candidiasis, including invasive burn wound infections has yet to be accurately determined in any modern clinical trials (19,20). However, in those patients with severe and extensive infections, approximately 70% of those patients eventually develop either candidemia or invasive candidiasis (3).

A recent prospective study by Chen et al. evaluated the epidemiology of *Candida* in a tertiary care burn unit in Detroit (21). In their study, the authors evaluated both the environment and the patients prospectively over a period of six months. Twenty-two patients were followed during their hospitalization in the burn unit (ranging from 7 to 95 days, average length of stay was 30 days). During their stay, cultures were obtained weekly from at least three different surfaces. While almost 45% of the patients admitted to the burn unit were colonized at baseline, 66% of the patients were colonized by week 2, and 77% were culture positive by week 3. During the study period, 19 of the 22 patients were eventually colonized with *Candida*. A total of 100

Candida isolates were recovered from patients, 10 yeast isolates from environmental surfaces, and four yeast isolates from the burn unit staff. In contrast to *Candida* epidemiologic studies performed in medical and surgical ICUs, where *C. albicans* and *C. glabrata* tend to be the most commonly isolated species in the burn unit among the 19 patients colonized by *Candida*, 58% were colonized by *C. parapsilosis*, while 40% were colonized by *C. albicans*. Additionally, of the health care workers (HCW) surveyed, 42% were colonized with *Candida* spp. Seven of the HCW were colonized by *C. parapsilosis*, while three were colonized by *C. albicans*. Five environmental surfaces were culture positive for yeast, three were colonized with *C. parapsilosis*, one with *C. albicans*, and one surface had *Rhodotorula rubra*. Interestingly, after genomic karyotyping was performed on all of the isolates, significant sharing of isolates was found among the patients, the environmental surfaces, and the HCW. The results are similar to the prior epidemiologic studies frequently demonstrating a cross-contamination or colonization between patient, environment, and HCW in the burn units (22,23). In contrast, numerous epidemiologic studies performed in medical ICUs and surgical ICUs describe *C. albicans* and *C. glabrata* as the most common *Candida* spp. found on patients in these units. This increased frequency of *C. parapsilosis* isolates in burn units as a cause of either colonization or invasive infection has been previously described by several investigators (24,25). In addition to the difference in *Candida* spp. distribution, Chen et al. also described significant degrees of fluconazole and caspofungin/micafungin resistance among the *C. parapsilosis* isolates recovered from the patients. However, the investigators were not able to identify any other antifungal resistance among any of the *Candida* spp. recovered from either the HCW or the burn unit environment.

In a separate study of candidiasis in burn units, Dube et al. described candidemias and colonization in their unit due to nystatin-resistant, amphotericin-susceptible *C. rugosa* (26). The increase in infection by these isolates appeared to be associated to the use of topical nystatin dressings being utilized in their burn unit. In fact, the incidence of infection from these resistant isolates increased from 0.36% to 5.25%. After the withdrawal of nystatin dressings from the burn unit, the frequency of candidemia due to these isolates also decreased.

Although *C. parapsilosis* and *C. albicans* appear to be the predominant pathogens found in burn units, prior epidemiologic studies have also described candidemias and invasive candidiasis due to the other common *Candida* spp. such as *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. kefyr*, *C. guilliermondii*, and *C. lusitanae* (3,13,15). These few epidemiologic studies identify the complex nature of *Candida* epidemiology in the burn unit and the significant differences in *Candida* colonization between burn unit patients and patients in other ICUs (27).

Unfortunately, few epidemiologic studies in burn units have evaluated the incidence or the epidemiology of invasive fungal infections due to molds. In the 1990s, the distribution of invasive fungal infections due to molds in various burn units was to be approximately 60% to 70% due to *Aspergillus* and *Fusarium* spp., 10% due to *Zygomycetes*, and approximately 5% due to *Alternaria* and *Curvuleria* (12–14,17,28).

RISK FACTORS

Acute thermal injuries induce acute and chronic inflammatory responses. As with most fungal infections, host defects play a major role in the development of fungal colonization and subsequent infection. In addition to the conventional risk factors well recognized and associated with invasive fungal infections, there are several unique risk factors in the burn patients that place them at high risk for developing invasive fungal infections (12). This includes loss of the gastrointestinal (GI) barrier function, due to either stress-related mucosal ulcers or catecholamine-induced vasoconstriction of the mesenteric circulation leading to a decrease in total blood flow, especially within the villi where there is a higher resting oxygen consumption. Additionally, in burn patients there appears to be an increase in fungal and bacterial translocation from the GI tract due to alterations in the microbial microflora from frequent use of broad-spectrum antimicrobials and because of a generalized increase in capillary permeability resulting in edema (29–33). Furthermore, since all severely burned patients have a compromised immune function, there are well-described alterations inducing humoral and cellular suppression along with the increased production of immunosuppressive prostaglandins from stimulated macrophages. According to Abraham, the immunodeficiencies found in burn patients can be divided into two different types (34). Deficiencies in the inflammatory response and neutrophil function increases

Table 2 Risk Factors Associated with Fungal Infections in Burn Patients

-
- Increased fungal translocation
 - Increase fungal colonization rates
 - Alteration in microbial flora
 - Broad-spectrum antimicrobials
 - Mechanical ventilation
 - Increased capillary permeability resulting in generalized edema and increased translocation rates
 - Increased cortisol levels leading to increased translocation
 - Loss of barriers
 - Compromised gastrointestinal barrier
 - Stress-induced mucosal ulcerations and lesions
 - Catecholamine-induced vasoconstriction of splanchnic blood flow, especially in the distal villi with high oxygen consumption resulting in necrosis
 - Gastrointestinal surgery
 - Compromised cutaneous barriers
 - Central venous catheters
 - Avascular, necrotic wounds
 - Large open wounds with beds of neovascularized tissue
 - Compromised immunologic function
 - Increased prostaglandin production by altered macrophages
 - Alteration in cell-mediated immune function
 - Alteration in humoral immune function
-

the accessibility of microorganisms to the host through damaged epithelial and endothelial barriers and once the organisms have gained access to the damaged surfaces the alterations in the T- and B-cell function also allow invasion of microorganisms (29,35,36) (Table 2).

The burn patient also has other possible sources of invasive infection such as extensive open wounds and beds of neovascularized tissues that may lead to subeschar colonization with microorganisms and eventual systemic dissemination.

Candida spp. also contain their own well-recognized virulence factors. Some of the primary virulence factors especially involved in thermal injury include surface molecules that permit increased adherence, especially to diseased tissue, acid proteases, intrinsic proteolytic activity, and phospholipase production (37,38).

CLINICAL MANIFESTATIONS OF INVASIVE FUNGAL INFECTIONS

Eventually, most burn wounds become colonized with bacteria and frequently with yeast (39,40). The invasive fungal infections found in burn patients may be divided into either invasive fungal burn wound infections or systemic fungal infections, the latter, generally present as in any high-risk host.

Invasive Burn Wound Infections

Invasive burn wound infections due to either *Candida* spp., *Aspergillus* spp., or almost any opportunistic fungal pathogen are the most common and most important emerging causes of late-onset morbidity and mortality in patients with severe burns and severely compromised immune systems. In addition, for unknown reasons, children are at an even greater risk for developing burn wound fungal infections (1,41). Although burn wounds are sterile immediately after the thermal injury, these wounds eventually become colonized with the hospital-associated microorganisms, including fungi. As with bacterial wound infections, fungal burn wound infections tend to have a late-onset, generally occurring after the second week of thermal injury, and are usually diagnosed after a period of burn wound colonization with the yeast or molds that are normally seen in the patient's surrounding environment. The main concern regarding colonized and infected wounds is not only the fact that they have a propensity to heal much slower, but that they are also a significant source of systemic infection and can rapidly lead to the development of invasive fungal infections (42,43). Furthermore, fungal colonization and wound infection will also prevent adequate wound healing and skin grafting (42,43).

Although there are no classic or typical manifestations associated with an invasive burn wound infection, it is not uncommon for the edges of the wound to darken initially in the periphery and subsequently throughout the entire wound area, with an appearance similar to ecthyma gangrenosum.

Occasionally, burn wounds may also become infected with different genera of *Zygomycetes*, especially with *Rhizopus* or *Mucor* spp. (44). Zygomycosis is an increasingly common cause of life-threatening fungal infection in compromised host. Although infections due to these molds are not very common, they are important to diagnose early because of their ability to spread rapidly across different facial tissue planes as well as their ability to invade the local vasculature producing diffuse small vessel thrombosis resulting in local tissue necrosis and occasional systemic dissemination of the infection. The infections in the burn patient may be divided into primary and secondary. As with other forms of zygomycosis, cutaneous lesions are characterized by blood vessel invasion, black necrotic eschars, and gangrenous lesions. Fever and associated changes in the appearance of the burn wound may provide clues to the infection. Rarely superinfected burn wounds have also been associated with the use of elastoplast bandages (44). Hematogenous dissemination may also occur with spread to the skin producing zygomycotic nodules. Infections due to the *Zygomycetes* are extremely difficult to diagnose unless a biopsy and histopathological analysis of the affected site are performed. The management of these infections continues to be difficult and they continue to have extremely high mortality rates.

There are four main types of burn wound infections, burn wound impetigo, burn-related surgical wounds infection, burn wound cellulitis, and invasive infection in unexcised burn wounds. The latter are the burn wounds more frequently associated with the development of disseminated fungal infections, septic shock, multiorgan dysfunction, and death.

Diagnosis of Invasive Burn Wound Infections

The diagnosis of invasive wound infections is extremely difficult because of the fact that most burn wounds will eventually become colonized with opportunistic pathogens. Surface wound cultures are frequently performed in all burn patients. They are easy to do and are relatively inexpensive, but do not accurately reflect or predict the invading organism in invasive or systemic infections.

The diagnosis of burn wound infections can only reliably be made by histological examination of affected tissue along with microbiological examination and analysis of the tissue (45). It is essential to recognize the difference between the significance of a positive superficial wound culture and "true" infection. In most situations, fungal pathogens recovered from an open wound will be considered colonizers unless there is evidence that these pathogens have become invasive, or there are local signs or symptoms of infection. Unfortunately, to date, there are no significant clinical studies that have evaluated the correlation of wound organism colonization and their association with invasive burn wound infections. The clinical diagnosis of a burn wound infection relies on regular monitoring of vital signs and inspection of the wound surface. Local signs of infection with invasion include conversion of a partial thickness to a full-thickness wound, rapidly extending cellulitis of healthy tissue surrounding the injury, rapid eschar separation, and adjacent tissue necrosis.

Thus, the decision to initiate aggressive systemic antifungal therapy should be dictated by the probability that the patient has a true invasive burn wound infection and is not just colonized with fungi. The "gold standard" in making a diagnosis of an invasive burn wound infection still continues to be a tissue biopsy with microbiological cultures. The diagnosis of invasive infection is generally characterized by the finding on histological analysis showing invasion of organisms into the dermis beneath the eschar and surrounding healthy tissue. However, we do have to realize that invasive fungal infections frequently originate as an endogenous source, either from a heavily colonized wound or via a contaminated line.

Quantitative wound biopsies and cultures of burn wounds are frequently performed and are beneficial when the microorganism counts are greater than 100,000 cfu's/g of tissue. This quantitation has been utilized to predict bacterial invasive wounds, and not invasive wounds due to fungi (12). Additionally, the sensitivity of the quantitative cultures decreases later on in the course of a burn wound after the burn eschar begins to separate or is excised. However, in

one study, 87% of patients with quantitative wound cultures of $>100,000$ cfu/g of tissue went on to develop an invasive wound infection with associated sepsis.

In addition to the local manifestations of infected burn wounds, it is not uncommon in burn patients to be otherwise asymptomatic. In essence, it is not uncommon for patients to be afebrile and have nonspecific signs or symptoms of active infection despite having an invasive wound infection.

Candida can also produce either candidemia or disseminated candidiasis as is typically manifested in patients hospitalized SICU or MICUs and with similar risk factors. The manifestations of candidemia and systemic candidiasis are similar to those manifestations seen in other high-risk patients, except that the burn patients are typically hyperdynamic and this may consistently mimic a systemic inflammatory response syndrome or sepsis. It is also important to note that although blood cultures may be helpful in the diagnosis of candidemia, the blood culture positivity rate even in highly colonized patients may be no more than 60%. In contrast to other high-risk groups, candidemia in the burn unit population has been associated with mortality rates of 50% to 77%, substantially higher than the mortality rates (30–40%) observed with other high-risk groups.

Although in burn patients, infections due to molds are not as common as infections due to *Candida*, if the infection disseminates, the systemic manifestations are similar to those seen in other immunocompromised host. However, the most common site of infection in these patients is the burn wound and eschar, instead of the respiratory tract, the central nervous system, or the sinuses as has been described in compromised hosts such as those seen in with neutropenia, patients on corticosteroids, stem cell transplants, or solid transplant recipients.

TREATMENT

Even though it is virtually inevitable that most burn wounds will be colonized by several different kinds of microorganisms, it is not uncommon in some of the smaller burn wounds that these organisms may remain in low enough numbers with topical therapy alone and heal successfully. However, in most situations the organisms will continue to multiply, invade the affected necrotic tissues, especially those with minimal blood flow (eschar), subsequently damaging the affected tissues, delaying wound healing, and occasionally causing infected burn wounds and a systemic fungal infection. The potential for colonizing fungi to produce harmful effects is generally influenced by the degree of immunosuppression of the host, the number of fungi in the wound, and the amount of blood flow to the wound and the surrounding area. In all patients, it is imperative to attempt to differentiate between contamination of a wound, colonization of a wound, localized infection, and systemic infection (45). In the normal host with either wound contamination or wound colonization, they may not require antimicrobial or antifungal therapy. However, in burn wounds that are colonized with fungi, not only is there a risk for developing a systemic infection, but the organisms in the wound can also lead to wound deterioration and significantly impair wound healing because of the production of metalloproteinases and their detrimental effect on granulation tissue.

It is also important to recognize that the improper care and management of infected burn wounds may actually lead to increase in the size and depth of the original burn wound. In many situations, the treatment of invasive burn wounds due to fungi are extremely difficult to manage and will require not only appropriate antimicrobial therapy, but also surgical debridement. Early, emergent, and aggressive wide surgical excision and debridement of infected burn wounds or eschar is one of the primary principles of treatment and may lead to an improved chance of survival (4,46). Antimicrobials alone will not completely eradicate the microorganisms from the burn wound. Excision of eschar removes the possible source of infection, but is also dangerous because it may induce bacteremia and fungemia during the surgical removal of heavily colonized or infected tissue. Wound infections developing in previously excised wounds may respond to systemic antimicrobials alone if there is a significant blood supply to the infected area.

Early Presumptive Therapy

As stated previously, given the difficulty in establishing a diagnosis of invasive candidiasis, it is imperative that the clinician be aware of the more common risk factors associated with

Candida colonization and subsequent infection. Thus, in addition to the appropriate initiation and use of antifungals in patients with documented evidence of invasive burn wounds and systemic infections, consideration must be given to the occasional use of antifungals prior to the development of "full blown" infection (46,47). There are several studies demonstrating that the use of antifungals early on, in selective high-risk patients, may decrease the overall mortality rate in patients (48). The main concern is how and when does the clinician make the decision to initiate systemic antifungal therapy when there is no good definitive evidence of active infection and there are no adequate studies as to when to initiate therapy.

PROPHYLAXIS

There is significant controversy regarding the prophylactic use of antimicrobials in burn patients. Careful and aggressive wound care and topical agents are adequate for wounds that do not demonstrate any evidence of infection or systemic sepsis. In several recent clinical trials, widespread application of an effective topical antimicrobial agent substantially reduces the colony-forming units on the burn wound surface and leads to a reduction in the incidence of burn wounds and systemic sepsis (4). However, the overuse of systemic or topical antimicrobials can also be detrimental. There is enough evidence to predict that the inappropriate usage of antimicrobials may lead to the development of resistance. This has been demonstrated by two different studies by Chen and Dube that have demonstrated the development of echinocandin and nystatin resistance with the use of either topical or systemic antifungals (21,26).

Recent studies have demonstrated that silver-based topical antimicrobials have a benefit over the use of combination therapy with an antibacterial and antifungal in infected wounds, especially infected burn wounds. Silver ions possess potent bactericidal and fungicidal properties due to their strong interaction with thiol groups present in the respiratory enzymes in cells (49,50). Silver nitrate is rarely used nowadays, because the deposition of silver discolors the wound bed, has the potential for direct toxicity, and is difficult to regulate the concentration of silver ions to the wounds. The newer nanocrystalline silver products (Acticoat AB) consist of nanocrystalline silver coating of two sheets of high-density polyethylene mesh (50). These products offer controlled and more prolonged silver ion release and sustained concentrations to the burn wounds without the risk of tissue damage and discomfort (49,50). In addition, these products also offer high and sustained concentrations of silver to the burn area with the hope of avoiding the induction of heavy metal resistance. Additionally, if the burn wound has minimal exudate, the dressing can remain in place from three to seven days without replacement and continued activity. These new silver dressings are quickly replacing silver-based creams in many burn centers.

The use of laminar air flow rooms in combination with optimal local wound care also appears to significantly decrease the incidence of both local and systemic fungal infections.

In conclusion, fungal infections in these high-risk, immunocompromised patients remain a common cause of morbidity and mortality. In this patient population, a high index of suspicion for cutaneous or invasive fungal infections in the high-risk patient, followed by careful evaluation and screening, and evaluation of the patient's environment of cross-infection along with early and appropriate antifungal therapy may help reduce the morbidity and mortality in burn patients. More importantly because of the differences in host and host characteristics, continued research with the new antifungals is mandatory.

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INTRODUCTION

Allergic bronchopulmonary aspergillosis (ABPA) is a lung disease caused by an immunologic response to the mold *Aspergillus fumigatus*, and rarely other *Aspergillus* species. The disease is mostly described in genetically susceptible asthmatics and cystic fibrosis (CF) patients. An exaggerated host immune response involving Th2 CD4+T-cells, IgE and IgG antibodies is central to the pathogenesis of ABPA. The diagnosis of ABPA is often difficult and requires the combination of clinical, radiographic, and laboratory criteria.

This chapter reviews the major pathologic manifestations of ABPA, clinical, radiographic, and laboratory features of the disease and current knowledge about its pathogenesis and treatment.

PREVALENCE

ABPA was first reported in 1890 and later described by Hinson et al. in 1952 (1) in 12 adult asthmatic patients with recurrent pulmonary infiltrates, eosinophilia (blood and sputum), and *Aspergillus* hyphae in their sputum. In 1965, the disease was recognized in two children with CF and since then it has been diagnosed with increasing frequency in CF. Despite being a well-recognized complication of CF and asthma, the exact prevalence of ABPA is not clearly known. It is estimated that the prevalence of ABPA ranges from 2% to 25% in patients with CF (2–5) and from 1% to 8% in patients with asthma (6–9). Discrepancies in reported prevalence rates are mainly due to the lack of accepted uniform criteria in the diagnosis of ABPA, variability in the laboratory tests for diagnosis, as well as underrecognition of the disease by clinicians (7). For instance, the Epidemiologic Study of Cystic Fibrosis (ESCF), a large multicenter, prospective, observational study of the natural history and clinical course of patients with CF in North America, reported a prevalence rate of only 2.2% (10). In this study, underdiagnosis and under-reporting were the most likely explanation for the lower-than-expected prevalence of ABPA in CF. Similarly, the Epidemiologic Registry of Cystic Fibrosis (ERCF) reported data for 12,447 patients with CF from 224 CF centers in nine European countries (5). The overall prevalence was 7.8%, with a range of 2% in Sweden to 14% in Belgium (5). The ERCF used diagnostic criteria of total IgE concentration of >1000 IU/mL with positive results of skin testing and presence of serum precipitins to *A. fumigatus* together with additional clinical or laboratory parameters. Besides asthma and CF, ABPA has been reported in patients presenting with other pulmonary conditions (11–13). ABPA has also been described in patients with hyper-IgE syndrome and chronic granulomatous disease (14).

PREDISPOSING FACTORS

Factors that have been associated with the development of ABPA include patients with airway disease such as cystic fibrosis and asthma, mucous hypersecretion, and impaired mucociliary clearance, HLADR2, IL-10 promoter polymorphisms, surfactant protein polymorphisms, and CFTR gene mutation (15). It is hypothesized that ABPA develops in asthmatic and CF patients due to a combination of genetic susceptibility factors including HLA-DR2 and DR5 restriction, single nucleotide polymorphisms (SNP) of the IL-4 receptor alpha chain (IL-4R α), and other genetic risk factors in particular IL-13, IL-10, and surfactant protein polymorphisms and CFTR gene mutations (15,16). In addition to genetic predisposition, epithelial cell activation in individuals with asthma or CF and impaired mucus clearance in CF patients (17) as well as proteolytic enzymes secreted by *Aspergillus* (18) have all been incriminated in the development of ABPA.

Genetic Predisposition

T-cell reactivity to *A. fumigatus* is an important step in the pathogenesis of ABPA, which influences sensitization to *A. fumigatus* antigens. MHC (major histocompatibility complex) class II molecules on antigen-presenting cells are required for the activation of antigen-specific T-cells. The CD4+Th2 lymphocytes from ABPA patients are restricted to six MHC class II HLD-R subtypes. Genetic studies suggest that HLA-DR molecules (DR2, DR5, and possibly, DR4 or DR7) are associated with increased susceptibility to ABPA, whereas HLA-DQ2 molecules are associated with resistance (19,20). Additionally, patients with ABPA who do not have CF have an uncommonly high frequency of mutations in the cystic fibrosis transmembrane conductor regulator (CFTR) gene indicating that CFTR gene mutations are involved in the development of ABPA (21,22). Marchand et al. found that the frequency of cystic CFTR gene mutations was high in patients with ABPA compared to those with allergic asthma, even though both groups showed normal sweat chloride concentrations (23). Several gene polymorphisms have also been found to be associated with ABPA. Saxena et al. identified an association between polymorphisms in the collagen region of pulmonary surfactant protein-A2 and a predisposition to ABPA and severity of the disease (24,25). Polymorphisms of the promoter region of the IL-10 gene were shown to be associated with *Aspergillus* colonization and the development of ABPA in patients with CF (26). Similarly, the presence of an IL-4R α single nucleotide polymorphism, principally ile75val, has been shown to be a possible genetic risk factor for ABPA (16).

Aspergillus Characteristics

It is worthwhile to look at the biology of *A. fumigatus* for a better understanding of the pathophysiology of ABPA. *A. fumigatus* is the most common *Aspergillus* species associated with ABPA. Other *Aspergillus* species such as *A. niger*, *A. flavus*, *A. nidulans*, *A. oryzae*, or *A. glaucus* have occasionally been incriminated in this disease (27–29). *A. fumigatus* is a ubiquitous mold found in the environment, decaying organic materials, soil spaces, wood chips, mown vegetation, basements/indoor air, walls, or ceilings, particularly when these environments contain moisture. In the mycelium phase, *Aspergillus* exists in the form of 7–10- μ m long, septate, uniform hyphae with dichotomous branching at an angle of 45°. Reproduction is characterized by the formation of conidiophores with terminal vesicles producing chains of spores. Several biological characteristics have been implicated in the pathogenesis of *A. fumigatus*. These include a spore size of 3 to 5 μ m, and a rapid growth rate and secretion of virulence factors. The small spores' sizes allow the spores to penetrate deeply into the lung and attach to the epithelium. Moreover, spores are thermoresistant (grow at temperatures ranging from 15 °C to 53 °C), capable of withstanding unusual atmospheric conditions and suboptimal host defenses because of the presence of hydrophobic protein coat layer composed of rodlet fascicles (30,31). Once the spores reach the airway epithelium, they germinate into hyphae (32). It was shown that hyphal extension and overall fungal mass increase in a logarithmic manner in ~24 hours before reaching a plateau (33). The hyphal growth is accelerated in the presence of glucocorticoids therapy. *A. fumigatus* has a doubling time of 48 minutes and a hyphal extension rate of 1 to 2 cm/hr when grown with hydrocortisone in vitro (33). Such characteristics might explain the severity of *Aspergillus* pulmonary invasion in the presence of steroids therapy. In addition to spores' size and growth rate, various virulence factors of *A. fumigatus* have been described to play a role in the severity of lung infection by disturbing bronchial epithelium integrity. For instance, conidia produce an inhibitor of the oxidative burst. The fungus also produces various proteases (34,35), ribotoxin (36,37), phospholipases (38), hemolysin (39), gliotoxin (40), and phthioic acid (41). Kauffman's group demonstrated that *Aspergillus* proteases promote pulmonary epithelial cell detachment (42). Furthermore, they showed that *Aspergillus* culture filtrates containing protease induce human bronchial cell lines to produce proinflammatory chemokines and cytokine such as IL-8, IL-6, and monocyte chemoattractant protein (MCP)-1. This protease activity allows for enhanced allergens exposure to the bronchoalveolar lymphoid tissue (BALT) immune system. Several proteases are immunogenic, including Asp f5, f10, f13, f15, and f18. Gliotoxin has been shown to reduce macrophage and neutrophil phagocytosis (40). Phthioic acid may contribute to granuloma formation, as it may do in tuberculosis. *Aspergillus* also produces a number of superoxide dismutases (43), catalases (24,44), and mannitol (45). These enzymes may

protect the fungus from damage from singlet oxygen, hydrogen peroxide, hydroxyl, and other free radicals produced by phagocytes. One superoxide dismutase (Asp f6) and catalase B are immunogenic.

PATHOPHYSIOLOGY OF ABPA

In the proposed model of the immunopathogenesis of ABPA, *A. fumigatus* spores, 3 to 5 μm in size, are inhaled into the bronchial airway, where they are trapped by the luminal mucus, germinate, and form mycelia (46). Hyphae can also be identified within the interstitia of the pulmonary parenchyma allowing for *A. fumigatus* allergens to be exposed to the respiratory epithelium and immune system. *A. fumigatus* mycelia release allergens that are processed by antigen-presenting cells bearing HLA-DR2 or -DR5 and presented to T-cells within the bronchoalveolar lymphoid tissue (BALT). The T-cell response to *Aspergillus* allergens becomes skewed toward a Th2 CD4⁺ cell response, with synthesis and secretion of cytokines IL-4, IL-5, and IL-13 (18).

A combination of factors may lead to a greater bronchial adherence of *Aspergillus* and high levels of *Aspergillus* antigen absorption in patients developing ABPA: proteolytic enzymes secreted by *Aspergillus* (17), epithelial cell activation in individuals with asthma or CF and impaired mucus clearance in CF cases (18). Indeed, *A. fumigatus* activates epithelial cells, resulting in the secretion of IL-6 and IL-8 and upregulation of epithelial cell detachment (42). Destruction of the epithelial cell barrier either by proteases from the fungus or by eosinophilic and neutrophilic inflammation is followed by repair mechanisms, resulting in the influx of serum proteins and extracellular matrix proteins to the lumen site of the epithelium (47). Because spores and mycelium of *A. fumigatus* have surface structures that are able to interact with extracellular matrix molecules, damage and concurrent repair mechanisms of the airway mucosa may facilitate the binding of *Aspergillus* to the damaged sites of the airways. The enhanced release of proteolytic enzymes and allergens on the epithelial surface induces a continuous inflammatory response and mast cell degranulation, resulting in severe and long-lasting periods of exacerbations of ABPA.

In addition to the induction of cytokine response in epithelial cells, it has been shown that proteases from *A. fumigatus* at higher concentrations also caused hypofunction of epithelial cells, even below the spontaneous cytokine production of epithelial cells; this is in contrast to proteases from other fungi, which do not reduce cytokine production (48). The depression of the epithelial responsiveness, which is specific for the elastase- and collagenase-containing extracts of *A. fumigatus*, may represent an additional virulence factor by preventing effective targeting by infiltrative phagocytic cells, due to the lower concentrations of chemokines in the direct environment of the fungus.

In brief, continuous release of antigens and allergens induces a strong activation of the Th2-type immunologic response, with very high production of total and specific IgE antibody, and an additional Th1 response, with formation of IgG and IgA antibodies to antigens of *A. fumigatus*. ABPA, compared to other atopic diseases, has increased sensitivity to IL-4 from Th2 CD4⁺ cells with increased B-cell CD23 and CD86 expressions, and increased IgE production (49,50). Furthermore, patients with ABPA develop IgE antibodies to the specific *Aspergillus* proteins Asp f2, Asp f4, and/or Asp f6, whereas atopic patients develop IgE antibodies to Asp f1 and/or Asp f3 (51–55). It is hypothesized that mycelial formation and secretion of proteins in ABPA is necessary to trigger these events, suggesting that the colonization in patients with ABPA is greater than that in *Aspergillus*-sensitive atopic patients. In ABPA, extremely elevated total serum IgE concentrations and elevated levels of IgE anti-*Aspergillus* antibody are manifest. This high level of total and specific anti-*Aspergillus* IgE antibody responses has been described by several groups (56–61). Although other *Aspergillus*-exposed patients develop IgE, IgG, and IgA anti-*Aspergillus* antibodies, there is a quantitative increase in IgE anti-*Aspergillus* antibodies in patients with ABPA. It was shown that B-cells obtained from patients with ABPA spontaneously synthesize increased amounts of IgE in vitro compared with that synthesized by *Aspergillus*-sensitized patients without ABPA, indicating in vivo activation of IgE immunoblasts (62). Greenberger and Patterson (59) further demonstrated that the total IgE in the systemic lymphoid tissue constituted only a fraction of the specific anti-*Aspergillus* IgE antibodies implying activation of clones of B-cells in the systemic immune system by CD4⁺ Th2 cells.

The consequences of this response are intense airway inflammation, airway damage, and remodeling with the development of bronchiectasis and fibrosis. This makes a timely diagnosis critical.

CLINICAL CHARACTERISTICS AND STAGES OF ABPA

The diagnosis of ABPA requires a high degree of clinical suspicion in the right clinical setting. Diagnostic criteria for ABPA have been standardized and require the following criteria (63–65): (i) history of asthma, (ii) chest roentgenographic infiltrates—current or in the past—may be detectable on CT when chest radiography is unremarkable, (iii) immediate cutaneous reactivity to *Aspergillus* species, (iv) elevated total serum IgE (>417 IU/mL or >1000 ng/mL), (v) serum precipitating antibodies to *A. fumigatus*, (vi) central bronchiectasis on chest CT, (vii) peripheral blood eosinophilia, and (viii) elevated serum IgE and/or IgG to *A. fumigatus*. Not all asthmatics with ABPA meet all these criteria, especially if they are diagnosed early or are taking systemic corticosteroids. For this reason, Greenberger and Patterson (65) proposed two sets of minimum criteria to diagnose ABPA in asthmatic patients: one set of guideline is for patients with central bronchiectasis and a second set for those without bronchiectasis. It has been suggested that the minimal essential criteria for diagnosis of ABPA include the following (65): (i) asthma, (ii) immediate cutaneous reactivity to *Aspergillus* species, (iii) elevated total serum IgE concentration (>417 IU/mL or >1000 ng/mL), (iv) elevated serum IgE to *A. fumigatus* and IgG to *A. fumigatus*, and (v) central bronchiectasis. The presence of precipitating antibodies to *A. fumigatus* was found to support the diagnosis of ABPA, and in patients with ABPA, total IgE levels were found to predict disease exacerbations. Even these criteria may be too rigid, because use of prednisone or other oral corticosteroid agents can reduce the total serum IgE concentration to <1000 ng/mL in some patients with ABPA.

Patterson et al. have also subdivided the clinical course of ABPA into five stages: stage I: acute, stage II: remission, stage III: exacerbation, stage IV: corticoid-dependent asthma and Stage V or end stage: fibrosis (63). Patients do not necessarily progress sequentially through each of the stages, and with an emphasis on early diagnosis, the diagnosis can often be made before an acute presentation occurs (stage I) or advanced fibrosis develops (stage V).

Although ABPA is not an uncommon complication of CF, the diagnosis in this setting is difficult. One reason is that several of the criteria used to diagnose ABPA are themselves common manifestations of CF. Recently, the Cystic Fibrosis Foundation has recommended its own set of classic and minimal criteria for the diagnosis of ABPA (46). These criteria focus on detection of exacerbations of ABPA that are responsible for a significant illness burden.

Screening for ABPA

The recommended initial screening test for patients with asthma is cutaneous testing for *Aspergillus* species (66,67). In the absence of positive cutaneous testing, ABPA can be eliminated from the differential diagnosis (68). However, positive reactivity is not specific for ABPA. The prevalence of skin reactivity to *Aspergillus* is 23% to 28% in patients with asthma (6,9) and 29% in patients with CF (4). Cutaneous skin reactions to *A. fumigatus* antigens have been found in 31% of patients with CF without ABPA (69). A positive skin prick test must therefore be followed by further serologic and radiologic testing to determine whether minimum diagnostic criteria are met (70). The Consensus Conference recommendations for screening for ABPA in CF advocate to maintain a high level of suspicion for ABPA in patients >6 years of age and to measure total serum IgE annually. Two scenarios are described: If total serum IgE is >500 kU/L, immediate cutaneous reactivity to *Aspergillus fumigatus* should be done and the diagnosis of ABPA should be considered based on the minimal criteria described above. If total serum IgE level is between 200 and 500 kU/L, it is recommended to repeat IgE level measurement and perform further diagnostic tests if suspicion is high.

Other Diagnostic Tests

In addition to the diagnostic criteria, several additional findings are consistent with a diagnosis of ABPA. Sputum from patients with ABPA might contain golden-brown mucus plugs, fungal mycelia, and/or large numbers of eosinophils (46).

However, the presence of *A. fumigatus* in sputum is not specific for ABPA. Chest radiographs and computed tomography aid with the diagnosis, detection of complication, and staging of ABPA. Infiltrates typically involve the middle or upper lobes and may be transient or permanent. Central bronchiectasis is one of the hallmarks of ABPA. It usually involves the inner two-thirds of the lung. High resolution CT scan is more sensitive than chest X-ray for the detection of pulmonary infiltrate or bronchiectasis. Pulmonary fibrosis, pneumothorax, and cavities occur during end-stage ABPA (71–73).

Treatment of ABPA

Therapy for ABPA consists of prophylaxis against and treatment of acute exacerbations, to prevent end-stage fibrotic disease (74). There are two strategies of treatment of ABPA. The first is the attenuation of the inflammation and immunologic response, for which corticosteroids are the mainstay of therapy (75). The second is the attenuation of the antigen burden arising from fungal colonization of the bronchial tree (76). The Infectious Disease Society of America recommends the combination of corticosteroids and itraconazole (Evidence level A–I) for the treatment of ABPA (77).

Treatment protocols emerged from uncontrolled series of patients responding to regimen of oral corticosteroids; however, dose regimen and duration have never been standardized. For instance, Wang et al. (78) proposed an oral steroid regimen based on experience with 25 patients with asthma and ABPA. Similar results were reported in 33 patients by Capewell et al. (79). In this study, long-term use of oral prednisolone in patients with ABPA resulted in long-term preservation of pulmonary function. Oral steroids have been adopted for use in patients with CF and ABPA despite the lack of controlled trial evaluating their effectiveness in CF. In general, oral steroids are indicated in all cases of ABPA exacerbation. An initial course of at least 0.5 mg/kg/day of prednisolone or equivalent to a maximum of 60 mg daily is given for one to two weeks followed by switching to a regimen of alternate day administration at the same dose for another one to two weeks and subsequent taper over two to three months until oral steroids are discontinued (80). Unfortunately, the use of oral steroids in ABPA is problematic due to the frequency of relapse after discontinuation, metabolic toxicities, and the lack of steroids effect on airway fungal burden. Therefore, alternative approaches to management of ABPA have been developed. Reducing the fungal burden in the respiratory tract might decrease antigenic stimulation, reduce inflammatory response, ameliorate symptoms, and possibly reduce the long-term risk of disease progression. In this regard, itraconazole has been suggested as a corticosteroid-sparing agent for the treatment of ABPA (81). Two double-blind, randomized, placebo-controlled trials for ABPA demonstrated that itraconazole (200 mg twice daily orally for 16 weeks) resulted in significant difference in the ability to ameliorate disease, as assessed by the reduction in corticosteroid dose, increased interval between corticosteroid courses, eosinophilic inflammatory parameters, and IgE concentration, as well as improvement in exercise tolerance and pulmonary function (82,83). Benefits continued and were realized in some initial nonresponders in a follow-up open-label study phase (83). Similar benefits of itraconazole were observed in patients with cystic fibrosis and ABPA (84). Despite combined use of oral steroids and itraconazole, many patients with ABPA present ongoing challenges. Yet, only small uncontrolled studies of alternative agents have been reported. Nebulized amphotericin B has been used in patients with ABPA and CF with apparent tolerance and clinical success (85). Voriconazole has also been used with some clinical success despite its side effects (86). In addition, both itraconazole and voriconazole have been used in some ABPA patients with CF as monotherapy with mixed results (87). High dose pulse steroids therapy has been tried in patients with CF and refractory ABPA with some promising outcome (88). No controlled trials of voriconazole, inhaled amphotericin, or IV pulse steroids have been published.

Several case reports and small series described the clinical benefit of the use of inhaled steroid in the treatment of ABPA in asthmatic patients (89–92). However, the only double-blind, placebo-controlled trial of inhaled steroids using beclomethasone at a dose of 400 µg daily for ABPA conducted by the British Thoracic Society failed to show a statistically significant benefit (93). Furthermore, there are now numerous reports of toxicity using budesonide in combination with itraconazole resulting in adrenal suppression in both asthma and CF patients with ABPA (94–98) owing to itraconazole inhibition of the hepatic cytochrome P4503A4, which

is responsible for metabolic detoxification of budesonide. The lack of clearly established benefit coupled with concerns for toxicity lead to great caution when considering use of inhaled steroids especially budesonide in combination with itraconazole to treat ABPA.

CONCLUSION

In conclusion, systemic corticosteroid in combination with itraconazole is now considered the standard of care for ABPA as suggested by the Infectious Disease Society of America. Further studies should focus on controlled trials of antifungal and immunomodulatory agents to spare patients from steroid side effects.

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30 Fungal Infections of the Genitourinary Tract

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INTRODUCTION

Fungal infections of the urinary tract encompass a broad variety of fungi including the endemic mycosis, *Cryptococcus* species, and opportunistic pathogens such as *Aspergillus* species (1–3). However, the overwhelming majority of fungal infections of the urinary tract are caused by *Candida* spp. The escalating use of broad-spectrum antimicrobial agents, corticosteroids, and immunosuppressive and cytotoxic drugs with the frequent use of indwelling urinary catheters have been implicated as risk factors for *Candida* urinary tract infections (UTI) (4). The presence of candiduria may signal diverse pathological states, including invasive renal parenchymal disease, fungal balls in obstructed ureters, superficial lower urinary tract infection, and lower urinary tract candidal colonization associated with urinary catheterization. Accordingly, the spectrums of clinical disease embrace asymptomatic candiduria, cystitis, pyelonephritis, and renal candidiasis. *Candida* spp. have the propensity to cause renal disease by either the hematogenous or the ascending route, which is the most common route of *Candida* UTI generally occurring in the setting of bladder instrumentation. Candiduria has always posed a diagnostic and therapeutic challenge as its presence does not always mean infection, and, therefore, may not require treatment. Unfortunately, there are no established diagnostic tests that reliably distinguish infection from colonization. Guidelines for the treatment of candiduria, based almost entirely on anecdotal reports and experts' opinion rather than on controlled clinical trials, have been suggested by the Infectious Diseases Society of America (IDSA) and the Mycoses Study Group (5).

The urinary tract is also susceptible to infection by another group of fungi, namely the endemic fungi including *Histoplasma*, *Blastomyces*, and *Coccidioides* spp. However, these pathogens are rarely responsible for the common clinical syndromes of urethritis, cystitis, and pyelonephritis. Instead, they occasionally cause prostatitis, epididymitis, chronic bladder inflammation or ulceration, ureteric obstruction, and chronic renal disease (1).

This chapter focuses on the pathogenesis and the spectrum of clinical manifestations of fungal UTIs. Special emphasis has been placed on the various therapeutic approaches.

GENITOURINARY CANDIDIASIS

Epidemiology

Candida is a frequent saprophytic inhabitant of the oral cavity, intestinal tract, external genitalia, and urethra; the mouth and gastrointestinal tract where 30% to 50% of normal individuals are colonized with *Candida* spp. Point prevalence studies indicate that 20% to 25% of healthy women have positive vaginal culture for *Candida albicans* (6). However, isolation of *Candida* cells from pure urine culture of healthy individuals is rather infrequent. In one study, funguria was found in only 1% of patients; half of them had diabetes mellitus and were receiving antibiotics (7). In contrast, candiduria is a common problem in hospitalized patients with indwelling urinary catheters occurring in up to 83% of patients with urinary tract drainage devices (4). *Candida* spp. are responsible for 10% to 15% of nosocomial UTI and up to 26.5% of UTIs in catheterized patients (8–11). *C. albicans* is the most common pathogen incriminated in UTIs in patients admitted to the intensive care unit (ICU) and it ranks second after *Escherichia coli* in non-ICU patients (12,13).

Microbiology

C. albicans is the most common fungal pathogen in UTIs accounting for more than half of the cases of fungal UTIs (4,14). *C. glabrata* accounts for 25% to 35% of infections whereas other *Candida* spp., including *C. tropicalis*, *C. krusei*, and *C. parapsilosis*, account for 8% to 28% of cases (1). *C. parapsilosis* is found more often in urine from neonates and is usually associated with systemic infection in this population (15). In about 10% of cases, more than one species of *Candida* can be isolated and candiduria can coexist with or follow bacteriuria (14). Since many hospital laboratories do not speciate yeasts when recovered from urinary culture unless requested to do so, changes in species trends cannot be easily tracked.

Pathogenesis and Risk Factors

Ascending infection is the most common route for infection of the urinary tract. Women have a shorter urethra and are more prone to develop UTI by the ascending route secondary to vulvovestibular colonization by *Candida* spp. The presence of urinary devices facilitates the introduction of the organism into the bladder. The pathogenesis of ascending infection with *Candida* spp. is not well known since no animal models exist to study this condition. Ascending infection might lead to upper urinary tract infection in the setting of vesicoureteral reflux or obstruction of the urinary flow resulting in pyelonephritis but rarely leading to disseminated infection such as candidemia. A fungus ball consisting of yeast cells, hyphal elements, epithelial and inflammatory cells can form in dilated areas of the urinary tract resulting in a complicated UTI (1,16).

Renal candidiasis is the result of hematogenous seeding of the renal parenchyma. The pathogenesis of hematogenous seeding of *Candida* spp. to the kidney is well established (16–19). Multiple microabscesses will develop over the cortex secondary to candidemia. *Candida* cells thereafter penetrate through the glomeruli into the proximal tubules to be shed in the urine (19,20). The presence of yeast in the urine might imply widespread dissemination to many organs. It is believed that *Candida* spp. express special tropism to the kidney. In an autopsy study, renal involvement was noted in 90% of patients dying from disseminated candidiasis. Multiple abscesses in the renal interstitium, the glomeruli, and peritubular vessels associated with papillary necrosis were seen in these autopsies (20,21).

Risk factors for *Candida* UTI are well described in the literature and include diabetes mellitus, previous use of broad-spectrum antibiotics, corticosteroids and cytotoxic agents, indwelling urinary catheters, old age, malignancy, prior surgical procedures, recent hospitalization, as well as structural and functional abnormalities of the urinary tract (22,23). Candiduria is a common finding in renal transplant recipients with an incidence rate reaching 11% in one study (24). Risk factors for *Candida* UTI in renal transplant recipients are similar to those in hospitalized patients who have not received a transplant. However, in these patients, non-*albicans* spp. are more frequently isolated from the urine. In one study, *C. glabrata* accounted for more than one half (53%) of *Candida* UTI, followed by *C. albicans* (35%) and *C. parapsilosis* (4%) (23). This increase in the incidence of *C. glabrata* could be attributed to the overuse of fluconazole prescribed for antifungal prophylaxis, which selects for the emergence of *C. glabrata* (24).

Diabetes mellitus is the single most common underlying disease noted in most studies about funguria (4,14). In one retrospective study, 30% of patients with fungal UTI were diabetic and in a prospective multicenter surveillance study in the United States, up to 40% of patients with funguria had diabetes (4,22). This association is related in part to insulin deficiency which affects the intracellular killing system including the myeloperoxidase, hydrogen peroxide, and the superoxide anion system of polymorphonuclear leukocytes leading to impairment of the phagocytic and fungicidal activity of neutrophils against *Candida* spp. Furthermore, the growth of *Candida* in urine is more enhanced when urinary levels of glucose exceed 150 mg/dL. In addition, diabetic female patients have a higher rate of *Candida* colonization of their perineum putting them at higher risks for *Candida* UTI. The most important predisposing factor for *Candida* UTI in the diabetic population is the presence of diabetic autonomic neuropathy leading to a neurogenic bladder that sets up a favorable milieu for fungal UTIs due to stasis of the urine, and in some cases to the frequent need for instrumentation and use of drainage devices.

Additional risk factors for the development of candiduria include the frequent use of broad-spectrum antibiotics. Antibiotics suppress the normal flora of the perineum, allowing

overgrowth of opportunistic fungi with subsequent ingress over the urethra and colonization or infection of the bladder. In one study, *Candida* was found in the urine of 93% of patients who had recently received antibiotics (14). No antibiotic is exempt from this complication. However, carbapenems and third-generation cephalosporin such as ceftazidime were found to be associated with the highest rates of *Candida* colonization (25). Several mechanisms have been incriminated for the association between antibiotic use and candiduria. Sulfonamides, for example, reduce the neutrophil intracellular killing mechanism of *Candida*. Whereas, tetracycline, doxycycline, and aminoglycosides have been shown to suppress neutrophil phagocytosis (26–29). Besides antibacterial agents, antifungal agents, such as fluconazole, have been shown to favor colonization with *C. glabrata* over *C. albicans* (22). The time between the onset of candiduria and the use of antibiotics is not well defined. Candiduria might occur during or immediately following antibiotic therapy (1). Indwelling urinary drainage devices play a major role in predisposition to candiduria. These devices include indwelling urethral catheters, suprapubic catheters, urethral stents, and nephrostomy tubes. They inevitably become colonized with time and serve as a portal of entry for microorganisms into the urinary system. As foreign bodies, they mechanically damage the urinary epithelium and the glycosaminoglycan layer and disrupt adequate antibacterial neutrophil function allowing yeast overgrowth. In one study, urinary tract drainage devices were present at the time of or within 30 days prior to funguria in 83% of patients (4). The underlying cause of catheter associated UTI is related to the formation of a pathogenic biofilm on the surface of the indwelling urinary catheter. A biofilm is a collection of microbial cells on a surface that is surrounded by an extracellular matrix made up of primarily polysaccharide. Formation of biofilm by *Candida* spp. has been demonstrated on a number of devices, including central venous catheters, joint devices, dialysis access devices, cardiovascular devices, urinary catheters, penile implants, voice prostheses, dentures, and ocular implants (30,31). Fluorescence and confocal scanning laser microscopy (CSLM) utilizing carbohydrate-specific dyes (e. g., calcofluor and concanavalin A, Con-A) indicated that the *C. albicans* biofilms are encased within a polysaccharide-rich extracellular matrix. *C. albicans* biofilm formation proceeds in an organized fashion through the early, intermediate, and maturation phases of development. Similar stages of development and the presence of extracellular polysaccharide matrix have also been reported for bacterial biofilms (32–34). In a scanning electron microscopy study of 50 urethral catheters, which had been indwelling for a mean of 35 days, 44 catheters had evidence of biofilm formation. Biofilm ranged from 3 to 490 μm in depth and had visible bacterial cells up to 400 cells (35). Like their bacterial counterparts, biofilm-grown *C. albicans* cells are highly resistant to antimicrobials, which explain the high relapse rate of candiduria in the setting of urinary device. Multifactorial mechanisms of resistance in fungal biofilms have been proposed including the physiological state of fungal cells, the steric hindrance, or barrier function of extracellular matrix, the overexpression of drug efflux pumps, the variations in fungal membrane sterol composition, and different developmental phases. Such broad spectrum of defense is effective against many types of antifungal agents and would explain the difficulty in eradicating candiduria in the presence of urinary catheters (36–38).

It is important to note that all the aforementioned risk factors for candiduria also predispose a patient to bacteriuria. In fact, candiduria per se is almost invariably preceded by bacteriuria. Specific factors that predispose a patient to candiduria but not bacteriuria, other than duration of hospitalization, have not been identified. More studies are needed to elucidate such factors.

Clinical Manifestations

Asymptomatic Candiduria

Asymptomatic candiduria is the hallmark of hospitalized, debilitated elderly patients with indwelling urinary catheters (16). Most patients with candiduria have no urinary symptoms. Asymptomatic candiduria is usually a benign condition that rarely leads to candidemia except in the setting of urinary tract obstruction (39). Conversely, asymptomatic candiduria might be an early predictor or a sign of hematogenous dissemination in critically and chronically ill patients associated with high mortality rate if left untreated (40).

Finding of *Candida* spp. in urine in asymptomatic patients is often challenging for the clinicians. Such finding may represent either contamination, colonization, or a true infection. Contamination can usually be differentiated from urinary tract colonization or UTI by repeating urine culture to see if yeasts persist. In older women, to eliminate potential contamination by perineal flora, it is often necessary to obtain other samples of urine by sterile bladder catheterization. If the second specimen yields no growth of yeast, contamination by the perineal flora is the likely cause of candiduria and no further action should be undertaken (16). Diagnostic criteria differentiating between colonization and infection in patients with asymptomatic candiduria are lacking. Pyuria and quantitative cultures of urine have proved to be of little value in separating infection from colonization. Infection occurs at any colony count ranging between 10 and 10⁶ colony forming units (CFU/mL). Kozinn et al. showed that in patients without indwelling urinary catheters, colony counts of more than 10,000 CFU/mL of urine were associated with infection rather than colonization (41). However, quantitative urine colony counts are of much less value in the catheterized patients. Furthermore, the presence of pyuria in catheterized patients has not been shown to be helpful in differentiating infection from colonization, as an indwelling catheter may in itself lead to pyuria from mechanical irritation of the bladder mucosa. On the other hand, it has been suggested that the presence of pseudohyphae could distinguish yeasts causing infection from those colonizing the bladder, but this theory has not been proven (16). In fact, some *Candida* spp. such as *C. glabrata* cannot make pseudohyphae, and *C. albicans* can be induced to form pseudohyphae merely by varying the pH and nutrients in the urine. In the 1970s, it was thought that antibody-coated yeasts in the urine could be used as a marker for infection (42–44). However, this was shown to be a nonspecific finding present in most urine samples tested including those who had no evidence on autopsy of invasive *Candida* spp. in their urinary tract (42).

This diagnostic dilemma directly affects the clinician's decision to treat or not to treat when *Candida* spp. are found in the urine of an asymptomatic patient. It also influences the interpretation of the results of such treatment. Given the benign nature of asymptomatic candiduria, and the infrequency of secondary candidemia there is evidence now to suggest that the most effective treatment modality in patients with asymptomatic candiduria is risk factors modification (10,39). This approach includes control of diabetes, removal of indwelling catheters, and discontinuation of antibiotics whenever possible. In a prospective, multicenter, placebo-controlled study comparing the efficacy of fluconazole versus placebo in the eradication of candiduria in asymptomatic patients, Sobel et al. found that asymptomatic candiduria resolved with catheter removal in 41% of hospitalized, catheterized patients (15). After a new catheter was inserted, untreated candiduria resolved in 20% of patients. Although, high short-term rates of eradication of candiduria occurred in patients who received fluconazole therapy, the rate of candiduria two weeks after stopping treatment were similar in the fluconazole and placebo arms (15). In another study, funguria cleared in 75% of untreated patients compared to 35% of patients who underwent catheter removal, and 50% of patients who received antifungal therapy (4). Moreover, there is no evidence that patients benefit from therapy and relapse is frequent. Several open randomized trials comparing amphotericin B bladder irrigation and oral fluconazole in the treatment of candiduria have shown similar rates of candiduria recurrence one month after therapy (45–47). However, not all experts agree that persistent asymptomatic candiduria in noncatheterized subjects can simply be observed. They believe that persistent candiduria in noncatheterized subjects should be investigated, since the likelihood of obstruction and stasis is high. The IDSA has issued guidelines for the treatment of candiduria (5). Because of the increased risk of disseminated disease, they recommend to have the following groups of patients with candiduria treated with antifungal agents: all symptomatic patients, infants with very low birth weights, patients undergoing genitourinary procedures, patients with neutropenia, and renal transplant recipients. These recommendations are based on only moderate evidence [clinical experience, opinions of respected authorities, descriptive studies, or reports of expert committees (grade III B)].

Cystitis

Patients usually present with signs and symptoms of bladder irritation including dysuria, hematuria, frequency, urgency, and suprapubic pain. Cystoscopy may reveal soft, white

elevated patches with friable mucosa (1). *Candida* cystitis can lead to the formation of fungus ball in the bladder and emphysematous cystitis. Symptomatic cystitis is rare in catheterized and noncatheterized patients as the bladder is usually resistant to *Candida* invasion. In symptomatic patients, candiduria represents true infection; therefore, treatment is recommended. However, many patients with *Candida* cystitis have concomitant bacterial UTI making it difficult to determine whether the symptoms are attributable to *Candida*. Despite these uncertainties, patients with signs and symptoms of cystitis and with only yeast found in culture should be considered as candidates for treatment (5). Several antifungal agents have been used for the treatment of *Candida* cystitis mainly fluconazole, amphotericin B bladder irrigation, and flucytosine (48,49).

Fluconazole, administered orally, remains the first-line treatment regimen for patients with *Candida* UTI because of its favorable pharmacokinetic and safety profiles. Fluconazole is a water-soluble triazole that has an oral bioavailability of more than 90%, and is excreted unchanged in the urine to a large degree (80%), with urinary concentration reaching 10 times that of blood (50). Published experience with other azoles such as itraconazole and voriconazole is limited since these agents undergo a high degree of hepatic metabolism and achieve limited concentrations in the urinary tract (50). The IDSA recommends the use of oral fluconazole in the treatment of *Candida* cystitis at 200 mg daily for 7 to 14 days (5).

Continuous bladder irrigation with amphotericin B through an indwelling urinary catheter is an old modality of treatment of *Candida* cystitis first described in 1960 (51). The eradication rate of candiduria using this therapeutic approach varied from 43% to 100% depending on the study (52,53). The optimal dose and/or concentration of amphotericin B in bladder irrigation have not been defined yet. Concentrations in most reports ranged from 5 to 50 mg/L (53). Two randomized trials have compared different concentrations of amphotericin B in bladder irrigation used for the treatment of funguria (46,54–56). The first one compared concentrations of 5 mg/L, 100 mg/L, and 200 mg/L, each administered three times daily for three days (46). Although, on day 1, the efficacy rates for elimination of funguria (~80% to 85% for all treatment groups) were significantly better for amphotericin B bladder irrigations than for placebo, by day 10, clearance rates for the three concentrations fell to 42.9%, 68.4%, and 68.2% respectively. Another randomized study comparing concentrations of 10 mg/L and 50 mg/L administered by continuous irrigation for 72 hours to 26 patients reported efficacy rates of 67% and 100%, respectively (54). Descriptions of drug administration range from intermittent instillation (up to three times daily with medication dwell times of two to three hours) to continuous irrigation (usually via a 3-way Foley catheter) (53). In spite of all these studies, the optimal method of delivery for amphotericin B bladder irrigation has not been established yet. A randomized, prospective study compared the efficacy on day 1 of treatment delivered as continuous irrigation of 50 mg/L for 48 hours with that of intermittent instillation of 10 mg/100 mL three times daily (55). Eight of 10 patients in the continuous infusion group had clearance of fungus from the urine at 72 hours, compared with 3 of 10 patients in the intermittent treatment group. By day 7, relapse was seen in two patients in the continuous treatment group.

Moreover, the optimal duration of therapy of amphotericin B bladder irrigation is unknown. A single dose has shown limited success for patients without apparent upper tract disease (56) and may not be significantly different from drug delivery for seven days (45). Two days regimen has also been evaluated, with efficacy rates of 75% reported in one study (57). Despite the lack of solid evidence for the use of amphotericin B bladder irrigation for the treatment of *Candida* cystitis, such treatment strategy is still recommended by some authorities in clinical practice (58). Adverse effects associated with amphotericin B bladder irrigation have not been frequent and include hematuria, lower abdominal cramping, bladder discomfort, dysuria, and burning during irrigation (54,55).

A single dose of amphotericin B deoxycholate in dosages of 0.3 to 1 mg/kg/day for one to seven days given intravenously has also been proposed for treatment of funguria but requires parenteral administration and has increased adverse effects compared to fluconazole (59). Finally, flucytosine has been successfully used in the treatment of urinary candidiasis with response rates of over 90% (60). It has excellent activity against non-*albicans* *Candida* spp., especially *C. glabrata*. Flucytosine achieves a high concentration in the urine of patients who do not have advanced renal failure. Its limitation includes bone marrow, hepatic and gastrointestinal toxicity, de novo resistance (25%) observed in *C. albicans* strains, and a rapid

tendency to acquired resistance when used as monotherapy. Its use should only be restricted to cases of azole-resistant strains at a dose of 25 mg/kg four times daily given orally for 7 to 14 days (5).

Pyelonephritis

Patients with infection of the upper urinary tract or pyelonephritis present with fever, leukocytosis, abdominal pain, and costovertebral angle tenderness. *Candida* pyelonephritis occurs almost invariably in the setting of urinary obstruction, particularly in patients with diabetes and nephrolithiasis. Candidemia is a rare complication reported only in 3% to 10% of cases (39,40). Fungal balls (bezoars) formation is the most serious complication of *Candida* pyelonephritis. They are commonly found in the pelvis or upper ureters. Fungal balls are fortunately rare, and their presence is suggested by signs of ureteral obstruction associated with candiduria. Renal colic may occur with the passage of fungal stones, which are portions of fungus balls. Excretory urography or retrograde pyelography shows a filling defect in the collecting system. Other complications of *Candida* pyelonephritis include papillary necrosis and local suppurative diseases resulting in pyonephrosis and perinephric or intrarenal abscess formation.

Upper urinary tract infections usually require systemic antifungal therapy as well as immediate investigation and imaging of the urinary system to exclude obstruction, papillary necrosis, and fungus-ball formation. Amphotericin B bladder irrigation has no role in the treatment of patients with pyelonephritis. Systemic antifungal therapy with either parenteral amphotericin B deoxycholate at >0.6 mg/kg/day, or fluconazole at 6 mg/kg/day is recommended (5). Flucytosine is occasionally combined with amphotericin B to cover for resistant *Candida* spp. (60). Efficacy data on the use of the lipid formulation of amphotericin B is lacking. Their use in the treatment of pyelonephritis is not advisable because their structure, which protects the kidney from the toxic effect of amphotericin B, may impair their urinary excretion (61).

Adequate drainage of the urinary tract system by placement of a nephrostomy tube as well as surgical removal of fungus balls is always essential. Irrigation of nephrostomy tube with either amphotericin B or fluconazole is advisable in some cases to achieve high concentrations of antifungal agent at the site of infection. Long-term drainage is often needed after clearance of candiduria.

Published experience with the echinocandin caspofungin in the treatment of ascending fungal UTI is limited (62). One of the major pharmacokinetic disadvantages of echinocandins is the poor glomerular filtration or tubular secretion resulting in subtherapeutic antifungal concentration in the urine. Accordingly, despite their favorable in vitro activity, echinocandins are not considered among the preferred agents for the treatment of candiduria. However, the echinocandins achieve a high tissue concentration independent of glomerular filtration making them a suitable alternative for the treatment of renal candidiasis caused by resistant non-*albicans* strain in the absence of urinary tract obstruction (63,64).

Renal Candidiasis

Renal candidiasis develops almost exclusively secondary to hematogenous seeding of the kidney during an episode of candidemia. Patients present with high-grade fever, dynamic instability, and renal insufficiency. Candiduria along with a subtle decline in urinary function is often the only diagnostic clue of disseminated candidiasis. Other clinical features include skin rash and endophthalmitis. Candidemia is detected in 50% of cases making the diagnosis difficult to establish. Moreover, finding of candidal casts in the urine is a specific diagnostic marker for detection of renal candidiasis; however, this marker is a very insensitive tool (20). The diagnosis of renal candidiasis relies on clinical presentation mostly. Hematogenous renal candidiasis should be treated with high dose systemic amphotericin B at >0.6 mg/kg/day or fluconazole at 400 mg/day. Lipid formulations of amphotericin B, although less nephrotoxic than the standard deoxycholate form, have not been shown to be superior to the latter and are not currently regarded as first-line therapy for disseminated candidiasis (5).

Epididymitis, Orchitis, and Prostatitis

Epididymo-orchitis and prostatitis caused by *Candida* spp. are an uncommon manifestation of *Candida* urinary tract disease (65–76). They are mostly described in diabetic and HIV patients, or

following bladder instrumentation. Epididymo-orchitis usually results from retrograde spread of UTI via the ejaculatory duct and vas deferens. Epididymal infections are rarely caused by hematogenous dissemination of systemic fungal infection (67). Both *C. albicans* and *C. glabrata* were reported in the setting of epididymo-orchitis. Patients present with scrotal swelling and pain typical of epididymo-orchitis, which is bilateral and complicated in most cases (71). Failure to isolate a causative bacterium in association with the presence of candiduria and risk factors for candidal infection is highly suggestive of a diagnosis of *Candida* epididymo-orchitis. Percutaneous or open drainage should be considered in complicated cases to obtain specimens for diagnostic microbiology and histology (76). Treatment requires the combination of orchiectomy or surgical drainage and systemic antifungal therapy according to the species susceptibility pattern. Oral fluconazole at 200 to 400 mg/day is the preferred regimen. However, in case of fluconazole-resistant *Candida* spp., amphotericin B at 0.5 mg/kg/day with or without flucytosine would be an appropriate choice (76).

Isolated *Candida* prostaticitis is extremely rare (74,75,77). It occurs usually secondary to systemic infections in immunosuppressed patients and is complicated by prostatic abscess formation. Patients present with irritative voiding syndrome, perineal pain, fever, and acute urinary retention. Surgical drainage in case of abscess formation along with systemic antifungal therapy is recommended.

GENITOURINARY TRACT ASPERGILLOSIS

Aspergillosis of the genitourinary tract is an uncommon infection. It has been mainly reported in patients with malignancies, diabetes, intravenous drug use, chronic alcoholism, as well as in renal transplant recipients (78–82). Three disease patterns of genitourinary aspergillosis have been described in the literature. The first and most common pattern results from hematogenous spread of disseminated *Aspergillus* infection and is associated with multiorgan involvement including kidney and prostate. The primary site of infection in such cases is either the lungs or the skin (2). The second pattern is the result of a panurothelial ascending infection, involving the urethra, bladder, ureters, prostate, and kidneys and is secondary to iatrogenic bladder instrumentation (83,84). The third pattern consists of a localized parenchymal necrosis with an obstructing fungus ball (bezoars) formation that can be seen in any part of the urinary tract. This pattern is mostly reported in patients with diabetes, HIV infection, and renal transplant recipients where corticosteroid therapy is a major risk factor (81,84).

Patients with genitourinary tract aspergillosis usually present with fever and microscopic hematuria. Localizing symptoms such as flank pain and dysuria are uncommon. Urine culture is invariably negative making the diagnosis of urinary tract aspergillosis difficult in the absence of clinical suspicion. Repeated large volume urine culture on sabouraud dextrose medium or brain–heart enriched infusion broth has been suggested. However, the yield is poor because *Aspergillus* organisms have a propensity for tissue attachment and invasion. Tissue culture or pathological examination showing the fungal hyphae in renal tissue remain the standard diagnostic modality (85,86).

Management of urinary tract aspergillosis requires the combination of medical and surgical interventions (87–92). Systemic amphotericin B alone or combined with flucytosine is the first line treatment (84). Irrigation with amphotericin B or a liposomal preparation of amphotericin B through a urinary catheter or percutaneous nephrostomy tube has been tried successfully (87). Open surgical drainage is indicated for obstructing fungus ball (81). Infection limited to the prostate could be surgically treated without additional antifungal therapy (92). Earlier in vitro data suggesting that rifampin potentiates the activity of amphotericin B against *Aspergillus* spp. have not been proven in clinical studies (93). Data supporting the use of itraconazole in the treatment of genitourinary aspergillosis are scanty since the drug is poorly excreted in the urine. The new azoles such as voriconazole and posaconazole and the echinocandins have excellent in vitro and in vivo activity against a variety of *Aspergillus* infections. However, their use in the treatment of genitourinary aspergillosis has not been validated. Voriconazole undergoes extensive liver metabolism and less than 2% of its active metabolite is excreted in the urine, making it a less suitable antifungal agent for the treatment of urinary tract aspergillosis (94). The pharmacokinetic properties of posaconazole and caspofungin preclude their use in the treatment of genitourinary aspergillosis. Posaconazole is primarily degraded in the liver to inactive

metabolites and excreted in the feces, and caspofungin undergoes primarily liver metabolism as stated previously (95). Clinical evidence for their success in the treatment of such infections is still awaited.

CRYPTOCOCCUS INFECTIONS OF THE GENITOURINARY TRACT

Cryptococcosis is an infection caused by *Cryptococcus neoformans*, a yeast commonly found in the soil, mostly in pigeon excreta. The disease is acquired by inhalation of the organism, which may then disseminate to the meninges, lymph nodes, skin, and the genitourinary tract. Therefore, infection of the urinary tract is almost always equivalent to systemic infection. Individuals at risk include patients with the acquired immunodeficiency syndrome (AIDS), as well as patients with other immunocompromising conditions such as hematological malignancies, collagen vascular diseases, diabetes mellitus, renal transplantation, and corticosteroids therapy (96–101). Cryptococcal infection of the urinary tract can present as pyelonephritis, prostatitis, or as occult cryptococcal UTI without a rise in serum cryptococcal antigen. Prostatic cryptococcosis is usually asymptomatic. The prostate gland is considered a site for yeast sequestration after an occult or treated disseminated cryptococcal infection. It has been recognized as a potential clinical site for sanctuary of *C. neoformans* from antifungal treatment. It is an important reservoir for relapse of cryptococcosis in patients who have a high burden of yeasts (101). A latent *C. neoformans* infection of the prostate has even been found to spread into the blood during prostatic surgery (91,102). Diagnosis of prostatic cryptococcosis can be achieved by using ultrasound-guided prostatic biopsy with fungal culture or histopathology of prostatic tissue. High levels of prostate-specific antigen (PSA), which may represent prostatic inflammation, were recognized in prostatic cryptococcosis in a patient who underwent renal transplantation (92,103). After initial induction antifungal treatment of cryptococcal meningoencephalitis in patients who have AIDS, urine or seminal fluid cultures may still be positive for yeasts (104). Therefore, the prostate might be an important site for relapsed infections if therapy is discontinued early and immune reconstitution has not occurred.

The treatment of genitourinary cryptococcal infection necessitates systemic antifungal therapy with amphotericin B or fluconazole. It has been suggested that antifungal therapy should continue for six weeks if the patient is immunocompetent. However, in AIDS patients a lifetime suppressive therapy with fluconazole is recommended. The use of flucytosine in combination with amphotericin B has been proposed based on the response rates of patients with cryptococcal meningitis treated with this combination regimen (105).

HISTOPLASMOSIS OF THE GENITOURINARY TRACT

Histoplasma capsulatum infection is usually a benign self-limited respiratory disease that is most commonly seen in areas of endemicity in the upper Mississippi River and Ohio River valleys. Most of these infections are clinically silent and resolve without consequences (106). Disseminated disease is an uncommon but well-recognized sequelae of histoplasmosis that occurs primarily in patients with impaired cellular defenses or after introduction of a large inoculum (107). Genitourinary and prostatic involvement with disseminated histoplasmosis rarely occurs. Most cases were only identified at autopsy (108,109). Of note, many large series reported no evidence of *Histoplasma* prostatitis, including a large review of 102 cases of disseminated histoplasmosis from Vanderbilt (106). Most patients with prostatic histoplasmosis described in the literature had no identifiable immunodeficiency; however, the disease is currently being reported more and more in patients with HIV infection or AIDS where all cases were detected at the stage of prostatic abscess (110–112). Well-characterized relapses of cryptococcal disease from prostatic reservoirs raise questions about this form of histoplasmosis functioning in the same manner. Treatment data regarding disseminated histoplasmosis involving the prostate are limited. In the genitourinary tract, surgery may not be an option because of the possibility of further dissemination with surgical intervention (113). Treatment is indicated for all patients with progressive disseminated histoplasmosis. In immunocompetent hosts and immunocompromised hosts without AIDS, Amphotericin B at 0.7 to 1.0 mg/kg/day is recommended for patients who are sufficiently ill to require hospitalization (114). Experience using the lipid formulations of amphotericin B for treating histoplasmosis has not been reported. Most patients respond quickly to amphotericin B and can then be switched to itraconazole to continue the

therapeutic course. Itraconazole, 200 mg once or twice daily for 6 to 18 months, is the treatment of choice for patients with mild or only moderately severe symptoms who do not require hospitalization, and for continuation of therapy in those whose condition has improved in response to amphotericin B. Fluconazole should be used only in patients who cannot take itraconazole (115). The fluconazole dosage should be at least 400 mg daily in immunocompetent individuals and 800 mg daily in those with severe immunosuppressive conditions. *H. capsulatum* may develop resistance to fluconazole during therapy, leading to relapse, thus necessitating careful follow-up assessment, including measurement of *H. capsulatum* antigen concentration in blood and urine (116). In patients with AIDS, treatment is divided into an initial 12-week intensive phase to induce a remission in the clinical illness followed by a chronic maintenance phase to prevent relapse. A similar approach may be appropriate in other patients without AIDS who have relapsed after appropriate courses of therapy. For induction therapy, amphotericin B is recommended for patients who are sufficiently ill to require hospitalization. Amphotericin B can be replaced with itraconazole, 200 mg twice daily (when the patient no longer requires hospitalization or IV therapy), to complete a 12-week total course of induction therapy. Itraconazole 200 mg three times daily for three days and then twice daily for 12 weeks is the treatment of choice for patients who have mild or moderately severe symptoms and do not require hospitalization. For maintenance therapy, the treatment of choice is itraconazole 200 mg once or twice daily for life (grade AII level of evidence). Antigen concentrations in serum and urine should be monitored every three to six months to provide evidence that maintenance therapy is continuing to suppress the progression of infection.

BLASTOMYCOSIS OF THE GENITOURINARY TRACT

Blastomyces dermatitidis is a dimorphic fungus existing in the mycelial form in soil and in the yeast form in tissue. All systemic blastomycosis infections arise from inhalation of spores aerosolized from soil. The skin, bone, genitourinary, and central nervous systems are the most commonly involved extrapulmonary sites. Genitourinary involvement by *B. dermatitidis* occurs secondary to hematogenous dissemination where the lung is usually the primary site of infection. Blastomycosis is a relatively uncommon source of genitourinary disease; however, it remains an important diagnostic consideration particularly in endemic areas including the Mississippi, Missouri, Ohio River valleys, and the Great Lakes region (117). Genitourinary involvement has been noted in 45% of cases (118). The prostate and epididymis are most frequently affected, but renal, testicular, and preputial disease has also been reported. Genitourinary symptoms may be the primary complaint in 2% of systemic cases (119). Definitive diagnosis is made by culture or histological identification of the characteristic budding yeast form. Because they lack both sensitivity and specificity, serological tests are generally not helpful for diagnosing blastomycosis. A negative serological test should never be used to rule out disease, nor should a positive titer be an indication to start treatment.

Patients with life-threatening disseminated disease should be treated with amphotericin B (0.7–1 mg/kg/day; total dose, 1.5–2.5 g). Therapy for some patients may be switched to itraconazole after clinical stabilization with amphotericin B. Patients with mild-to-moderate disseminated blastomycosis should be treated with itraconazole (200–400 mg/day) for at least six months. Ketoconazole and fluconazole, both at dosages of 400 to 800 mg/day, are alternatives to itraconazole (120). Although, voriconazole has been successfully used in the treatment of cerebral blastomycosis, data about its efficacy in the treatment of genitourinary tract blastomycosis is lacking (121,122). One study showed that high dose posaconazole is effective in curing pulmonary blastomycosis in a murine model; however, this antifungal has not been studied in humans with *B. dermatitidis* infection (123).

COCCIDIOIDOMYCOSIS OF THE GENITOURINARY TRACT

Coccidioidomycosis is a fungal infection endemic to the southwestern United States (124). Humans acquire infection by inhalation of airborne fungal spores of *Coccidioides immitis*. Clinical manifestations are typically a flu-like illness or self-limited pneumonia. Immunocompromised patients are at increased risk for disseminated disease which most commonly involves the meninges, bones, joints, or skin. The genitourinary tract is infrequently affected. An autopsy

series of 214 patients with disseminated Coccidioidomycosis showed kidney involvement in 57 (27%), prostatic infection in 4 (1.8%), and epididymal involvement in 2 (0.9%) patients (125–127). Kidneys are the sixth most common site of dissemination. Patients with coccidioidal prostatitis may be asymptomatic or may present with obstructive symptoms. Most patients with epididymal infection present with scrotal swelling, epididymal induration, and tenderness. Urinalysis is often abnormal showing pyuria and hematuria. Urine culture is helpful for establishing the diagnosis of genitourinary coccidioidomycosis. *C. immitis* grows well on most commonly used media. The yield of urine culture can be increased by prostatic massage. Definitive diagnosis requires pathologic tissue examination in which characteristic endospore-containing spherules are seen. The IDSA has published guidelines for the management of coccidioidomycosis. A daily dose of 0.5 to 1.5 mg/kg of intravenous amphotericin B has been recommended in patients with severe or rapidly progressive disease. For less acute infections, 400 to 800 mg of oral fluconazole daily or 400 mg of itraconazole daily is effective. Extrapulmonary and nonmeningeal infections are generally treated medically but surgical debridement is an adjunctive measure in some cases. The treatment of isolated genital tract diseases has not been well defined. Surgical resection alone of the involved genital organ with no antifungal therapy in immunocompetent patients would probably be sufficient (128).

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An essential guide for all infectious disease specialists, dermatologists, and critical care specialists dealing with the rising number of fungal infections worldwide, ***Antifungal Therapy*** presents practical strategies for the safe and effective management of these infections.

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- **comprehensive content**—this stand-alone guide to the complete scope of antifungal therapy, including its history, future, drug classes, and evidence-based outcomes, supplies the knowledge clinicians need to develop individualized treatment plans and improve patient care
- **expert guidance**—nearly 30 internationally recognized contributors provide readers with reliable advice and insights on dosing, choosing the right drug, and managing complications, based on years of experience in the field
- **detailed management strategies**—with practical information on how to care for various types of risk groups, including unique patient populations, and how to tackle both common and rare infections, ensures that physicians are prepared to handle most clinical situations

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