Fungal Infections in the Immunocompromised Patient

edited by
John R. Wingard
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Fungal Infections in the Immunocompromised Patient
INFECTION DISEASE AND THERAPY

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Fungal Infections in the Immunocompromised Patient

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Preface

Opportunistic infections have always been accompaniments of medical conditions that compromise host defenses. Because of the severe morbidity and mortality that result from such infectious complications, these pose substantial challenges for the clinician who cares for such individuals. With medical progress, the number of immunocompromised patients is steadily climbing. Moreover the types of host defense compromises are changing. As transplant practices evolve, as critical care procedures advance, and as HIV management strategies change, the spectrum of opportunistic pathogens shifts.

Initially, bacterial infections were most problematic. As strategies to control bacterial infections improved, the herpesviruses came to increasing attention of clinicians. Herpes simplex and varicella zoster virus cause considerable morbidity and occasional mortality. Cytomegalovirus (CMV) became recognized as a major killer of transplant recipients, but morbidity declined due to advances in rapid diagnostics and the introduction of effective antiviral agents such as acyclovir and ganciclovir.

Today, the invasive fungal pathogens have seized center stage from the above historically important opportunistic pathogens. During the 1980s the rate of nosocomial invasive fungal disease in U.S. hospitals doubled with no sign of slowing during the 1990s. Candida has become the fourth leading bloodstream isolate in U.S. hospitals, surpassing many historically important bacterial pathogens. Estimates are that the United States spends approximately one billion dollars annually for Candida infections, and more than $650 million annually for Aspergillus disease. However, accurate diagnostics and effective therapies have lagged for this emerging group of opportunists. As survival from bacteria and the herpesviruses has improved, more immunocompromised patients are now living to develop infection from fungi.

Candida is the most common genus of fungal pathogens. C. albicans long was recognized as a cause of mucosal disease of the mouth, esophagus, and vagina in patients with T-cell deficiency, as seen in patients with HIV infection, those treated with corticosteroids or other potent immunosuppressive drugs, and patients with lymphoreticular neoplasms. Fungemia is especially problematic in cancer patients with chemotherapy-induced myelosuppression, blood and marrow transplant recipients, and patients in critical care units on multiple antibiotics with venous, bladder, or endotracheal catheters. With the increasing use of potent immunosuppressive
purine analogues, such as fludarabine, pentostatin, and cladribine, and anti-T and anti-B cell antibodies (e.g., rituximab, alemtuzumab, and anti-thymocyte globulin) in the management of hematolymphoreticular malignancies, increasing emphasis on chemotherapy dose intensity in oncologic practice, and the growth in critical care, the number of patients at risk for fungal diseases is growing.

In recent years, the non-albicans Candida species have become increasingly recognized as fungal pathogens in immunocompromised patients. In cancer patients, C. tropicalis and C. glabrata are especially problematic. In critical care patients, the rates of C. glabrata infections are climbing. A variety of risk factors for different Candida species has been identified.

Aspergillus species have been the chief non-Candida fungal opportunistic pathogens. The major portals of entry for these airborne organisms are the nasal passages, sinuses, and respiratory tract, in contrast to the gastrointestinal tract for Candida organisms. In bone marrow transplant recipients and in patients with acute leukemia, the mortality is in excess of 75%.

Mold pathogens other than Aspergillus are increasing. The agents of mucormycosis are quite difficult to culture and the syndromes caused by these infections are indistinguishable from those caused by Aspergillus species; response to therapy is poor. Fusarium, a soil fungus, has been historically impervious to most therapeutics. Scedosporium, another emerging pathogen, is being increasingly reported in blood and marrow transplant recipients.

Difficulty in accurate diagnosis has been a tremendous impediment hindering therapeutic advances. Noninvasive techniques have been limited for most opportunistic fungal pathogens. Blood culture techniques have improved for detection of Candida, but still are not foolproof. Aspergillus is poorly diagnosed by bronchoscopy, and reliable diagnosis requires a thoracotomy, an invasive procedure which is quite dangerous in many of the patients that are suspected to be infected. There is an urgent need for noninvasive, rapid, accurate diagnostics. Antigen detection assays have been helpful aids to diagnosis for only a few fungal pathogens, such as Cryptococcus and Histoplasma. Recent studies suggest that antigen detection assays (such as galactomannan and glucan) and PCR methods for detecting fungal antigens may finally yield promising tools for a broader array of fungal pathogens and these will be discussed in the book.

Antifungal therapy had been quite limited in the past. The gold standard of therapy has been amphotericin B, a polyene antifungal agent with considerable toxicity. This concern was somewhat eased by the development of lipid formulations of amphotericin B, permitting delivery of high doses of amphotericin B with substantially less toxicity. The introduction of antifungal azoles offered considerable promise, but because of limited spectrum of activity and poor or erratic bioavailability, little progress was realized until fluconazole was introduced a decade ago. With excellent bioavailability, little toxicity, and both oral and intravenous formulations, fluconazole was quickly embraced by clinicians treating immunocompromised patients with suspected or proven Candida infection. The only blemish of fluconazole is a limited spectrum of activity: excellent activity against many yeast pathogens, but none against mold pathogens. Selection of fluconazole resistant yeasts was also a concern, especially in patients with advanced HIV disease failing anti-retroviral therapy.

New antifungal agents have been introduced into the clinical arena and more are arriving. Caspofungin, a member of a unique class of agents, the echinocandins, was licensed several years ago. This class of agents acts on the fungal cell wall, interfering
with the biosynthesis of beta glucan, the main constituent of the fungal cell wall, whereas both polyenes and azoles act on a constituent of the fungal cell membrane, namely ergosterol. With excellent activity against the two most frequent invasive fungal pathogens, Candida and Aspergillus, and an outstanding safety profile, caspofungin has rapidly become widely used. Anidulafungin and micafungin are also on the horizon. New extended spectrum azoles have been introduced and others are in development. One, voriconazole, has been shown to be more effective than amphotericin B as first-line therapy of invasive aspergillosis, in a randomized trial. The drug is now available worldwide. New oral and intravenous formulations of itraconazole were approved to expand that agent’s utility in clinical practice. Other broad spectrum azoles, such as posaconazole, are in clinical trials.

Even with effective and safe therapeutics, treatment is frequently started late during the course of infection, when the burden of organisms is high and the likelihood of therapeutic success low. This accounts for much of the extraordinarily high mortality. Accordingly, considerable attention has been paid to different antifungal strategies. Prophylaxis and empiric therapy have been evaluated in groups of immunocompromised patients at high risk for fungal infection. Today, there are good evidence-based data to support use of a broad array of antifungal agents and strategies for different patient settings.

Considerable progress has been achieved during the past decade. The cumulative mortality from Candida infections is finally beginning to recede. However, the collective mortality from aspergillosis continues to climb. Moreover, infection rates from fungi also are increasing. More work is needed. Yet, with new diagnostics and the expanding array of antifungals, the future looks bright.

The goal of this book is to provide an up-to-date overview of the fungal syndromes in immunocompromised patients, describe the shifts in fungal pathogens and the reasons behind them, indicate the setting in which they cause illness and the risk factors for infection, cover the pros and cons of current and emerging diagnostic measures, and discuss treatment modalities and strategies. The book is divided into five sections to cover the above topics, with individual chapters devoted to specific syndromes, infections, and settings.

This book is targeted to the clinician caring for immunocompromised patients at risk for invasive fungal infections. This includes transplant clinicians, critical care specialists, oncologists, stem cell transplant specialists, and infectious disease physicians. Both academic physicians and practitioners will find this book informative. Clinical microbiologists, mycologists, and individuals with research interests in developing new antimicrobial agents will also find very useful information related to their respective fields.

An international group of expert clinicians who have defined many of the pertinent issues in fungal epidemiologic studies and clinical trials have contributed to this effort. The authors review the published data, offer critical insights as to the interpretation of the literature, and provide timely summaries of the current state of knowledge. We are truly grateful for their hard work in making this project happen.

John R. Wingard
Elias J. Anaissie
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1 Overview of Host Defenses: Innate and Acquired Immunity

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I. INTRODUCTION

Invasive fungal infections remain a serious challenge to clinicians caring for immunocompromised patients. The proportion of vulnerable patients is increasing, paralleling the increased use of immunosuppressive therapies and the more effective supportive care in high-risk populations.

Fungi responsible for these infections can be separated into two groups: the pathogenic and the opportunistic. The true pathogenic fungi cause self-limited disease in normal hosts but may cause devastating infections in compromised patients. Examples of such pathogens include Cryptococcus neoformans and the endemic fungi Histoplasma capsulatum, Coccidioides immitis, Paracoccidioides brasiliensis, and Blastomyces dermatitidis. These infections may remain in a latent state only to recur when the patient is immunosuppressed. Opportunistic fungi rarely cause serious disease in normal hosts but are responsible for life-threatening infection in the setting of weakened host defenses. These fungi include Candida spp. and Aspergillus spp. and less commonly Zygomycete spp., Trichosporon spp., Fusarium spp., and Pseudoallescheria boydii among others.

Innate and acquired immunities work together as part of an integrated host immune response to prevent fungal infections (Fig. 1).

II. INNATE IMMUNITY

Innate immunity, also called natural or native immunity, consists of cellular and biochemical defense mechanisms that are in place before the exposure to the offending pathogen and, thus, can rapidly respond to prevent infection. This response remains unchanged upon reexposure to the pathogen.
The principal components of the innate immunity include physical barriers, soluble components and cell membrane receptors, the complement system, and the phagocytes.

**A. Physical Barriers**

Intact epithelium, fatty acids, mucus, and cilia act as physical barriers to invading pathogens. In the airways, the mechanism of cough helps to eliminate potential pathogens.

**B. Soluble Components**

Antimicrobial peptides (lysozymes, lactoferrin, secretory leukoprotease inhibitors, and defensins) are present in mucosal secretions. In the respiratory tract, these substances inhibit the entry of fungi through the epithelial barriers by disrupting crucial microbial structures, sequestering essential nutrients, interfering with microbial metabolism, and inhibiting microbial replication. Defensins are peptides produced by epithelial cells and phagocytes (neutrophil azurophilic granules) and possess broad antimicrobial activity, including fungi. Defensins also act as chemotactic factors for mononuclear cells (a-defensins) and T-cells (b-defensins) and stimulate the release of interleukin-8 (IL-8), a potent neutrophil chemotactic agent from epithelial cells.

**Figure 1** (Caption on Facing Page)
Various antimicrobial substances attack particular microbial targets and co-operate to enhance antimicrobial defenses (1) (Table 1).

**C. Recognition of the Pathogen Through Pattern-Recognition Receptors**

If microbes penetrate the epithelial barrier, defense mechanisms act to distinguish self from the potentially harmful pathogens through “pattern-recognition receptors” (PRRs) that are soluble molecules or present on cellular membranes.

1. **Cellular Membrane PRRs**

The PRRs that are present on the cellular membrane are the toll-like receptors (TLRs), mannose, and Fc receptors (Fig. 2).

TLRs are a family of membrane proteins present in phagocytes and epithelial cells that recognize microbial sugars. The interaction of TLRs with the pathogen-associated molecular patterns (PAMPs) activates host immune cells leading to the production of microbicidal peptides and proteins, cytokines, induction of respiratory response.

**Figure 1** (Facing Page) Immune response to airborne fungi. Protective immunity against airborne fungi developed in two stages shown in sequence: Immediate by innate immunity (upper part) and delayed by acquired immunity (lower part). Immediate: (I) Three lines of defense against airborne fungi: epithelial barrier, soluble factors (antimicrobial and opsonizing molecules), and phagocytes. (II) If the spores invade the epithelium, the epithelial cells respond with chemokines production that activate immune cells: (a) Neutrophils attack intracellular and extracellular fungi; (b) macrophages attack intracellular fungi and produce proinflammatory cytokines such as IL-1, TNF, and IL-6; (c) NK cells secrete IFN-γ; and (d) DCs phagocytose the fungi and process and present fungal antigens to naive or memory T-cells. They also secrete IL-12, IL-18, and IFN-γ. A proinflammatory environment is thus created. (III) Proinflammatory cytokines upregulate the expression of adherence molecules in the recruitment of neutrophils and monocytes to the site of infection. Delayed (IV) (a) DCs process the antigens and migrate to the lymph nodes. (b) naive and memory T-cells also migrate from the circulation to lymph nodes. (V) DCs present the fungal antigen to naive and memory lymphocytes. T lymphocytes are specifically activated, which secrete IL-2 and promote the proliferation of B cell, CD4+, and CD8+ T-cells. (VI) Specific B and T-cells migrate through lymphatic vessels, thoracic duct, and general circulation and leave the circulation driven by chemoattractant released at the site of infection. (VII) In a proinflammatory environment, specific T lymphocytes polarize to type 1 and secrete IFN-γ. IFN-γ stimulates neutrophils and macrophages for a more effective killing of the pathogen. B cells produce (IgG), which opsonizes the pathogen for better phagocytosis. (VIII) Effective killing and clearance of the pathogen. Resolution of the infection. In contrast, a deleterious immune response to airborne fungal pathogens may develop in immunocompromised hosts. Immediate: Damaged epithelium, decreased number or function of phagocytes, or decrease in the concentration of antimicrobial and opsonizing molecules allow fungi to invade the host, reach the parenchyma and proliferate. Decreased number and function of phagocytes result in fungal proliferation and infection. Poor recruitment of inflammatory cells deprives the host from a strong phagocytic defense. A proinflammatory environment is not present. Delayed: Suppressed DC function results in inadequate antigen presentation to T-cells and little T-cell proliferation. The specific T-cells that migrate to the site of infection reach an environment devoid of proinflammatory cytokines. Without the proinflammatory cytokines (IL-12 and IFN-γ), the polarization of lymphocytes is to type 2 with production of IL-4 and IL-5 by T-cells and IgE from B cells. This type of response results in proliferation of fungi and progressive infection.
<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Source</th>
<th>Probable mechanism of action</th>
<th>Known fungal target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defensins</td>
<td>Neutrophils (azurophilic granules), epithelial cells</td>
<td>Disruption of microbial membrane, chemotaxis, complement modulation</td>
<td>Candida spp. (126–128), Histoplasma capsulatum, Cryptococcus neoformans (129,130)</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Neutrophils (azurophilic and specific granules), monocytes, macrophages, epithelial cells</td>
<td>Cleavage of glycosidic links in fungal membrane</td>
<td>Candida spp. (131)</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Neutrophils (specific granules)</td>
<td>Iron sequestration</td>
<td>Candida spp.</td>
</tr>
<tr>
<td>Histatins</td>
<td>Salivary glands</td>
<td>Binding to metals (Zn and Cu)</td>
<td>Candida spp., Aspergillus spp. C. neoformans, Saccharomyces cerevisiae (132)</td>
</tr>
<tr>
<td>Antileukoproteases</td>
<td>Respiratory and genital epithelial cells</td>
<td>Protease inhibition</td>
<td>Candida spp., Aspergillus spp. (133)</td>
</tr>
<tr>
<td>Peroxydases</td>
<td>Neutrophils, monocytes, eosinophils, epithelial cells</td>
<td>Generation of microbicidal hypohalites from hydrogen</td>
<td>Aspergillus spp., Candida spp. (29,134)</td>
</tr>
</tbody>
</table>
burst, and phagocyte degranulation. Fungal PAMPs on the fungal cell wall include polysaccharides as α- and β-glucans, galactomannans, chitin, and zymosan (2).

TLR4 and TLR2 receptors interact with CD14 and Dectin-1 receptor, respectively (3). Dectin-1 mediates the biological effects of fungal-derived β-glucans and zymosan and acts with TLR2 receptor to produce tumor necrosis factor (TNF) in response to fungi (4).

Our understanding of the role of TLRs in immunity against fungi is rapidly evolving. Most studies suggest that TLR2 and TLR4 are involved in the production of proinflammatory cytokines in response to Aspergillus fumigatus conidia and hyphae (2,5,6). In one study, however, TLR2 binding to hyphae from A. fumigatus resulted in an anti-inflammatory cytokine response (7). Studies with C. neoformans and Candida albicans further support the proinflammatory cytokine response induced by TLR2 and TLR4 (7,8).

Mannose receptors present on phagocytic membranes bind to fucose and mannos (absent in mammalian cells but abundant in fungi) and trigger phagocytosis. Chemokines bind to G-protein-coupled receptors and stimulate chemotaxis.

2. Soluble PRRs
Soluble PRRs are present in body fluids and on mucosal surfaces and include two collectins: mannose-binding lectins (MBLs) and surfactant protein-A (SP) and SP-D;
ficolins, and pentraxin. These soluble PRRs recognize unique sugar patterns on microbes and act as opsonins presenting the microbe to the phagocyte directly or through complement activation (9–12) (Table 2).

D. Complement System

The complement system consists of plasma proteins normally present in an inactive state; when activated, the complement system plays a key role in opsonization, chemotaxis, and killing of microbes (Fig. 3). The proteins of the complement system are activated along an enzymatic cascade of sequential proteolysis resulting in bioactive molecules that facilitate opsonization, osmotic lysis of targeted cells, and recruitment of phagocytic cells. There are three major pathways of complement activation: the classical pathway in which activation results from binding of complement split Clq to IgG or IgM bound to an antigen; the alternative pathway, which is activated without the need for such antibodies, and the lectin pathway, which is activated by a plasma lectin that binds to mannose residues on microbes. The common event in complement activation is the cleavage of the complement protein C3, leading to C3b and C3a. C3b binds to the microbial surface and participates in the activation of C5, splitting it into C5b and C5a. C5b is followed by deposition of C6, C7, and C8, and the assembling the membrane attack complex (MAC) with a polymer of C9 inserted in the cell membrane of the microbe leading to microbial lysis. The opsonization of pathogens is mediated by C3b or C4b, which promotes phagocytosis by the cell bearing the appropriate receptors.

C3a, C4a, and C5a stimulate inflammation through chemotaxis and activating neutrophils and mast cells. MAC formation has not been found to play an important role against fungi as the fungal wall may block the formation of this complex, but another mechanism of complement system plays a role in host defense against certain fungi. Fungi are potent activators of the alternative and lectin pathway.

The phagocytic cell membrane has complement receptors (CRs) of four different types. Type 1 receptor, CR1 promotes phagocytosis of C3b- and C4b-coated particles and acts synergistically with the Fc \( \gamma \) receptor. Type 2 receptor, CR2 stimulates humoral immune response. Type 3 receptor, CR3 also called Mac-1, is an integrin composed of two chains (CD11b, CD18), is expressed on phagocytes and natural killer (NK) cells and promotes phagocytosis of C3-coated pathogens. CR3 also interacts with intercellular adhesion molecule (ICAM)-1 from endothelial cells during the recruitment of leukocytes. Type 4 receptor, CR4 (CD11c, CD18), is similar to CR3 and has similar functions but is more abundant on dendritic cells (DCs).

C3 acts as a major opsonin for \( C. neoformans \) in the \( C. neoformans \) naive host, whereas C5a seems to play a protective role against infections caused by \( C. albicans \), \( A. fumigatus \), \( C. neoformans \), and \( Saccharomyces cerevisiae \) but not against dimorphic fungi (\( H. capsulatum \), \( C. immitis \), or \( P. brasiliensis \)) (13–15).

E. Phagocytes

The effector cells of the innate immunity are neutrophils, circulating monocytes, and tissue-based macrophages. As described earlier, these cells express surface receptors: mannose receptors, Dectin-1 receptors and TLRs that recognize pathogens, chemokine receptors and CRs for migration to the site of infection, cytokine receptors to respond to cytokines, mannose, and CRs and Fc receptors to increase phagocytosis (Fig. 2). Neutrophils and monocytes are recruited from the blood to sites of infection
### Table 2: Pattern Recognition Receptors and Their Fungal Interaction

<table>
<thead>
<tr>
<th>Origin</th>
<th>Mechanism of Action</th>
<th>Fungal pathogen</th>
<th>Biological correlates</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBL (collectin)</td>
<td>Binding to MASP and activation of the complement system to increase phagocytosis</td>
<td>Acapsular <em>C. neoformans</em> <em>Candida</em> spp. <em>Aspergillus</em> spp. <em>Pneumocystis carinii</em></td>
<td>Decreased MBL levels is associated with decreased opsonization; increased infections in patients receiving chemotherapy (135) and those with cystic fibrosis; recurrent vulvovaginal candidiasis (26); chronic necrotizing pulmonary aspergillosis (28)</td>
</tr>
<tr>
<td>SP-A and SP-D</td>
<td>Opsonization. Stimulation of chemotaxis, phagocytosis, production of proinflammatory cytokines, and fungal killing by phagocytes. Inhibition of IgE-mediated response to fungi</td>
<td><em>Pneumocystis carinii</em>, acapsular <em>C. neoformans</em>, <em>A. fumigatus</em> (increase phagocytosis), <em>Candida albicans</em> (SP-A: anti-inflammatory effect on pulmonary alveolar macrophages) (136)</td>
<td>Decreased in patients with cystic fibrosis, adult respiratory distress syndrome, smokers, asthma, and conditions associated with increased risk for pneumonia. Increased in patients with interstitial lung diseases (137).</td>
</tr>
<tr>
<td>Ficolins</td>
<td>Binding to MASP and activation of the complement system to increase phagocytosis</td>
<td>Bind to acapsular <em>C. neoformans</em></td>
<td>None documented</td>
</tr>
<tr>
<td>Long Pentraxin 3</td>
<td>Binding to C1q to increase phagocytosis</td>
<td><em>Aspergillus fumigatus</em>, <em>A. flavus</em>, <em>A. niger</em></td>
<td>Pentraxin deficient (−/−) mice have increased mortality when challenged with <em>Aspergillus</em> spores. Administration of Pentraxin 3 to these animals increases survival</td>
</tr>
</tbody>
</table>

Abbreviations: MASP, Mannose-binding protein-associated serine protease; MBL, mannose-binding lectin; SP-A, surfactant protein-A; SP-D, surfactant protein-D.
by binding to adhesion molecules on the endothelial cells and by chemoattractants produced in response to infection (Fig. 4).

There are three families of adhesion molecules: selectins, integrins, and immunoglobulin (Ig) superfamily adhesion molecules. Selectins are found on all type of leukocytes. Selectins participate in the process of leukocyte rolling along vascular endothelium, whereas integrins and the Ig superfamily adhesion molecules are important for stopping leukocyte rolling and mediating leukocyte aggregation and trans-endothelial migration. The integrins include the very-late antigen (VLA) molecules, leukocyte function-associated antigen (LFA)-1, and Mac-1. The Ig superfamily adhesion molecules include the ICAMs, vascular adhesion molecules (VCAM), LFA-2 and LFA-3, and platelet endothelial cell adhesion molecule (PECAM)-1.

Resident tissue macrophages that recognize microbes secrete cytokines such as TNF, IL-1, and chemokines. TNF and IL-1 act on the endothelial cells of postcapillary venules adjacent to the infection and induce the expression of selectins by

**Figure 3** Complement system. Three pathways of complement activation lead to opsonization, chemotaxis, and the formation of the membrane attack complex (MAC). Classical pathway: Several molecules of IgG (or one molecule of IgM) bound to the fungal surface and bind to C1q, C1r, and C1s. Activated C1 leads to cleavage of C4 and C2 in C4a and C4b, C2a, and C2b. C4b and C2a have the function of C3 convertase. C3 convertase cleaves C3, leading further to C3a and C3b. Lectin pathway: Mannan-binding lectin (MBL) with a similar structure to C1q binds to the surface of fungi and to the MBL-associated serum proteases (MASP) and can cleave C4 or C2 in a fashion similar to that of the classical pathway. Alternative pathway: Antibodies are not required in this pathway. C3 cleaves spontaneously, binds to Factor B, and is cleaved by Factor D resulting in C3bBb, which has the function of a C3 convertase, analogous to the C4bC2a complex of the classical pathway (shown in gray as egg-shaped drawings). Properdin stabilizes the C3-convertase cleaves. The addition of C3b to the C3 convertases from classical or alternative pathway leads to C5 convertase that cleave C5 into C5a and C5b. C5b initiates the formation of the MAC that will cause lysis of the organism, although the importance for fungi is not clear. C3a and C5a are chemotactic molecules that favor inflammation. C3b and C4b are opsonins that will favor phagocytosis.
endothelial cells. Lymphocytes, monocytes, and neutrophils express ligands that bind to the endothelial selectins and cause leukocytes to roll along the endothelial surface.

TNF and IL-1 also induce endothelial expression of ligands for integrins, mainly VCAM-1, the ligand for the VLA-4 integrin, and ICAM-1, the ligand for the LFA-1 and Mac-1 integrin. Leukocytes express the integrin in low-affinity fashion. Chemokines and IFN-γ produced at the site of the infection are released into the bloodstream, bind to endothelial cells, and stimulate leukocytes to enhance the expression of leukocyte integrins. The binding of leukocyte integrins and endothelial cell ligands results in a firm binding of leukocytes to the endothelial interface. The leukocytes are attracted by PECAM-1 molecules, which are abundant in the interendothelial junction and migrate through the interendothelial spaces to the site of infection.

**Figure 4** Recruitment and migration of inflammatory cells to site of fungal infection. (I) Secretion of cytokines: Macrophages secrete cytokines, IL-1, and TNF, which increase the expression of selectins on endothelial cells (E and P) and leukocytes (L) and the expression of the corresponding ligand on the opposite cell. Endothelial cells secrete chemokines as IL-8, which attract neutrophils and monocyte chemotactic protein (MCP)-1 to attract monocytes. (II) Rolling adhesion: The light attachment of leukocytes to endothelial cells is disrupted by the forces of the circulating blood resulting in a rolling motion for leukocytes. (III) Firm adhesion: Chemokines (IL-8 and MCP-1) and IFN-γ increase the expression of integrins on leukocytes (LFA-1, Mac-1) and their corresponding ligands on endothelial cells resulting in a change in the shape of leukocytes and their firm adhesion to the vascular wall. (IV) Extravasation and recruitment of phagocytes: Platelet-endothelial cell adhesion molecules (PECAMs) are upregulated on leukocytes and endothelial cells. PECAMs guide the extravasation of the leukocytes to the site of infection with further recruitment in the presence of C5a (chemoattractant to neutrophils) and C3a (chemoattractant to macrophages).
1. **Neutrophils**

Neutrophils are the most abundant phagocytic cells and the earliest to be recruited to the infectious foci. They contain azurophilic and specific granules in their cytoplasm (Fig. 5). The azurophilic granules are similar to lysosomes filled with myeloperoxidase, a variety of proteolytic enzymes, including acid hydrolase, glucuronidase, mannosidase, arylsulfatase, cathepsin, elastase, lysozyme, collagenase, elastase, protease, and antimicrobial peptides such as defensins and bactericidal permeability-increasing protein. The specific granules contain lactoferrin, lysozymes, monocyte chemotactic factor, C3 and C5 cleaving proteases, membrane-bound components of

![Figure 5](image)

**Figure 5** Neutrophil activation: phagocytosis and killing. (I) Membrane receptors recognize the presence of the pathogen and activate the neutrophil. (II) Phagocytosis begins. (III) The phagocytosed particles form a phagosome, which attracts the azurophilic granules. (IV) A phagolysosome is formed from the fusion of the phagosome with azurophilic granules. The organism is attacked within the phagolysosome by oxidative and nonoxidative mechanisms. Oxidative-dependent mechanisms consist of the respiratory burst performed by the oxidase complex, resulting in toxic reactive oxygen intermediates (ROIs). Hydrogen peroxide (H₂O₂) is a substrate for myeloperoxidase (MPO) with formation of hydrochlorus acid (HOC1), a very potent antimicrobial compound. Defensins and proteolytic enzymes attack the pathogen in an oxygen-independent manner. (V) The pathogen is digested in the phagolysosome by proteolytic enzymes. (VI) The phagolysosome degranulates its content, mainly to the extracellular space, but can also form a vacuole in the phagocyte. (II') Upon cell activation, the specific granules migrate toward the cell membrane and fuse with it. (III') The specific granules degranulate to the extracellular space delivering microbicidal molecules to extracellular fungi. (IV') Exposure of membrane-bound receptors (complement, immunoglobulin, chemokine receptors) and the oxidase complex delivers ROIs to the extracellular fungi.
NADPH oxidase system, cytochrome-\(b_{558}\) and membrane-bound receptors, CR3, CR4, and the chemotactic receptor for C5a.

After the phagocytes recognize the pathogen, they ingest it through phagocytosis. In this process, they form vesicles called phagosomes, the contents of which bind to lysosomes forming the phagolysosomes in which pathogens can be killed. In the phagolysosomes, the molecular \(O_2\) is converted to reactive oxygen intermediates (ROIs) by the oxidase system in a process called respiratory burst. The ROIs are highly reactive oxidizing agents that destroy pathogens. Superoxide is one of the ROIs that can be converted to hydrogen peroxide and used by myeloperoxidase to produce more microbicidal products. Neutrophils also have nonoxidative killing mechanisms mediated by enzymes and defensins contained in their granules.

Azurophilic granules fuse with the phagosome and deliver high concentration of lytic proteins to the microbes while specific granules fuse preferentially with the plasma membrane releasing their contents in the extracellular space. This brings to the cell surface various functionally important membrane structures such as integrins, receptors for chemokines and opsonins, and the oxidase system.

Neutrophils play a major role in the defense against Candida spp. and Aspergillus spp. The normal number of circulating neutrophils is between 1500 and 2000 cells/mm\(^3\), and when the number drops below 100 cells/mL, the risk for fungal infections increases. There are several categories of impairments of neutrophils.

a. **Neutropenia.** Patients can have a deficient number of neutrophils as a consequence of myelosuppressive antineoplastic chemotherapy, induction of autoantibodies, or genetic disorders. The hereditary forms of neutropenia can be severe such as Kostmann’s syndrome, which is a rare autosomal recessive disorder with granulocyte-maturation arrest and early death, or benign such as cyclic or familial neutropenia. Patients with invasive fungal infections remain a major complication among neutropenic cancer patients and stem cell transplant recipients. The most common pathogens of fungal infections are Aspergillus spp. and Candida spp., mucormycosis and the emerging fungal pathogens Fusarium spp., Scedosporium spp., and Trichosporon spp. The likelihood of fungal infection not only correlates with the depth and duration of the neutropenia, but also with the presence of neutrophil dysfunction resulting from suppression of acquired immunity by drugs (glucocorticoids) or the presence of graft versus host disease. The severity of fungal infections in profoundly neutropenic patients with hematological malignancies is reflected by the poor survival rate, unless marrow recovery is complete (16,17)

Hematopoietic growth factors, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulate proliferation, maturation, and activation of phagocytes (Table 3). These cytokines are administered as prophylaxis to shorten the duration of neutropenia and as preemptive therapy at the onset of febrile neutropenia (18–21). Another strategy is the use of granulocyte transfusions from G-CSF and corticosteroid-stimulated donors. This approach has been used for treatment and for prophylaxis with variable results. Success appears to correlate with the higher numbers of granulocytes transfused and the host-performance status (22,23).

b. **Functional Defects of Neutrophils.** Chronic granulomatous disease (CGD): It is a hereditary disease, which can be autosomal or crosslinked, resulting from a defect in the cytochrome-\(b_{558}\) with failure to generate a respiratory burst in response to pathogens. CGD patients suffer from infections caused by catalase positive microorganisms, such as Staphylococcus aureus, Pseudomonas spp., Salmonella spp.,...
Table 3  Cytokines and Fungal Immunity: Protective and Deleterious Effects

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Source</th>
<th>Functional effect</th>
<th>Effect on fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytokines with a protective effect on fungal immunity</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Tumor necrosis factor (TNF)</td>
<td>Monocytes and macrophages</td>
<td>Stimulates and recruits neutrophils, monocytes, and lymphocytes. Increases expression of adhesion molecules and chemokine secretion. Stimulates IL-1 secretion by macrophages. Increases killing activity of phagocytes in large amounts, produces systemic effects: fever, increases liver production of acute phase reactants: C reactive protein, fibrinogen, and serum amyloid A protein (ESR). High levels produce hypotension and shock, reduces synthesis of lipoprotein lipase and causes cachexia, it is more typically produced in a type 1 response but can be present in a type 2 response (138). Critical for protecting mice against infection by Aspergillus spp. (139), Cryptococcus neoformans (99,100) H. capsulatum (140). Increases the fungicidal activity of neutrophils against hyphae and phagocytic activity of macrophages against conidia of Aspergillus spp. (100,141) Use of anti-TNF in humans has been complicated with aspergillosis and histoplasmosis (114,115,142).</td>
<td></td>
</tr>
<tr>
<td>Interferon-γ (IFN-γ)</td>
<td>NK cells in innate immunity T lymphocytes (in adaptive immunity)</td>
<td>Activates killing mechanisms of phagocytes, promotes Th1 polarization of the immune response and inhibits Th2 differentiation, stimulates production of IgG involved in opsonization for phagocytosis.</td>
<td>Enhances neutrophil and monocyte fungicidal effect against yeasts and molds (143–146). Increases in vitro neutrophil fungal damage against Aspergillus hyphae in CGD patients (147). Exhibits synergistic effect with Amphotericin B against cryptococcosis in SCID mice (148). Critical in protection against invasive aspergillosis (139) and histoplasmosis (111,140,149) in mice but not against candidiasis (150). Increases neutrophil antifungal activity against A. fumigatus, Candida albicans, Rhizopus arrhizus, Fusarium solani (143,145,146,153,154). Improves antifungal damage by phagocytes of HIV+ patients against A. fumigatus (33,155). Increases in vitro</td>
</tr>
</tbody>
</table>
DC1, and polarizes to a Th2 response during mobilization and when used to accelerate engraftment after bone marrow transplant (151, 152).

- **Granulocyte-macrophage colony-stimulating factor (GM-CSF)**
  - Monocytes, macrophages, endothelial cells, fibroblasts
  - Promotes differentiation of multipotential hematopoietic progenitor cells, activates neutrophils, eosinophils, monocytes, and macrophages. Improves phagocytosis, oxidative metabolism, release of chemotactic factors, and antigen presentation. Can be produced in the context of a Th1 or Th2 response. Inhibits IL-12 production by mouse DCs (157). Induces Th2 differentiation (158).

- **Interleukin (IL)-1**
  - Macrophages, neutrophils, epithelial and endothelial cells
  - Stimulates acute phase plasma proteins, induces central fever and cachexia

- **IL-2**
  - T lymphocytes
  - Promotes T- and B-cell proliferation.

- **IL-6**
  - Monocytes, macrophages, T lymphocytes, endothelial cells, fibroblasts
  - Stimulates the acute phase response (innate immunity). Promotes B-cell proliferation (adaptive immunity)

- **IL-12**
  - Monocytes, macrophages and DCs
  - Induces Th1 immune response, stimulates production of IFN-γ from T and NK cells. Increases the cytolytic activity of CD8+ and NK cells.

- Phagocytic fungal damage against *Candida* spp., *A. fumigatus*, and *R. arrhizus* after in vivo administration (153). Reverses the corticosteroid-mediated detrimental effect on antifungal activity of neutrophils (35). Improves the outcome of mice with acute disseminated candidiasis but not those with subacute or chronic candidiasis (156).

Activates monocytes and macrophages antifungal activity against *C. albicans*, *Fusarium solani*, *A. fumigatus* (145), *H. capsulatum*, and *C. neoformans*. Prevents the deleterious effect of corticosteroids on monocyte-mediated antifungal activity and cytokine production (39, 159).

It is part of the proinflammatory response against *Aspergillus* spp. *Candida* spp., and *C. neoformans* (160–163).

IL-2-activated NK cells inhibit *C. albicans* yeast growth in vitro (164).

Enhances resistance to invasive aspergillosis and candidiasis in mice (165, 166).


(Continued)
<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Source</th>
<th>Functional effect</th>
<th>Effect on fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-15</td>
<td>Bone marrow stroma cells, gut and skin epithelia, macrophages</td>
<td>Stimulates production of IL-8 from neutrophils and monocytes and macrophages. Stimulates production of IFN-γ from NK cells, favors Th1 polarization.</td>
<td>Increases the oxidative antifungal activity of neutrophils against <em>A. fumigatus</em> and induces IL-8 release in vitro after exposure to <em>A. fumigatus</em> and <em>A. flavus</em> (169). Increases oxidative antifungal activity of monocytes against <em>C. albicans</em> (170). Critical for cytotoxic T lymphocyte activity against <em>C. neoformans</em> (105).</td>
</tr>
<tr>
<td>IL-18</td>
<td>Monocytes, macrophages, DCs, and fibroblasts</td>
<td>Synergizes IL-12 by stimulating IFN-γ production by T-cells mediating a Th1 response. IL-18 + IL-10 in the absence of IFN-γ stimulate secretion of IL-13, which is a type 2 cytokine</td>
<td>Improves the antifungal activity of murine peritoneal exudates against <em>C. neoformans</em> (171). Critical for an effective clearance of <em>A. fumigatus</em> conidia in the context of allergic fungal airway disease in a murine model (172). Protects mice against disseminated candidiasis through endogenous IFN-γ (173).</td>
</tr>
<tr>
<td>IL-8 and CXCL chemokines</td>
<td>Monocytes, macrophages, endothelial cells, epithelial cells, T-cells</td>
<td>Recruits neutrophils</td>
<td>Important in chemotaxis against aspergillosis (174)</td>
</tr>
<tr>
<td>CCL chemokines</td>
<td>Monocytes, macrophages, endothelial cells, epithelial cells, T-cells</td>
<td>Chemoattractant to monocytes, chemoattractant and activator to eosinophils and basophils</td>
<td>Monocyte chemotactic protein-1 (MCP-1) is necessary for clearance of <em>C. neoformans</em> and <em>Aspergillus</em> from murine lungs (46,102,103) MIP-1α is necessary for the development of a Th1 response to <em>C. neoformans</em></td>
</tr>
<tr>
<td><strong>Cytokines with deleterious effect on fungal immunity</strong></td>
<td></td>
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<tr>
<td>IL-4</td>
<td>T lymphocytes, mast cells and basophils</td>
<td>Promotes B- and T-cell proliferation and production of IgGl and IgE. Inhibits macrophage activation, antagonizes Th1 development</td>
<td>Plays a role in the pathogenesis of allergic aspergillosis (175). IL-4-deficient mice are more resistant to invasive aspergillosis and cryptococcosis than wild-type mice (78,176). Impairs pulmonary clearance of <em>H. capsulatum</em> in mice (177).</td>
</tr>
<tr>
<td>IL-5</td>
<td>T lymphocytes and mast cells</td>
<td>Promotes production of IgM, IgA, and IgE, eosinophil expansion, and activation of eosinophils. Favors allergic bronchopulmonary aspergillosis (175,178,179).</td>
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</tr>
<tr>
<td>IL-10</td>
<td>Monocytes, macrophages, CD4+ T-cells, B cells, keratinocytes</td>
<td>Anti-inflammatory effect, inhibits proinflammatory cytokine secretion. Decreases phagocytosis, oxidative burst, intracellular killing and antigen presentation to T-cell, decreases inflammation in a T&lt;sub&gt;H2&lt;/sub&gt; context, upregulates perforin expression and the Fas/Fas ligand system by cytotoxic T lymphocytes. Decreases antifungal activity of monocytes against Aspergillus hyphae in vitro (180). Detrimental in invasive aspergillosis in a murine model (80). Beneficial effect in mice with allergic pulmonary aspergillosis (82). Increased levels in non-neutropenic patients with invasive aspergillosis correlates with poor outcome (79).</td>
<td></td>
</tr>
<tr>
<td>IL-25</td>
<td>Lymphocytes</td>
<td>Induces airway hyper-reactivity, induces type 2 cytokine response, activates eosinophils. Produced in response to Aspergillus spp. in the airway of animals. May mediate allergic diseases related to Aspergillus spp. (181).</td>
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</tbody>
</table>
Aspergillus spp., and Candida spp. (24). Antibiotics and IFN-γ prevent serious infections in these patients (25). Neutrophils of CGD patients improve their antiaspergilar activity when the patient is treated with IFN-γ (9).

**Leukocyte adhesion deficiency (LAD):** It is an autosomal recessive disorder in which the number of neutrophils may be increased; neutrophils, however, are unable to migrate to the site of infection. LAD-1 is associated with a defect in integrins, which affects the neutrophil’s ability to adhere firmly to the endovascular endothelium, and LAD-2 is related to a defect in selectins, which affects the neutrophil’s capacity to roll over the endothelium. These disorders cause early death as a result of life-threatening infections, mostly bacterial.

**Chediak–Higashi syndrome (CHS):** It is an autosomal recessive disorder affecting neutrophils and other cells. The lysosomes are giant and dysmorphic. In these patients, leukocyte counts are decreased and neutrophils have poor chemotactic response and delayed degranulation of phagolysosomes resulting in deficient and delayed microbial killing and severe infections.

**Job’s syndrome:** This syndrome is characterized by defective neutrophil chemotactic response, recurrent cutaneous bacterial infections, and mucocutaneous candidiasis.

**Abnormal phagocytosis because of decreased MBL:** Patients with low levels of MBL are at increased risk for infections particularly after cytotoxic chemotherapy. Low levels of MBL have been associated with recurrent vulvovaginal candidiasis, and polymorphism of the MBL gene was described in patients with chronic necrotizing pulmonary aspergillosis (26–28).

**Myeloperoxidase deficiency:** Neutrophils with MPO deficiency have delayed microbial killing, though without an apparent increase in incidence of infections in humans. Myeloperoxidase deficiency produces a delay in the clearance of A. fumigatus and decreased cytotoxicity to C. albicans, C. tropicalis, and Trichosporon asahii in experimental murine infections (29).

**Specific granule deficiency:** A rare congenital disease characterized by dysfunction of the neutrophils resulting in recurrent severe bacterial infections, but uncommon fungal infections, starting from early infancy. The disease is characterized by abnormal migration and decreased killing activity of neutrophils lacking defensins, lactoferrin, BPI, and collagenase. Eosinophils, monocyte, and macrophages are also affected.

c. Functional Defects of Neutrophils in Systemic Conditions. Several examples of this are notable:

1. Diabetes mellitus: Neutrophils in diabetic patients have decreased chemotaxis, phagocytic capacity, and killing capacity, especially during hyperglycemia (30,31).
2. AIDS: Neutrophils from patients with advanced AIDS have decreased migration, phagocytosis, and killing when stimulated by encapsulated C. neoformans in vitro (32). Neutrophils from HIV-infected patients with low CD4+ lymphocyte counts also have decreased antifungal activity against A. fumigatus in vitro that can be partially corrected with G-CSF pretreatment (33).
3. **Glucocorticoid therapy:** Glucocorticoids affect multiple neutrophil functions: suppress migration, phagocytosis, oxidative burst and free radical generation, degranulation, nitric oxide release, and cytokine production, although neutrophil survival is increased (decrease apoptosis). In vitro, A. fumigatus is stimulated in the presence of pharmacological doses of
hydrocortisone (34). IFN-γ and G-CSF prevent the suppression of *A. fumigatus* hyphal damage by neutrophils induced by glucocorticoids (35).

2. **Mononuclear Phagocytes**

Resident tissue macrophages encounter the pathogen upon initial infection, and their central role is to inhibit it and kill it. Monocytes are also recruited from the circulation to the site of infection attracted by chemokines. Alveolar and interstitial macrophages from the lungs play a very important role against fungi because most fungal infections are acquired through the airborne route.

Mononuclear phagocytes kill pathogens by oxygen-dependent and oxygen-independent mechanisms, as described with neutrophils. In the oxidative antimicrobial killing, oxygen is converted into ROIs by the oxidase system. In addition to ROIs, macrophages produce nitric oxide (NO), which can combine with hydrogen peroxide or superoxide and generate more potent antimicrobial molecules.

Macrophages produce cytokines including TNF, IL-β, IL-6, IL-12 and chemokines such as MCP-1 and possess the machinery required for antigen presentation.

Macrophages live longer than neutrophils and persist longer in the site of the infection. G-CSF, GM-CSF, M-CSF, and IFN-γ enhance the antifungal activity of phagocytes by augmenting oxidative and nonoxidative mechanisms to damage the membrane and cell wall of fungi (Table 3).

Of note, antifungal agents affecting the integrity of the fungal cell membrane and wall have a synergistic and/or additive effect with phagocytes against fungi with increased fungal damage in vitro (36) and in animal models of murine aspergillosis (37).

Macrophages are the main effector cells against *H. capsulatum, C. neoformans*, and *Penicillium marneffei* yeast cells but they require cytokine activation to destroy these fungi.

Glucocorticoids induce reversible monocytopenia and impair the maturation, chemotaxis, migration, and phagocytosis and cytokine production by monocytes. The killing effect of alveolar macrophages against *A. fumigatus* conidia is mediated through ROIs. This effect is reduced by corticosteroids and may be responsible of the increased susceptibility to invasive infections (38). GM-CSF prevents in vitro the inhibitory effect of glucocorticoids on cytokine production by alveolar macrophages challenged with *Aspergillus* conidia (39) (Table 3).

In contrast to the damaging effect of phagocytes on fungal cells, fungi have their own protective mechanisms against phagocytes. Spores of *Aspergillus* spp. inhibit phagocytosis in vitro (40,41), and toxins such as gliotoxin and proteases produced by *Aspergillus* spp. and other pathogenic fungi inhibit neutrophil activation, respiratory burst, and chemotaxis (41–43). Unidentified products from *A. fumigatus* also inhibit the effect of macrophages on conidial germination and hyphal damage by neutrophils (44).

3. **NK Cells**

NK cells are lymphocytes that can secrete IFN-γ, recognize cells lacking the major histocompatibility complex (MHC) class I molecule with resulting NK-cell activation and killing of these cells. NK cells are especially important for defense against viruses, but can also kill cells infected with other intracellular pathogens.

NK cells are activated by cytokines, mainly IL-15 and IL-12 and high levels of IL-2. Through the secretion of IFN-γ, NK cells activate macrophages and favor polarization of T-cells to type 1 response. NK cells kill target cells (infected cells, tumor cells), by creating pores in the cellular membranes with perforins, and indu-
cing apoptosis of target cell through granzymes. Apoptosis can also result from a Fas–Fas ligand activation. NK cells play a role against *C. neoformans* and *A. fumigatus* through secretion of IFN-γ (45,46).

4. Platelets
Platelets aggregate and are able to damage non-albicans *Candida* spp. and *A. fumigatus* (47,48) in in vitro assays. Whether this is clinically important is unclear.

III. ACQUIRED IMMUNITY

Adaptive, acquired, or specific immunity, like innate immunity, is stimulated by exposure to infectious agents. However, adaptive immunity is characterized by its extraordinary capacity to distinguish among different, even closely related agents, and its ability to generate an enhanced immune response through the production of memory cells after exposure to a particular agent. The main characteristics of acquired immunity are its specificity and its memory allowing response to a previously known antigen.

There are two types of acquired immunity: humoral and cell-mediated.

A. Humoral Immunity
Humoral immunity is mediated by antibodies secreted by B lymphocytes and can be transferred by serum from an immunized individual to a nonimmunized one. Antibodies bind to the antigens of extracellular microbes and function to neutralize and eliminate these microbes. The elimination of different types of microbes requires several effector mechanisms, which are mediated by distinct classes, or isotypes of antibodies. B lymphocytes populate peripheral lymphoid tissues, which are the sites of interaction with foreign antigens. Antigen binds to the membrane IgM and IgD on naive cells and activates these cells. B cells proliferate in response to the antigen, resulting in clonal expansion of antigen-specific cells and differentiation into effector cells that actively secrete antibodies and into memory cells. The B cell internalizes the antigen and processes it. If the antigen is a protein, the antibody response requires CD4+ helper T lymphocytes that recognize the antigen and play an essential role in further activation of B cells (Fig. 8B). Polysaccharides and lipid antigens activate B cells through a T-independent pathway.

The IgM and IgD are two chains, and Igα and Igβ are the receptors for the antigen. They form the B-cell receptor complex (similar to T-cell receptor (TCR) with CD3 and ζ in T-cells). Once the antigen is recognized, the complex starts the first signal of activation and the second signal is provided by complement: C3b bound on the surface of the microbe is degraded to C3d, and this binds to CR2 (type 2 CR), CD19, and CD81. These three receptors represent the coreceptor complex, and when the microbe is bound to the BCR and the complement is bound to this coreceptor complex, the B-cell activation begins. Activated B cells upregulate the expression of class II MHC, costimulatory molecules, and chemokine and cytokine receptors. They process the antigen into peptides that bind to the MHC class II molecule. The complex of class II MHC and peptide migrates toward CD4 lymphocytes and results in stimulation of B-cell clonal expansion, isotype switching, and differentiation in memory B cells (Fig. 8B). When the memory cells are exposed to the antigen again, the secondary response develops faster and produces larger amounts of antibodies.

Humoral immunity plays a limited role in host defense against fungi. B-cell depleted mice are susceptible to systemic but not to mucosal candidiasis (49).
Vaccination of mice with a liposome-mannan vaccine elicits monoclonal antibodies that are protective against disseminated and vaginal candidiasis. The passive administration of these antibodies is also protective against these infections. Protection is elicited by binding of these antibodies to complement (activating the classical pathway) with opsonization of the yeast cell for faster and more effective phagocytosis (50–52). Mycograb, a human monoclonal antibody against *Candida* heat shock protein (Hsp) 90, is present in patients with disseminated candidiasis, and its presence correlates with recovery from infection. This antibody is protective against murine disseminated candidiasis and acts synergistically with amphotericin B against *albicans* and *non-albicans Candida* spp. This action may be mediated by decreasing the adherence of circulating yeast cells to tissues (53,54). MAb C7, a monoclonal antibody against a protein epitope from a mannoprotein, has three mechanisms of protection against candidiasis: decreasing the adherence of *Candida* to epithelial cells, inhibiting germination, and damaging the yeast by direct candidacidal effect. This antibody is also fungicidal against *C. neoformans*, *A. fumigatus*, and *Scedosporium prolificans*, mimicking the activities of a killer toxin (52). An anti-idiotypic antibody to antibodies recognizing killer toxin and mimicking natural antikiller toxin receptor is protective against experimental candidiasis, cryptococcosis, aspergillosis, and pneumocystosis (55).

Antibodies against *C. neoformans* can be protective, nonprotective, or deleterious, depending on dose, antibody isotype, inoculum size, and availability of T-cells and mediators of cell immunity (56). Vaccines with polysaccharide conjugate and peptide vaccines that elicit antibodies to the capsule are protective against experimental Cryptococcosis. In addition, monoclonal antibodies against glucuronoxylomannan (GXM), a capsule component, protect against *C. neoformans*, probably by facilitation of cellular response (57,58).

Humoral immunity does not appear to play an important role in the defense against infections by other fungi such as *Aspergillus* spp, *H. capsulatum*, or *Coccidioides immitis*, despite the role that antibodies against these pathogens play in diagnosis.

### B. Cell-mediated Immunity

Cell-mediated immunity is the most important mechanism of acquired immunity against fungal pathogens. In cell-mediated immunity, T lymphocytes recognize the antigen and act to destroy the micro-organism and/or infected cell. Its mode of action relies to a great degree on stimulating phagocytic immune response.

The principal cells of the cell-mediated immune system are the antigen-presenting cells (APCs) and lymphocytes. The most highly specialized APCs are DCs, although monocytes, macrophages, and B lymphocytes may also act as APCs. DCs are the best at stimulating naive T-cells while DCs, macrophages, and B cells can stimulate specific T-cells previously primed for the same antigen.

The adaptive immune response starts when the antigen is presented to the T-cell, and the T-cell recognizes it. The T-cell responds with proliferation leading to clonal expansion and activation. The activated T-cells stimulate the response, which will eliminate the pathogen through stimulation of phagocytic cells. Once the elimination of the pathogen is achieved, the homeostasis is reached by a decrease of the immune response. Most of the immune cells die because of the lack of antigen stimulation, but some cells are preserved as memory cells that will respond faster than naive cells during a second encounter with the same pathogen (Fig 6).
1. **Antigen Recognition**

To present the antigen to T-cells, the APCs, mainly immature DCs, have to uptake and process the antigen. Immature DCs are located in the skin and mucosa (gastrointestinal, respiratory tract, genitourinary tract) where they capture microbial protein antigens, process them, and transport them to the draining lymph nodes where they present them to T-cells. During this process, DCs become mature (Fig. 1–IV).

The process is different according to the source of the antigen, which can be extra- or intracellular (Fig. 7):

a. Extracellular antigens are attracted by the APCs through receptors such as mannose, Fc, complement, and TLR. The APCs endocytose the antigen to process it into peptides (antigen processing) and present the peptides bound to MHC to T-cells (antigen presentation) to initiate a T-cell response to the given antigen (priming).

DCs and macrophages also can phagocytose the pathogen; the phagosome then fusing with the lysosome to form a phagolysosome.
The pathogen is destroyed and results in formation of proteic antigens and peptides. In B cells, protein antigens bind to BCR and coreceptor on the cell surface, which induce endocytosis of the antigen.

Endocytosed proteins in the DC, macrophage, or B cell reside in the
endosome where proteolytic enzymes degrade them into peptides that will be assembled with the MHC class II molecule. MHC class II molecules are synthesized in the endoplasmic reticulum where they bind to an invariant chain. The invariant chain stabilizes the MHC class II molecule and guides its movement toward the endosome. The endosome and the vesicle containing the MHC class II molecule fuse together, and the new vesicle is called MIIC. In the MIIC, the invariant chain is released from the MHC class II molecule and the peptides replace the invariant chain to form the complex to be presented on the APC cell surface to the CD4+ T-cell.

b. Intracellular antigens are those produced by virally infected cells, mutated proteins produced by tumor cell, or less frequently, produced by an intracellular pathogen other than virus (Fig. 7C). The proteins from the cytosol are processed by the proteasome resulting in peptides. These peptides are transported into the endoplasmic reticulum by a transporter associated with antigen presentation (TAP). In the endoplasmic reticulum, the peptide binds the MHC class I molecule. The complex MHC class I–peptide moves toward the Golgi complex and is transported to the cell surface by exocytic vesicles. Once on the surface, the antigen is presented to a CD8+ cell. Any nucleated cell having the MHC class I molecule can present intracellular antigens to CD8+ cells.

During antigen processing, DCs lose their adhesion molecules to epithelial cells and express a chemokine receptor called CCR7 (receptor for CC chemokine) that is specific for lymph node chemokines. These chemokines attract DCs bearing CCR7 receptor to the lymph node. Because naive T-cells also express CCR7, they are also attracted to the lymph node where they will encounter the DCs with resulting antigen presentation to naive T-cells.

In order to respond, the T-cell has to recognize the MHC molecule, and the peptide from the MHC–peptide complex. Naive T-cells will accept any peptide, but memory specific T-cells will only respond to the same antigen recognized during prior contact. This gives T-cells a dual MHC and antigen specificity.

The activation of T-cells requires two signals: the antigen itself triggers the first signal and a second signal is induced by microbial products or cytokines produced in response to a microbial product. The requirement for a second signal triggered by microbes or cytokines ensures that immune responses are triggered only when needed and not against harmless substances, including self-antigens (Fig. 8).

The T-cells are first attracted by adhesion receptors and their ligands. Once in contact, the T-cells recognize the MHC–peptide complex in the APC through the TCR. The presence of the CD4+ and CD8+ molecules on the surface of T-cells is indispensable for the lymphocyte to respond, initiate the first-signal transduction event leading to cell maturation and promotion of adhesion of the TCR to the MHC–peptide complex. Once the peptide is recognized, CD3 and ζ can lead to functional activation of the T-cell. CD2 binds to LFA-3 and is important for both adhesion and activation.

The second signal is provided by the costimulatory APC molecules B7-1 (CD80) and B7-2 (CD86). These molecules bind to CD28 on the lymphocyte surface, which results in an antiapoptotic signal to cells, proliferation and production of cytokines, and growth factors. The expression of B7 is upregulated by microbial substances such as endotoxins, and by cytokines produced by the innate immune reaction to an antigen. Activated T-cells also express CD40 ligand, a molecule that
binds CD40 expressed on APC and also upregulates B7. After lymphocyte activation, another receptor, the CTLA-4 (CD 152) is expressed on T-cells and binds to B7 receptors to deactivate the lymphocyte and terminate the TCR. CTLA-4 plays an important role in self-tolerance.

### 2. Cell Activation

After the first encounter with the antigen, naive T-cells get activated and respond with proliferation, differentiation, and synthesis of new proteins (Fig. IV). Prolifera-
tion, mediated by IL-2 in an autocrine pathway, results in expansion of the T-cell pool and differentiation into memory and effector T-cells. Each antigen originates a clone of cells that will only recognize that given antigen presented by the MHC molecule. Upon subsequent exposures to this antigen, the specific pre-existing clone will be activated.

Effector T-cells migrate to the site where the antigen is located (Fig. VI). This migration is not antigen specific, but once in the inflamed tissue, only lymphocytes specific for the attracting antigen will stay, whereas the other lymphocytes may return to circulation. At this stage, CD4+ lymphocytes are called T helper precursor. When they get to the site of inflammation, they become effector CD4+ cells and can produce two subsets of cytokines, T helper 1 (T\textsubscript{H1}) or T helper 2 (T\textsubscript{H2}) also called type 1 and type 2 immunity because the response involves not only T helper lymphocytes, but also other cells. The polarization to T\textsubscript{H1} or T\textsubscript{H2} is determined by the type of antigen and APCs, by the presence of hormones, and most importantly by the cytokine environment (Fig. 9). The presence of IL-12 produced by monocytes and DCs at the site of the infection will polarize toward a T\textsubscript{H1} response whereas during the lack of IL-12, the presence of IL-4 will induce a T\textsubscript{H2} response. When the APC is a B cell, T\textsubscript{H2} ensues, but a T\textsubscript{H1} response occurs when the APC is a macrophage. DCs can induce a T\textsubscript{H1} or T\textsubscript{H2} response.

The type of antigen also influences the response with bacteria and fungi stimulating T\textsubscript{H1} and helminths stimulating T\textsubscript{H2}. A T\textsubscript{H2} response also occurs in the presence of a high antigen burden (including fungi and bacteria). Thus, reducing the fungal burden by antifungal agents favors T\textsubscript{H1} polarization, while the administration of glucocorticoids or anti-IL-2 agents (cyclosporine, tacrolimus) favors type 2 immunity, even when responding to antigens that normally would elicit type 1 immune response. Estrogens decrease the activation of macrophages by increasing the production of hepatic inhibitory substance Apo-I. On the other hand, testosterone decreases the production of the inhibitory Apo-I, favoring T-cell–macrophage contact and the subsequent macrophage activation (59), although the role of hormones in cell-mediated immunity is still not well understood (60). Pregnancy is associated with abrogation of cell-mediated immunity and a T\textsubscript{H2} immune response. Fungal dissemination during pregnancy has been described with *H. capsulatum* and *C. immitis* (61,62). Acute stress favors type 1 response while chronic stress favors type 2 response (63).

The hallmark of type 1 response is the production of IFN-\(\gamma\), which is the most potent stimulus for the macrophage’s ability to kill intracellular pathogens including fungi. Activated macrophages increase inflammation through secretion of TNF, IL-1, chemokines, prostaglandins, leukotriens, and platelet-activating factors. Finally, macrophages remove dead tissue and facilitate damage repair.

Type 2 response is characterized by the production of IL-4, IL-5, IL-9, and IL-13. Effector cells are B lymphocytes and plasma cells with production of IgE antibodies that opsonize helminths. IL-5 activates eosinophils that bind to the opsonized helminths and destroy them. The relative proportions of T\textsubscript{H1}/T\textsubscript{H2} subsets induced during an immune response are major determinants of the protective functions and pathologic consequences of the response to a pathogen. Once a type of response is established, T\textsubscript{H1} favors its own expansion by an autocrine mechanism with T\textsubscript{H1} response expanded by IFN-\(\gamma\) and IL-2 while T\textsubscript{H2} response is perpetuated by IL-4.

Lymphocytes that are not completely polarized are called T\textsubscript{0} and they can simultaneously produce IFN-\(\gamma\) and IL-10.
A positive correlation has been found between the occurrence of TH1 cell responses and resistance to experimental infections by various fungi including *C. immitis*, *P. brasiliensis*, *H. capsulatum*, *B. dermatitidis*, *C. neoformans*, *A. fumigatus*, and *C. albicans* (64). A type 2 response is associated with fungal proliferation and invasive infection or an allergic reaction (65–68).
The effector CD8+ subset differentiates into Cytolytic T lymphocytes (CTLs) with the ability to kill infected cells (mainly virally infected cells) and tumor cells bearing a specific MHC–antigen complex.

The principal mechanism of CTL killing is through the delivery of granules containing perforins and granzymes. Perforins form aqueous channels in the membrane of the target cell through which granzymes penetrate into the cell and activate caspasess, which in turn lead to apoptosis. Caspase activation with cell death can also result from the binding of Fas ligand to T-cells. The role of cytotoxicity in fungal infections is not clear. CD8+ T-cells also produce IFN-γ.

3. Homeostasis

After the elimination of the pathogen, the immune system returns to its basal resting state, in large part because of the apoptosis of antigen-stimulated lymphocytes. The survival of these lymphocytes depends on antigens and antigen-induced growth factors. The elimination of the antigen by the immune response deprives these lymphocytes of their essential survival stimuli leading to their death.

The immune response can be suppressed by T regulatory cells (Tr, CD4+ CD25+) through direct contact with APCs and/or naive T-cells and through secretion of IL-10 and transforming growth factor β (TGF-β), which inhibit TH1. The role of regulatory cells is to prevent an exaggerated T-cell-mediated response that may result in severe tissue damage. Tr cells may prevent complete eradication of the infection thus allowing the induction of long-term immunity, a mechanism described with candidiasis (69). Tr lymphocytes are also considered tolerance inducers.

4. Memory

Memory T-cells are a population of antigen-specific T lymphocytes that survive for long periods of time even after elimination of the antigen and get rapidly activated when encountering the same antigen.

Memory T-cells accumulate through life and account for about 50% of the adult T-cells. Memory T-cells present in peripheral tissues exhibit a rapid response to encounter with the antigen and are called T effector memory (TEM) while the slower responding T central memory (TCM) are present predominantly in lymphoid organs. TCM is CCR7-high memory and TEM is CCR7-low memory (70,71).

a. Compromised Cell-Mediated Immunity and Fungal Infections. Patients with altered cell immunity include those with AIDS, the recipients of stem cell and organ transplantation, and patients receiving corticosteroids and other immunosuppressive drugs (OKT3, azathioprine, methotrexate, mycophenolate mofetil, tacrolimus, cyclosporine, rapamycin/sirolimus), anti-TNF and anti-IL-2 antibodies.

Patients with altered cell-mediated immunity are susceptible to infections caused by various fungi including H. capsulatum, C. neoformans, Pneumocystis carinii, and P. marneffei and dermatophytes including Aspergillus spp. and Candida spp.

IV. HOST DEFENSES AGAINST THE MOST COMMON FUNGI IN IMMUNOCOMPROMISED HOSTS

A summary of the host defenses against the most common fungal pathogens affecting immunocompromised hosts is shown in Table 4.
Table 4  Host Defenses Against the Most Common Fungi in the Immunosuppressed Host

<table>
<thead>
<tr>
<th></th>
<th>Molds</th>
<th>Candida spp.</th>
<th>Cryptococcus neoformans</th>
<th>Dimorphic fungi</th>
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</thead>
<tbody>
<tr>
<td><strong>Soluble factors</strong></td>
<td>Airway opsonizing and microbicidal substances improve phagocytosis</td>
<td>Lysozyme and Histatin 5 has anticanadial activity (182,183)</td>
<td>Airway opsonizing and microbicidal substances improve phagocytosis</td>
<td>Defensins are fungistatic against <em>Histoplasma capsulatum</em> (130)</td>
</tr>
<tr>
<td><strong>Complement</strong></td>
<td>Opsonization, chemotaxis and phagocytosis (13)</td>
<td>C5-deficient mice with disseminated candidiasis have a worse outcome (15)</td>
<td>Opsonization, chemotaxis, and phagocytosis (13)</td>
<td><em>Histoplasma capsulatum</em> can activate the alternative pathway, although importance for host defense is unknown.</td>
</tr>
<tr>
<td><strong>Neutrophils</strong></td>
<td>Hyphal destruction by oxidative and nonoxidative mechanisms.</td>
<td>Critical role. <em>Candida</em> hyphae damaged by oxidative and nonoxidative mechanisms (184)</td>
<td>Limited role. Neutropenic mice survive longer than controls (98)</td>
<td>Neutrophils can destroy <em>H. capsulatum</em> through the contents of azurophilic granules (130).</td>
</tr>
<tr>
<td></td>
<td>Function and number are critical in host defense (16,73)</td>
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<td></td>
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<td></td>
<td>White blood cell transfusion useful for neutropenic patients with mold infections (22,23).</td>
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<tr>
<td><strong>Macrophages</strong></td>
<td>Conidial phagocytosis and destruction, secretion of inflammatory cytokines and chemokines (74,75)</td>
<td>Phagocytes are the effector cells but need T-cell augmentation. Macrophages are indispensable for antifungal killing (185).</td>
<td>Fungal phagocytosis and destruction, secretion of inflammatory cytokines and chemokines. However, yeasts can multiply in the macrophage and kill in the absence of macrophage activation by cytokines (101).</td>
<td><em>Histoplasma capsulatum</em>: Fungal phagocytosis and destruction, secretion of inflammatory cytokines and chemokines (109), but the fungus can multiply in the macrophage and kill in the absence of macrophage activation by cytokines (186). <em>Penicillium marneffei</em>: Fungal phagocytosis and destruction, secretion of inflammatory cytokines and chemokines (120)</td>
</tr>
<tr>
<td><strong>NK cells</strong></td>
<td>Production of IFN-γ favoring a type 1 response. Increased mortality of NK cell-depleted (neutropenic) mice (46)</td>
<td>Production of IFN-γ after exposure to <em>Candida</em> spp. yeasts (164)</td>
<td>Production of IFN-γ favoring a type 1 response (45)</td>
<td>Production of IFN-γ favoring a type 1 response (113). May have cytotoxic activity because perforin-deficient mice have increased mortality (112)</td>
</tr>
</tbody>
</table>

*(Continued)*
### Table 4  Host Defenses Against the Most Common Fungi in the Immunosuppressed Host (Continued)

<table>
<thead>
<tr>
<th>Molds</th>
<th>Candida spp.</th>
<th>Cryptococcus neoformans</th>
<th>Dimorphic fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCs</td>
<td>Phagocytosis of fungi (conidia and hyphae), migration to lymph nodes, antigen presentation to T-cells, production of IL-12 and induction of a type 1 response (76)</td>
<td>T&lt;sub&gt;H1&lt;/sub&gt; response after stimulation with yeasts, germ-tube forms and hyphae of <em>Candida albicans</em> (187). In another study, exposure to conidia results in T&lt;sub&gt;H1&lt;/sub&gt; response whereas exposure to hyphae leads to T&lt;sub&gt;H2&lt;/sub&gt; response (188)</td>
<td>Phagocytosis of fungi, migration to lymph nodes, antigen presentation to T-cells, production of IL-12, and induction of a type 1 response (189). The fungal capsule interferes with phagocytosis, but antcapsular antibodies improve phagocytosis through Fc receptors (190)</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; cells</td>
<td>Production of IFN-γ and polarization towards a T&lt;sub&gt;H1&lt;/sub&gt; protective response.</td>
<td>Production of IFN-γ and polarization toward a T&lt;sub&gt;H1&lt;/sub&gt; protective response. Critical role in protection against disseminated and meningeal cryptococcosis (107,196–198)</td>
<td><em>Histoplasma capsulatum</em>: Production of IFN-γ and polarization towards a T&lt;sub&gt;H1&lt;/sub&gt; protective response. Critical role in protection in primary infection (111,118). CD4&lt;sup&gt;+&lt;/sup&gt; cells from spleen of immunized mice transfer protection to animals (199,200).</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt; cells</td>
<td>Role unknown</td>
<td>Decrease tissue damage in experimental candidiasis (203). Probable protective role of local CD8&lt;sup&gt;+&lt;/sup&gt; lymphocytes in experimental vaginal candidiasis (194,195)</td>
<td>In secondary infection, either CD4 or CD8 is necessary for survival (111). CD8&lt;sup&gt;+&lt;/sup&gt; may have cytotoxic role (112). Depletion of CD4 and CD8 in mice worsens experimental infections (CD4 depletion alone is worse than CD8 depletion) (205)</td>
</tr>
<tr>
<td>Type 1 response</td>
<td>Type 1 cells and cytokines (Table 3) critical for protection in animal models of infection (75,77) IFN-γ as adjuvant</td>
<td>Critical role for protection in animals (87). Failure to develop type 1 response may be the cause of CDC after recovery from</td>
<td><em>Histoplasma capsulatum</em>: Type 1 cells and cytokines (Table 3) critical for protection in animal models (107). IFN-γ adjuvant therapy is under evaluation in</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Paracoccidioides brasiliensis</em>: Type 1</td>
</tr>
</tbody>
</table>

*Histoplasma capsulatum*: Phagocytosis of yeast form, migration to lymph nodes, antigen presentation to T-cells, production of IL-12, and induction of a type 1 response. *Coccidioides immitis*: DC pulsed by a *Coccidioides* lyssate mature and induce a specific T<sub>H1</sub> response in vitro (191).
therapy for invasive infections in non-neutropenic patients

**Type 2 response**

- Type 2 cells and cytokines (Table 3) deleterious in animal models of infection. Plays a role in allergic complications, asthma, and allergic broncho-pulmonary aspergillosis (179,209).
- Deleterious in animal models of candidiasis (87). Pseudohyphae generate type 2 response (210).

**Antibodies**

- Not protective
- Protective role in experimental vulvovaginal, disseminated and systemic candidiasis(211,50,51,53,54)

**Vaccines**

- Partial protection shown in experimental aspergillosis with vaccines based on DCs, recombinant Aspergillus antigen, and Aspergillus filtrate (83,85,86)
- Partial protection against experimental disseminated candidiasis in immunosuppressed mice using a Candida membrane antigen (212–214) or DC-based vaccine (215)
- Partial protection in experimental cryptococcosis with various vaccines: culture filtrates (107), recombinant proteins (108), capsular polysaccharide mimotope (57) and APC-based (pulsed with a capsular glucuronoxylmanaran) (198).

- Histoplasma capsulatum: A monoclonal antibody against a yeast surface protein increases phagocytosis and intracellular killing by macrophages and prolongs animal survival (116)

- Partial protection in experimental histoplasmosis with various vaccines: recombinant protein H (117) and Hsp 60 (118). Coccidioides immitis: Partial protection in experimental coccidioidomycosis with various vaccines (216–220).
A. Molds

Most of the information related to immunity against molds is based on work on *Aspergillus* spp. After inhalation in the respiratory tract of a healthy individual, conidia are efficiently eliminated by the innate mucosal local defense. Alveolar macrophages selectively kill conidia (38,72), get activated in response to fungi, and release pro-inflammatory cytokines and chemokines that will result in a second wave of phagocyte recruitment and fungal phagocytosis. Conidia that escape the first line of cellular defense may germinate and grow as hyphae. Protection against hyphae is mediated by neutrophils (73), which is illustrated by the strong clinical association of severe neutropenia and defect of the oxidative killing of neutrophil with invasive aspergillosis (16,24). NK cells may also play a role as depletion of NK cells in neutropenic mice with invasive aspergillosis results in increased fungal burden and mortality (46,74,75).

APCs, mainly DCs, activate B and T-cells resulting in humoral and cellular response. DCs transport the antigen to the draining lymph nodes and present the antigen to T-cells. T-cells will result activated, will proliferate, and migrate to the infected tissue, where they will be polarized to a TH1 or TH2 response (76). The presence of IL-18 upregulates the IL-12 receptor on T-cells driving the response to a protective TH1 type, with production of IFN-γ, which recruits and stimulates the phagocytic and fungicidal activity of neutrophils and macrophages (75,77). Patients who cannot develop a TH1 response are vulnerable to invasive aspergillosis.

Type 2 response is associated with increased susceptibility to invasive aspergillosis in animal models (65,78). Higher serum levels of IL-10 were found in patients with progressive invasive aspergillosis, where as patients with good response to treatment have lower or undetectable levels (79). However, IL-10 is necessary to limit tissue damage and to the development of regulatory CD4⁺CD25⁺ T-cells that may lead to long-lasting memory lymphocytes and antifungal immunity (80,81).

Atopic patients initiate a type 2 response characterized by IL-4 and IL-5 cytokine production. IL-4 leads to production by B cells of IgE antibodies against *Aspergillus* antigens, followed by increased sensitization of mast cells. IL-5 recruits eosinophils from the bone marrow to the airways. These patients develop *Aspergillus*-associated asthma or the more complicated manifestation, allergic bronchopulmonary aspergillosis (ABPA). The continuous presence of the fungus on the epithelial surface will result in abundant liberation of antigens eliciting strong antibody production. IL-10 reduces the inflammation seen in animal models of ABPA (82). Studies in animals suggest that it could be possible to drive the immune defense to a beneficial TH1 type even in immunosuppressed animals by using adoptive transfer of antigen specific CD4⁺ T-cells producing IFN-γ and IL-2 or by conidial RNA-transfected DCs. Vaccination with *Aspergillus* filtrates or a recombinant antigen with cytosine phosphate guanosine oligodeoxynucleotides as adjuvant promotes a dominant TH1 response with protective effect in experimental aspergillosis (83–86).

B. Yeasts

1. *Candida* spp.

*Candida* spp. colonize the gastrointestinal and genital tract of the normal host. The innate and adaptive immunities regulate candidal resistance to prevent progression from simple mucosal colonization to symptomatic infection. Phagocytic cells are...
the effector cells (neutrophils and mononuclear phagocytes), but intact T-cells are critical for an effective antifungal activity of phagocytes. TH1 is considered the protective mechanism of defense, and IFN-\(\gamma\) the key cytokine in resistance to \textit{Candida} spp. (87).

IL-10 and the development of regulatory Tr cells are necessary for long-lasting protection. These cells dampen the type 1-mediated antifungal resistance, avoiding inflammatory pathology at the expense of fungal persistence as a commensal. Thus, preventing reactivation rather than complete sterilization of ubiquitous fungal pathogens may represent the ultimate expectation of vaccine-based strategies (69).

The most important manifestations of candidiasis include the following:

- **Acute disseminated candidiasis**: Neutropenia, neutrophil dysfunction and breakage of the mucosal barrier are the main risk factors for disseminated infections (88). At least two routes of entry are thought to predispose to invasive disease in immunocompromised hosts: translocation from the gastrointestinal tract and infection via intravascular catheters (89).

- **Chronic disseminated candidiasis (CDC)**: develops after marrow recovery following myelosuppressive therapy. Myeloid cells expressing CD11b can inhibit T-cells. These cells are abundant at sites of intensive hematopoiesis and in tumor-bearing hosts. Inhibition of lymphocytes by these cells may be responsible for the failure to develop a type 1 response to overcome the infection. The inhibitory effect of suppressor granulocytes appears to be mediated by CD80/CD28. CD80 is upregulated in neutrophils after exposure to hyphae from \textit{Candida}. It was demonstrated that mice with overwhelming \textit{Candida} infection have an expanded population of granulocytes expressing CD11b and CD80, which suggests that these neutrophils act as myeloid suppressor cells, skewing the adaptive immunity to a type 2, non protective response (90).

- **Mucosal candidiasis**: defense against this infection relies on cell-mediated immunity. As a consequence, mucosal candidiasis is common in patients with AIDS. Of interest, patients with deficit in IFN-\(\gamma\) or IL-12 receptor are not susceptible to this infection.

- **Chronic mucocutaneous candidiasis (CMC)**: is a chronic, debilitating disease secondary to a failure to develop TH1 response to \textit{Candida}, leading to persistence of fungi in the skin, nails, and mucosa (91). In CMC patients, the innate immunity and antibody response are normal but a defect in the cell-mediated immunity with a switch to a TH2 type of cytokine response against \textit{Candida} antigens is present (91–93).

- **Vulvovaginal candidiasis** develop in immunocompetent women, which can be acute, chronic, or recurrent, sometimes very difficult to treat. Local response is more important than the systemic immune response in the defense of this form of infection.

2. \textit{Cryptococcus neoformans}

Cryptococcosis is a rare disease in individuals with normal host defenses, despite the presence of relatively common asymptomatic infection (94). Cell-mediated immunity is critical in protecting against cryptococcal infections because deficit in T-cells is the most common risk factor associated with cryptococcosis (95–97).

\textit{Cryptococcus neoformans} is acquired via the respiratory tract and the normal host develops immunity against it during self-limited, asymptomatic infection.
Alveolar macrophages are the first cells to encounter cryptococcal yeasts and are able to kill the fungi. TNF and chemokines are essential for the recruitment of inflammatory cells and CD4+ T lymphocytes. NK cells are important in the development of a Th1 response (45). Neutrophils can efficiently ingest and kill C. neoformans in vitro, but their role in the clinical setting is not important (98). DCs process and present the antigen to T-cells and generate the protective Th1 response. IFN-γ stimulates macrophages to finally eliminate the fungi. Failure to produce TNF or IL-12 is associated with increased levels of IL-4 and IL-5, pulmonary eosinophilia, and persistence of fungi (99–103). CD8+ cells can be cytotoxic against C. neoformans, but the clinical significance of this response is not clear. CD8+ cells are dependent on CD4+ signals (104,105).

The capsule and melanin of C. neoformans downregulate the immune response, and encapsulated C. neoformans are more protected from phagocytosis than non-encapsulated variants. Monoclonal antibodies against GXM can reduce fungal burden in infected mice. The antibodies can enhance host defense against C. neoformans by promoting more effective cellular immunity. However, studies in animal models have shown that the effect depends on dose, type of antibody, and inoculum size (56). Passive antibody-mediated protection against C. neoformans requires both type 1 and type 2-associated cytokines (106).

Animal studies using vaccines with different antigens from C. neoformans confer protection to animals by inducing Th1 response (107,108).

C. Dimorphic Fungi

Sporothrix schenckii and agents of chromomycosis have worldwide distribution, but diseases are common only in tropical and subtropical areas and are secondary to traumatic inoculation with subcutaneous and osteoarticular involvement. More invasive and disseminated infections occur in patients with AIDS or those receiving immunosuppressive therapy.

Histoplasma capsulatum, B. dermatitidis, C. immitis, and P. brasiliensis cause endemic infections, which can be life-threatening in patients with defects in cellular-mediated immunity. Acquisition of infection is through inhalation of airborne fungi, although P. brasiliensis is usually acquired through cutaneous inoculation.

1. Histoplasma Capsulatum

Normal host may develop self-limited respiratory or systemic symptoms of histoplasmosis but immunocompromised host, such as patients with AIDS or patients receiving immunosuppressive therapy for cancer, transplantation, and autoimmune diseases can develop disseminated infections.

Histoplasma capsulatum is a facultative intracellular parasite that lives in macrophages. Macrophages are the principal effector cells in host resistance. The yeast can replicate inside the macrophage and inhibit the fusion of lysosomes with phagosomes until host resistance develops with the subsequent activation of macrophages to destroy the yeast and eradicate the infection (109). TNF, IL-12, GM-CSF, and induction of CD4+ T-cell type 1 response are critical for the successful killing of the intracellular yeast during primary infection. In secondary infection, CD4+ or CD8+ cells are necessary for survival, and TNF becomes the key regulator of host resistance (110–113). The development of disseminated histoplasmosis among recipients of TNF antibodies highlights the importance of TNF in the protection against
this infection (114,115). Antibodies to the cell wall of *H. capsulatum* can also have a role. They opsonize the yeast and increase macrophage phagocytosis and killing and are associated with increased IL-4, IL-6, and IFN-γ in murine lungs (116).

Protective vaccines have been developed that induce type 1 cytokine response in experimental pulmonary histoplasmosis (117). Recombinant Hsp60 from *H. capsulatum* also confers protection to mice mediated by CD4+ cells and production of IFN-γ and IL-12 (118).

2. *Penicillium Marneffei*

*Penicillium marneffei* is another dimorphic fungi that can cause disseminated infections in immunocompromised patients, specially in AIDS patients in southeast Asia (119). This observation suggests that cell-mediated immunity is the main host defense against these fungi.

*Penicillium marneffei* conidia reach the lungs and are ingested by macrophages, where they multiply as yeast.

Macrophages can kill the yeast by release of nitric oxide, but macrophage activation by T-cell derived cytokines, mainly IFN-γ, is critical for this activity (120).

D. Dermatophytosis and Other Cutaneous Fungal Infections

The normal skin is not suitable for the development of fungi, because of the presence of bacteria and keratin. Keratinocytes are the most common cells in the epidermis, not only form a physical barrier to micro-organisms, but also mediate immune reactions. They secrete cytokines that regulate the immune response including basic fibroblast growth factor, platelet-derived growth factors, transforming growth factor β, and TNF, IL-1, IL-3, IL-6, IL-7, IL-8, GM-CSF, G-CSF, and M-CSF. Keratinocytes express constitutively MHC class I and express MHC class II molecules when exposed to IFN-γ produced by infiltrating lymphocytes.

Local cutaneous conditions (warmth, moisture, and occlusion) are important factors in the development of dermatophytosis. Underlying diseases such as diabetes, or immunosuppressive therapy, and peripheral vascular diseases are associated with chronic infections. If the fungi progress beyond the stratum corneum of the skin, neutrophils and macrophages will be recruited to the infection site and initiate the inflammatory response (121). Endothelial cells participate in wound healing, angiogenesis, production of clotting factors, and secretion of cytokines and chemokines to recruit the immune cells. DCs are distributed in the dermis and epidermis while T-cells are mainly beneath the dermal–epidermal junction. DCs migrate with the antigen to lymph nodes where they initiate activation and clonal expansion of T-cells that migrate back to the dermis and epidermis by homing to cytokine-activated microvascular endothelial cells in the area. At the infection site, the T-cells can be restimulated to continue clonal expansion and generation of more effector T-cells to eliminate the micro-organisms. When the infection is cleared, some of memory T-cells may stay locally. Antibodies against fungal agents of the skin are present in the circulation in higher levels in patients who have cutaneous fungal infection.

Dermatophytes and *Candida* spp. hydrolyze keratin and facilitate the development of fungal infection in the stratum corneum where the cells of the immune system are barely present. The dermatophytes generally cause infections confined to the skin, but *C. albicans*, *Malassezia furfur* (the causative agent of tinea versicolor or pityriasis), *Trichosporon beigeli* (the causative agent of White piedra), and
T. pullulans are able to disseminate in patients with defective immunity (122). Most patients with chronic dermatophytosis are relatively intact immunologically. It is possible that an IgE response in some patients with chronic dermatophytes may inhibit the development of protective cell-mediated immune response (123).

The CD4+ lymphocytes and activated lymphocytes from peripheral blood of patients with dermatophytosis are significantly lower than in healthy individuals suggesting a role for cell-mediated immunity (124). Adequate cell-mediated immune response to Malassezia antigens has been demonstrated in noninfected human subjects, whereas patients with tinea versicolor (Malassezia infection) appear to have deficient cell-mediated immune response to the same antigen. Dermatophytosis, pityriasis, and mucocutaneous candidiasis are common in AIDS patients, which also suggests the importance of cell-mediated immunity against these fungal infections (125).

V. CONCLUSION

Susceptibility for invasive fungal infection is a function of both the virulence capacity of the fungal organism and the integrity of host defenses. Impairment of normal host defenses can occur by disease or by treatment of a disease that results in unintended compromise of one or more of the elements of innate or acquired immunity. After exposure to a potential fungal pathogen, the likelihood that invasive infection will occur which results in illness depends on the type of host defense compromised, the degree and duration of compromise, and the importance of the compromised host defense element for control of the given potential pathogen. Advances in understanding of host innate and adaptive immunities have led to new insights into the risks for infection and new strategies to improve control of infection.

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I. INTRODUCTION

Solid organ transplantation has made remarkable progress over the past three decades, evolving from a fascinating experiment in human immunobiology into the most effective means of rehabilitating patients with end-stage organ dysfunction of a variety of types. Today, at the best transplant centers, more than 90% long-term patient and allograft survival are being achieved following kidney, heart, and liver transplantation, with about 75% of lung-transplant recipients achieving these positive results. Because of these successes, the transplant community has been encouraged to “extend the envelope,” by bringing new forms of transplantation to the care of affected individuals. For example, in a number of transplant centers, bowel transplantation and transplantation of needed organs into patients with AIDS are now being explored in a responsible fashion (1–3).

The success that has occurred in organ transplantation has been achieved because of the progress in a number of areas in which multidisciplinary skills have been joined for the benefit of the patient (1):

1. **Technical success** in the conduct of the transplant operation itself, and the perioperative management of the endotracheal tube, vascular access catheters, and the variety of surgical drains that are required.
2. The appropriate use of **tissue typing** and sensitive cross-matching techniques to optimize the match of donor and recipient pretransplant and to
permit early recognition and treatment of post-transplant humoral responses that can threaten the allograft.

3. Careful evaluation of donor and recipient (in particular, eradicating all treatable infection prior to transplant) as well as meticulous care in the procurement and preservation of the donor organ.

4. Recognition of the clinical consequences of the impaired inflammatory response that exists in the transplant patient. This renders infection more occult and subtle, which often delays its recognition and compromises subsequent therapeutic efforts. Microbial load is often increased because of delay in diagnosis (as well as the amplification effect of immunosuppressive therapy), again making therapy more difficult (as well as more prolonged), and antimicrobial resistance more common. To prevent these events, early intervention is necessary and should trigger a more aggressive strategy in the use of high-resolution imaging studies and in the use of tissue sampling to effect early diagnosis and therapy (Fig. 1).

5. Precise, individualized management of the immunosuppressive regimen, on the one hand, effectively preventing or treating allograft rejection, and, on the other, minimizing the severe depression of a broad range of host defenses against infection that can be the consequence of overly aggressive immunosuppressive therapy.

6. Prevention of infection in the transplant patient rather than treatment of established disease is the goal. A time-honored example is the use of trimethoprim–sulfamethoxazole prophylaxis for the prevention of human pneumocystosis and other infections.

7. The integration of the prior two principles in clinical practice leads to a dynamic concept that we call the therapeutic prescription. The therapeutic prescription has two components: an immunosuppressive program to prevent and treat rejection, and an antimicrobial program to make it safe. Just as changes in the immunosuppressive program may be needed to deal with particular problems with the allograft, the ability to make changes in the antimicrobial program that are linked to the nature of the immunosuppression required is essential. The pre-emptive administration of ganciclovir to renal- or liver- transplant recipients who receive antilymphocyte antibody therapy to treat rejection is a well-studied example of this concept (4,5).
II. INVASIVE FUNGAL INFECTION IN SOLID ORGAN-TRANSPLANT PATIENTS

The range of organisms capable of causing significant infection in the organ-transplant recipient is quite broad, and these lend themselves to a simple classification system: true pathogens, sometime pathogens, and nonpathogens. True pathogens are the classic plagues of humankind (influenza, bubonic plague, smallpox, and others) that produce toxins and cross-tissue planes, and are able to evade the protection provided by innate immunity. Specific immunity or effective antimicrobial therapy are essential for their control. Sometime pathogens are those organisms that normally reside on mucocutaneous surfaces without clinical impact; injury to these surfaces provides access for these organisms to sites vulnerable to invasive infection (e.g., peritonitis after colonic perforation). Nonpathogens are those saprophytes that are ubiquitous in the environment and are kept in check by innate immune mechanisms and only cause disease in the significantly immunocompromised species (e.g., Aspergillus species, Pneumocystis jiroveci, zygomycetes, and a variety of other microbial species). The term opportunistic infection is applied to an invasive infection caused by a nonpathogen or to an infection caused by an organism that causes a trivial infection in the normal host but life threatening infection in the immunocompromised individual (e.g., candidal vaginitis vs. disseminated candidiasis). Transplant patients are subject to all three classes of infection, with amplification of a particular clinical syndrome being created by the immunosuppressed state (1,4).

In general, the fungal infections that occur in the transplant patient are caused by sometime and nonpathogenic organisms and can usefully be divided into three general categories:

1. The endemic, geographically restricted, systemic mycoses (caused by Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum, and others). These organisms share a number of characteristics in common: they are dimorphic in their growth pattern, with the mycelial or mold form present in the soil of endemic areas; in this state, they produce spores, which are infectious for humans. The spores or conidia are aerosolized when the soil is disturbed, making possible the inhalation of these infectious agents and their deposition in the lower respiratory tract. Conversion of the conidia to the invasive yeast form then occurs, with these events initiating the infective process (6,7).

   Innate immunity—as mediated by neutrophils, monocytes, and alveolar macrophages—can kill the conidia and block the conversion to the yeast form if the infecting inoculum is relatively small, but is overwhelmed by large inocula, such that invasive primary infection of the lung develops. In the transplant patient, there is abundant evidence of pulmonary disease as a consequence of these events with subsequent hematogenous dissemination. Over time, other forms of clinical infection may develop: reactivation of quiescent infection (again with the possibility of bloodstream seeding), which is not only more common among the immunocompromised, but also has a greater clinical impact than in the general population and reinfec-

   tion. Reinfection is perhaps the most interesting form of these infections: there is a prior minimally symptomatic infection that confers immunity on the individual. With the initiation of antirejection therapy post-transplant, the immunity is attenuated and re-exposure then leads to reinfection and the events that normally follow primary exposure (1,7).

2. Opportunistic Fungal Infection. The most common causes of invasive fungal infection in the transplant recipient are the commensal organisms present on the mucocutaneous surfaces of the body (Candida species) and the saprophytic organisms
that are ubiquitous in the environment (e.g., *Aspergillus*, *Cryptococcus*, and the zygomycetes). *Candida* species are present as part of the normal gastrointestinal flora in the vagina and on diseased skin. Overgrowth of these organisms is a critical first step in the pathogenesis of candidal infection. Factors that contribute to such overgrowth include decline in immune surveillance because of immunosuppression or neutropenia, diabetes mellitus, and other endocrinopathies, and the elimination of competing flora, especially anaerobes, by use of broad-spectrum antibiotics. Mucocutaneous candidal overgrowth can result in clinical entities such as oropharyngeal thrush, candidal esophagitis, vaginitis, intertrigo, paronychia, and onychomycosis (1,8).

Of greater clinical significance is mucocutaneous overgrowth associated with penetration of these surfaces. In transplant patients, this is usually because of technical factors: for example, contaminated vascular access lines or complicated liver-transplant operations in which *Candida* species from the gastrointestinal tract are spilled into devitalized tissue, hematomas, or ascites. If bloodstream invasion occurs, dissemination with the potential for metastatic infection occurs in more than half of transplant patients (1,8–10).

Invasive aspergillosis is the most fearsome of the opportunistic fungal infections occurring in transplant patients. It is acquired through inhalation of an aerosol laden with this mold, with the primary portal of entry being the lungs, sinuses, or damaged skin, although surgical site infections are encountered occasionally because of airborne or surgical supplies contamination. Exposure to this organism can occur in the hospital or in the community and is particularly associated with construction activities (1,9,10).

Cryptococcal infection and Mucorales infection also have pulmonary (and sinusual in the case of the Mucorales) portals of entry, with the potential for hematogenous spread and metastatic infection [e.g., the central nervous system (CNS) with *C. neoformans*] or contiguous spread (the Mucorales can cause bloodstream dissemination, but contiguous spread is more characteristic) (1,9,10).

### 3. New and Emerging Fungi

The nature of the fungal species causing invasive infection in the transplant patient is undergoing significant change. Although fluconazole-susceptible *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* remain the most common causes of invasive fungal infection in solid organ-transplant recipients, 2–10% of invasive fungal infections today are caused by such organisms as fluconazole-resistant *Candida* (both *albicans* and non-*albicans* strains), *Scedosporium*, *Trichosporon*, *Fusarium*, and other heretofore unusual organisms. The selective pressures of antifungal prevention strategies, the increased potency of modern immunosuppressive programs, and increased exposures (both in the hospital and in the community) undoubtedly have contributed to the growing importance of this category of infection. What is particularly important is that many of these organisms are poorly responsive to amphotericin B, requiring voriconazole or some other innovative therapy. This group of infections is almost assuredly going to continue to grow in importance over the next few years and will demand not only new therapies, but also new diagnostic approaches (1,11).

### III. RISK OF INVASIVE FUNGAL INFECTION IN THE SOLID ORGAN-TRANSPLANT RECIPIENT

The risk of invasive fungal infection in the solid organ transplant patient is largely determined by the interaction of three factors: technical-anatomic factors, environmental exposures, and the net state of immunosuppression.
A. Technical-Anatomic Factors in the Pathogenesis of Fungal Infection in the Solid Organ-Transplant Patient

The technical-anatomic factors that constitute a risk of infection are those that lead to devitalized tissue, fluid collections, and an ongoing need for invasive devices for vascular access, drainage catheters, and other foreign bodies that abridge or otherwise attenuate the primary mucocutaneous barriers to microbial invasion. Candida species are among the most common microbial pathogens that serve as secondary invaders of these technical mishaps. Indeed, the reports of >40% incidence of fungal infection in the early days of liver transplantation were largely because of these technical-anatomic considerations. Factors associated with a particularly high risk of post-transplant candidal infection include hyperglycemia, undrained fluid collections, need for re-exploration, hepatic artery thrombosis, the use of a choledochojunostomy biliary anastomosis, and a large blood-replacement requirement. Some centers recommend fluconazole prophylaxis for all liver-transplant recipients; we have restricted the use of fluconazole to those patients with one or more risk factors. Although no antimicrobial agent takes the place of technically perfect surgery, the use of fluconazole will improve outcomes further, such that the incidence of this type of fungal infection is now <2% in many centers (1,9,10,12–14).

The technical demands of liver transplantation easily explain the relatively high incidence of these infections, as they do for lung and pancreatic transplantation. In the case of diabetes, the organism burden is far higher on the skin and gastrointestinal mucosa of the individual, significantly adding to the risk of invasive fungal infections. As a general rule, the incidence of such events is determined by the complexity of the surgery involved—being most common following liver, lung, and pancreas transplants, somewhat less common following heart transplantation, and, least often, in association with the renal allograft (1).

Two uncommon problems with candidal infection in transplant patients may be encountered: infection and obstruction at the ureteropelvic junction in renal-transplant recipients (observed particularly in those with a neurogenic bladder), which results in obstructive uropathy, ascending pyelonephritis, and the possibility of bloodstream infection. A similar process occurs occasionally in the bile duct of liver-transplant recipients. Because of the difficulty in treating these entities once they are fully developed, we advocate pre-emptive antifungal therapy for those patients with microbiologic evidence of candidal colonization at either of these two sites (1).

If the patient is free of technical-anatomic abnormalities, the risk of infection, particularly opportunistic infection, is largely determined by the semi-quantitative relationship that exists between the environmental exposures and the net state of immunosuppression: if the epidemiologic exposure to a microbial agent is intense enough, even nonimmunosuppressed individuals can develop significant infection; conversely, if the net state of immunosuppression is great enough, then minimal exposure to non-invasive, commensal organisms can result in life-threatening infection (1,4,10).

B. Environmental Exposures of Importance in the Pathogenesis of Fungal Infection in the Solid Organ-Transplant Recipient

The inhalation of fungal conidia is usually the first step in establishing invasive pulmonary infection in the transplant patient. Whereas the endemic mycoses and cryptococcal infection are almost always acquired in the community, Aspergillus and Scedosporium species may be acquired either in the community or nosocomially (1).
The epidemiology of cryptococcal infection in transplant patients is very different from that of *Aspergillus* and other invasive molds. Asymptomatic primary infection of the lungs with *C. neoformans* is common in the general population, with latent infection controlled by a granulomatous response in a manner analogous to tuberculosis. Immunocompromise, as seen in transplant patients, those with AIDS, and patients with lymphoma under treatment, will result in reactivation of the infection in the lungs and systemic spread, particularly to CNS, skin, skeletal system, and prostate. It is believed that inhalation of infectious propagules of the organism from aerosols created from contaminated soil (particularly that enriched with pigeon and other avian excreta) is the source of the infection, but this concept has yet to be validated.

Acute, progressive primary disease in immunosuppressed patients exposed to a high density of organisms may occur but has not been documented (15–17). One exception is the report of vascular access-associated bloodstream infection (with hematogenous seeding of the lung) caused by *C. neoformans* that was found to spread from a pigeon roost located adjacent to the air intake for an unfiltered air conditioner (18).

In the community, acquisition of infection caused by the endemic mycoses, *Aspergillus*, and the other invasive molds is related to activities in which infectious aerosols are created. The most common scenario is exposure to construction activities in which older structures are being rehabilitated or the soil is being disturbed. The inhalation of these infectious agents then can lead directly to invasive infection or to colonization of the respiratory tract, with the degree of immunocompromise determining if invasive disease develops. There are two other situations that should be avoided by transplant patients because of the risk of invasive mold infection: marijuana use and gardening. Marijuana is commonly contaminated with *Aspergillus* species, and the smoking of contaminated marijuana can lead to invasive pulmonary infection; gardening in which the soil is being disturbed can likewise lead to invasive opportunistic infection because of the presence of a variety of *Aspergillus* species (as well as other organisms such as *Nocardia asteroides*). We have cared for a successful renal transplant patient (normal renal function, minimal maintenance immunosuppression, and 4 years post-transplant) who presented with disseminated infection due to *Aspergillus fumigatus*, *A. niger*, *A. flavus*, and *Nocardia asteroides*. All four agents were present on admission. This illness began ~1 week after a spending weekend tending to the patient’s rose bushes where considerable digging in the soil was involved. Samples of the soil from the patient’s rose garden yielded all of these organisms and other species of mold. Although the patient survived this infection with aggressive and extended therapy, considerable morbidity was encountered in what must be regarded as a preventable infection (1).

Nosocomial acquisition of the invasive molds, particularly *A. fumigatus* and *A. flavus*, and also including emerging agents such as *Scedosporium*, remains a significant problem for the transplant recipient, and is usually associated with hospital construction (1,19).

Water-borne aspergillosis associated with contaminated hospital water tanks or locally in patient room showers has been documented. Patients are exposed either by ingestion of conidia or by aerosolization of water droplets (20,21). The operative principle is that the transplant recipient, like other immunosuppressed hosts, is an epidemiologic “sentinel chicken” (1) within the hospital environment—any excess traffic in microbes will be seen first in this patient population, and constant surveillance is essential to prevent catastrophic outbreaks of life-threatening infection.
C. Net State of Immunosuppression in the Organ-Transplant Recipient

The net state of immunosuppression is a complex function determined by the interaction of a number of factors: the driving force is the dose, duration, and temporal sequence in which immunosuppressive drugs are being deployed; the presence of immunodeficiencies unrelated to therapy (e.g., those associated with the underlying disease, as well as newly acquired deficiencies like the acquired hypoglobulinemic state that has been linked to intensive therapy with tacrolimus and mycophenolate); compromise of mucocutaneous surfaces such that the barrier function is incomplete; degree of neutropenia; metabolic disorders (protein-calorie malnutrition, and perhaps diabetes and uremia); the two extremes in age; infection with one of the immunomodulating viruses [cytomegalovirus (CMV), Epstein–Barr virus (EBV), the hepatitis viruses, and the human immunodeficiency virus]; and, perhaps, race.

Modern immunosuppressive therapy is based on two general principles: multiple drugs that act by different mechanisms should be deployed in combination in order to achieve adequate control of the rejection process and to decrease toxicity and complicating infection. Of all the drugs that are employed, prednisone is thought to be associated with the greatest number of side effects (Table 1), and most regimens devised in the past two decades have been, in large part, designed to be steroid sparing. The most profound effect of currently used regimens is to block the microbe-specific cytotoxic T-cell response, thus putting the patient at special risk for such pathogens as certain classes of viruses, particularly the herpes group viruses, bacteria such as mycobacteria, Nocardia, and Listeria, and the fungi. Although the specific effects of different immunosuppressive agents in transplant recipients have been reviewed in some detail, as have the nature of the net state of immunosuppression, a few general points bear emphasis:

a. Although corticosteroids at high doses (e.g., pulse doses of >500mg intravenously to treat rejection) are immunosuppressive, the majority of their effects are

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Table 1  Effects of Corticosteroids on Inflammation and Immune Function*

<table>
<thead>
<tr>
<th>Anti-inflammatory effects</th>
<th>Immunosuppressive effects</th>
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<tbody>
<tr>
<td>Inhibition of proinflammatory cytokine production</td>
<td>Inhibit T-cell activation and proliferation</td>
</tr>
<tr>
<td>Increase in the circulating level of polymorphonuclear leukocytes (PMNs), but decrease in the accumulation of PMNs at site of tissue injury</td>
<td>Block clonal expansion in response to antigenic stimulation</td>
</tr>
<tr>
<td>Decrease the number of circulating lymphocytes and increase the ratio of B to T cells and CD8 to CD4 cells</td>
<td>Block IL-2 release, resulting in impaired cell-mediated immunity</td>
</tr>
<tr>
<td>Decrease the number of circulating monocytes, eosinophils, and basophils</td>
<td>Inhibit activation of immature B cells; little effect on recall</td>
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<tr>
<td>Inhibit all arachidonic acid metabolites, as well as platelet-activating factor (inflammatory mediators)</td>
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<tr>
<td>Decrease vascular permeability</td>
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<tr>
<td>Inhibit the inducible form of nitric-acid synthase</td>
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<td>Inhibit the mediators of vasodilatation</td>
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*Adapted from Ref. 1.
anti-inflammatory. The result, particularly with fungal infections, is not only to increase the incidence of opportunistic infections, but also to attenuate signs and symptoms, making clinical recognition of invasive disease difficult, increasing the organism burden, and mandating prolonged therapeutic courses (Fig. 1).

b. The immunomodulating viruses, most notably CMV, contribute significantly to the net state of immunosuppression, with 90% of opportunistic infections occurring in patients with active infection with one or more of these viruses. Indeed, occurrence of invasive fungal infection in a patient without viral replication is a clue to the presence of an unsuspected environmental exposure (1,4,10).

c. In addition to the direct immunosuppressive effects of the agents used, their particular effects on the reactivation of such viruses as CMV also help to determine the net state of immunosuppression. Agents like anti-lymphocyte antibodies (e.g., OKT3 and antithymocyte globulin) have a powerful effect in causing reactivation of latent virus. The cytotoxic agents azathioprine, mycophenolate, and cyclophosphamide have a moderate effect in this regard. In contrast, the calcineurin inhibitors (tacrolimus and cyclosporine), sirolimus, and prednisone have no ability to reactivate virus. However, once replicating virus is present, these agents, particularly the calcineurin inhibitors, have profound effects in increasing the risk of invasive fungal infections and of amplifying the infection if present. Candidemia and invasive aspergillosis are both significantly increased in patients with active CMV, and it is likely that other invasive fungal infections are as well (1,4,10).

d. Metabolic factors contribute to the net state of immunosuppression as well. In particular, protein-calorie malnutrition is important, with hyperglycemia and uremia likely to have an effect as well. If one segregates transplant patients on the basis of a serum albumin of greater than or less than 2.5 g/dL, there is a 10-fold difference in the incidence of opportunistic infection, including invasive fungal infection in those with lower albumin levels (1,4,10).

e. Genetically determined aspects of both innate and specific immunities probably control susceptibility to opportunistic infection. Racial background may be a surrogate marker for susceptibility. For example, when compared with Caucasians and Asians, African-Americans have a significantly lower incidence of cadaveric renal allograft survival; they also have a lower incidence of invasive infection. As all these groups receive essentially the same immunosuppressive program, these observations suggest that the net state of immunosuppression produced in African-Americans by standard anti-rejection programs is less than in other population groups. Therefore, unless other factors intervene, it would be expected that the incidence of invasive fungal infection in African-Americans subjected to standard immunosuppressive protocols is less than among other ethnic groups and that more intensive anti-rejection strategies would be safe in these individuals, perhaps improving the rate of allograft survival, emphasizing the importance of a judicious therapeutic prescription (22).

IV. TEMPORAL ASPECTS OF FUNGAL INFECTION IN THE SOLID ORGAN-TRANSPLANT RECIPIENT

There is an expected timetable of infection following organ transplantation. It is useful to divide the post-transplant course into three time periods: the first-month post-transplant, the period 1–6 months post-transplant; and the late period, >6 months post-transplant (Fig. 2). This timetable can be useful in three ways: in constructing a differential diagnosis in a patient who presents with a clinical infectious disease
syndrome; as a tool of infection control, as exceptions to the timetable are usually because of, heretofore, unrecognized environmental exposures; and as the cornerstone for prescribing cost-effective, focused preventative antimicrobial strategies (1,4,10).

A. Fungal Infection in the First Month Post-transplant

In the first month post-transplant, there are essentially three types of infection observed: active infection that was present pre-transplant and is now clinically apparent post-transplant; active infection that was conveyed through a contaminated allograft; and perioperative infection of the surgical wound, lungs, urinary tract, vascular access devices, and surgical drains (which account for >95% of infections in this time period). In terms of infection that was present prior to transplant, a significant minority of patients coming to lung, liver, or cardiac transplant (rarely, kidney recipients) have been recipients of immunosuppressive therapy in an attempt to control their underlying disease. As a result, these patients can present with invasive candidal, cryptococcal, *Aspergillus* infection, or other fungal infection early.
in the post-transplant period due to previously unrecognized disease. A cardinal rule of transplantation is that pre-existing infection should be eradicated prior to transplant. A corollary of this rule is that any patient who comes to transplant significantly immunosuppressed merits an evaluation for active infection, especially fungal infection. This evaluation includes blood cultures, cryptococcal antigen measurement, and a chest computed tomography (1,4,10).

Both histoplasmosis (23) and cryptococcosis (24) have rarely been transmitted via an allograft, representing dormant or active infection of the allograft acquired via the hematogenous route. Candidal contamination of the allograft as a consequence of terminal care of a cadaveric donor, the acquisition and transport of the allograft, and the handling of the organ is an uncommon cause of infection. Such contamination threatens the vascular suture line, with potentially grave consequences such as the formation and rupture of arterial pseudoaneurysms (1,4,10,25).

As noted, transplant patients are susceptible to the same infections that complicate comparable surgical procedures in nonimmunosuppressed individuals, although the consequences are usually greater in transplant patients. The occurrence of such infections is directly related to the skill with which the operation is performed, and how invasive care devices (endotracheal tubes, drains, and vascular access devices) are managed. 

Candida species infecting fluid collections, devitalized tissue or drainage catheters, and vascular access devices are the usual clinical problems caused by fungi in this time period. Antifungal prophylaxis with drugs such as fluconazole or an amphotericin B preparation can limit the impact of such infections, but nothing is as important as impeccable surgical and perioperative technical management. Antifungal prophylaxis is particularly useful in liver-transplant patients with one or more of the following risk factors: patients requiring re-exploration due to diabetes, a history of recent broad spectrum antibacterial therapy, and those with a choledochojejunostomy biliary anastomosis (1,4,10,12–14).

Notable by their absence during this time period are infections caused by opportunistic fungi such as Aspergillus, Cryptococcus, Scedosporium, etc., despite the fact that this is the time period when the highest daily doses of immunosuppression are prescribed. There are two important implications of these findings: if infection caused by one of these opportunistic fungi occurs in the first month, this is prima facie evidence of an unexpected environmental exposure that needs correction; it also emphasizes that the prime determinant of the net state of immuno suppression is sustained immunosuppression (“the area under the curve”), rather than the daily dose (1,4,10).

B. Fungal Infection 1 to 6 Months Post-transplant

Residual effects of infection acquired earlier may still be important at this time point. However, there are two types of clinical infections that are of particular relevance in this period. The major cause of infectious disease morbidity and mortality is the immunomodulating viruses, particularly CMV, EBV, and human herpesvirus 6 (HHV-6), which not only directly cause infectious disease syndromes, but also contribute significantly to the net state of immunosuppression. The combination of these effects and what is now sustained immunosuppression makes possible an array of opportunistic infections, including fungi such as Aspergillus species, Scedosporium, and Candida species, even in the absence of a particularly intense epidemiologic exposure. Prevention of fungal infection during this time period is accomplished by CMV prevention, provision of uncontaminated air (within the hospital, HEPA-filtered),
and avoidance of construction sites both inside and outside the hospital. Systemic antifungal therapy plays little role unless colonization is demonstrated, at which point pre-emptive therapy should be considered (1,4,9,10).

C. Fungal Infection More than 6 Months Post-transplant

The great majority of transplant patients in this time period are at relatively low risk of invasive fungal infection, unless an intense environmental exposure occurs. These are patients who have had a good outcome from transplantation: good allograft function, minimal maintenance immunosuppression (particularly, a prednisone dose of ≤10 mg/day), and no ongoing viral infection. Their biggest risk is from community-acquired viral infections such as influenza. Fungal infections seen in these patients are primarily mucocutaneous and can be effectively treated with topical or systemic therapy. The most common invasive fungal infection observed during this time period is that caused by *C. neoformans*, usually presenting as asymptomatic nodules on incidental chest imaging.

Approximately 10% of patients maintained on immunosuppression with a functioning allograft are at high risk for opportunistic infections of all types, but particularly fungal ones. These are patients who have had a relatively poor outcome from transplantation: borderline allograft function, too much acute and/or chronic immunosuppression and often, chronic viral infection. These “chronic n’er do wells” are at high risk for such fungal infections such as those caused by *C. neoformans*, *Aspergillus* species, the newly emerging fungi, and—if the epidemiologic history is appropriate—disseminated infection due to *Histoplasma capsulatum* or *Coccidioides immitis*. Prevention in these patients requires decreased immunosuppressive therapy, close supervision for possible environmental exposures, treatment of viral infection, and consideration of prophylactic azole administration (although guidelines for this last have not been established) (1,4,10,25).

V. FUNGAL INFECTIONS OF IMPORTANCE IN THE ORGAN-TRANSPLANT RECIPIENT

A. Candidiasis

*Candida* species are the most common causes of fungal infection in the organ-transplant recipient, causing a broad range of syndromes that range from the trivial to the life threatening. These organisms are part of the normal oral flora. Other sites of colonization are the skin, gastrointestinal tract, and the female genital tract. Candidal colonization is constantly being abetted by the ingestion of food and by exposure to the hospital environment. Of particular importance, the nosocomial spread of *Candida* species has been traced to the contaminated hands of hospital personnel. This mode of transmission has been particularly important in the acquisition of azole-resistant species (8–10,12–14,26).

**Oropharyngeal candidiasis** can take a variety of forms:

a. An acute exudative, pseudomembranous candidiasis.

b. Chronic atrophic stomatitis.

c. Chronic hyperplastic candidiasis (*Candida* leukoplakia).

d. Acute atrophic stomatitis.
Candida infection of the esophagus is not an uncommon finding in these immunocompromised individuals, often linked with oropharyngeal infection. Diagnosis is made by endoscopic evaluation, including biopsy. A rigorous approach to diagnosis is necessary as similar x-ray findings and even endoscopic appearance can be produced by herpetic, CMV, radiation, and reflux esophagitis. It is important to recognize that candidal esophagitis requires systemic antifungal therapy (oral or IV fluconazole being the drug of choice), with topical therapy being ineffective (1,8,12,26).

Urinary tract candidiasis is not uncommon in transplant patients, being made possible by instrumentation of the urinary tract (particularly an indwelling bladder catheter), diabetes, vulvovaginal infection, and the presence of a neurogenic bladder. Although hematogenous renal infection is well recognized as a consequence of bloodstream infection with Candida, the great majority of cases of urinary tract infection occur via the ascending route. A particularly virulent form of candiduria occurs when obstruction due to a fungal ball occurs at the ureterovesical junction, with resulting pyelonephritis and bloodstream invasion; papillary necrosis and/or renal cortical abscess can occur in the most severe of these cases. To prevent fungal balls from occurring, we advocate pre-emptive therapy of asymptomatic candiduria after removal of a bladder catheter and control of metabolic derangements. This is in direct contrast to the approach in nonimmunocompromised patients, where watchful waiting is advocated after catheter removal (1,27,28).

Candidemia and disseminated candidal infection occur most commonly because of infection of vascular access catheters, although intestinal translocation in critically ill patients can also occur. The importance of the vascular access device in the pathogenesis of this process is underlined by the observation that the response to systemic therapy is greatly improved in those patients whose catheters are removed promptly as therapy is initiated. In this setting, the maintenance of a bladder catheter and/or the insertion of a three-way bladder catheter to deliver antifungal washes are to be avoided. Once bloodstream infection occurs, metastatic infection can develop anywhere, producing renal infection, hepatosplenic disease, and eye disease (candidal endophthalmitis). It should be emphasized that metastatic infection to the skin can be the first sign of invasive candidal infection in as many as 20% of patients with disseminated disease (26,29,30).

Candidal pneumonia via the tracheobronchial route is vanishingly rare, whereas pulmonary invasion due to bloodstream infection is well recognized. This statement remains true even when the sputum has easily demonstrable candidal forms on microscopic exam and culture and even when blood cultures are positive. In this latter instance, the sequence of events is sputum to skin (with a marked increase in the skin microbial burden) to vascular access site, with this being the portal of entry to the bloodstream. The one situation in which Candida species in the sputum should be treated vigorously is the lung-transplant patient with a relatively fresh bronchial suture line. This site has a borderline blood supply, and the combination of organisms at the site and poor perfusion are synergistic in causing breakdown of the bronchial anastomosis. Hence, the principle that Candida in the sputum should be treated pre-emptively or to protect the suture line in this patient group (1,26,31).

Uncommon forms of invasive candidal infection. As previously noted, metastatic seeding of distant organs is not uncommon in transplant patients with candidemia. Hence, entities such as endocarditis, meningitis, osteomyelitis, septic arthritis, and other forms of focal infection result. Diagnosis of these entities requires the combination of state-of-the-art imaging techniques, surgical biopsy, and serial cultures. In
addition, surgical debridement or excision, in combination with appropriate antifungal therapy, can be particularly useful in the management of candidal endocarditis, osteomyelitis, and other focal lesions (1).

B. Aspergillosis

*Aspergillus* species are ubiquitous saprophytes in our environment, growing as a mold that produces the infective form, conidia, which is inhaled into the respiratory tract to initiate disease. Once inhaled, these conidia can produce a variety of clinical syndromes that are grouped into four categories of disease (32,33):

a. **Hypersensitivity syndromes.** Asthma, extrinsic allergic alveolitis, and allergic bronchopulmonary aspergillosis (ABPA) are all possible hypersensitivity responses to the inhalation of this organism. In addition, allergic sinusitis can occur, typically in individuals with other evidence of an allergic diathesis—allergic rhinitis, asthma, nasal polyps—in response to chronic colonization with *Aspergillus* species. ABPA is of particular importance, with a clinical syndrome of transient pulmonary infiltrates, asthma, sputum that contains brown particles, eosinophilia, and the presence of immunologic reactivity to *Aspergillus* antigens (as demonstrated by skin testing, the presence of precipitating antibodies, and *Aspergillus* specific IgE). If left untreated, central bronchiectasis and/or pulmonary fibrosis will develop. At present, the therapy of choice is the combination of corticosteroids and an anti-*Aspergillus* drug (e.g., itraconazole or voriconazole) (33–35).

b. **Colonization syndromes.** The best example of this manifestation of *Aspergillus* colonization is the formation of an “aspergilloma” or “fungal ball” at the site of such pre-existing lung cavities as those produced by tuberculosis, bronchiectasis, bullous emphysema, or sarcoidosis (Fig. 3). Indeed, sustained colonization of the lung usually connotes a significant abnormality of the tracheobronchial tree. One variant of tracheobronchial disease caused by *Aspergillus* species is obstructing bronchial aspergillosis in which exuberant colonization by these organisms obstruct the airway, with resultant atelectasis, and the production of a pseudomembranous tracheobronchitis. In transplantation, this is a particular problem among lung allograft recipients, particularly those patients with bronchial anastomotic problems or those who may need a bronchial stent because of breakdown of the anastomosis

![Figure 3](image-url) An aspergilloma in a bronchiectatic cavity in a patient with known history of sarcoidosis. (A) Conventional chest radiograph (posteroanterior view). (B) Computerized tomography scan of the chest of the same patient.
Aspergillomas consist of a meshwork of fungal hyphae held together by reactive debris (fibrin and inflammatory cells). Clinical symptoms resulting from the presence of an aspergilloma have usually been considered irritative: cough, sputum production, and hemoptysis. Erosion of an aspergilloma into an adjacent large blood vessel can be life threatening. However, increasingly, local invasion has been noted pathologically, and, in select patients, anti-\textit{Aspergillus} therapy can effect clinical improvement and avoidance of surgery in those whose overall lung function is poor.

c. \textit{Semi-invasive aspergillosis.} This is a relatively recently recognized entity characterized by slowly progressive necrosis of the involved lung in individuals not classically considered immunosuppressed, but who have underlying conditions such as diabetes, liver disease, or recent influenza. It has been suggested that a deficiency in mannose-binding protein may be the underlying defect responsible for this condition. Surgical excision under coverage of antifungal therapy appears to be the therapy of choice for this entity, as—unlike invasive aspergillosis in the truly immunosuppressed host—progression of this process is by contiguous spread, not by hematogenous dissemination (32,38,39).

d. \textit{Invasive aspergillosis.} This is the important form of aspergillosis for solid-organ transplant recipients. The great majority of cases involve the lung, with invasive sinusitis being the second most common portal of entry. Damaged skin is also susceptible to invasion by this organism. Studies of hematopoietic stem-cell transplant (HSCT) patients have shown that deficiencies of important host defenses put patients at high risk of invasive disease: a severe deficit in the number and function of polymorphonuclear leukocytes, and significant impairment in cell-mediated immunity. In the solid organ-transplant patient, the latter is the primary deficit that leads to this infection, particularly when infection with one of the immunomodulating viruses, especially CMV, is also present. Acquisition of this infection can occur either in the community or in the hospital and is associated with activities such as construction, the creation of a warm and wet environment (as with a leaking pipe in the ceiling), and even gardening. Two epidemiologic patterns have been identified within the hospital: domiciliary and nondomiciliary. In the domiciliary form, the infection is acquired by the inhalation of conidia laden air in the room or on the ward where the patient is housed. This usually results in the clustering of cases in time and space, and the hazard is relatively easily identified. Nondomiciliary exposures occur as patients travel through the hospital for essential procedures. Thus, outbreaks or single cases have been documented as resulting from exposures in a contaminated operating suite, the radiology suite, or while awaiting bronchoscopy or endomyocardial biopsy in an anteroom. Because of the nature of these exposures and the lack of clustering of cases, nondomiciliary hazards are often difficult to detect, with the best clue being the occurrence of this opportunistic infection at a time when the net state of immunosuppression is not great enough for such infection to occur without a particularly intense exposure (19,32,40).

This division of disease caused by \textit{Aspergillus} species is a clinically useful concept provided that the clinician recognizes that “cross-over” syndromes occur. Thus, invasive disease can occur as a result of steroid use to control allergic manifestations. A cavity caused by necrotizing infection can subsequently develop an aspergilloma. Increasingly, the possibility that some patients with what was regarded as “non-invasive” disease may benefit from antifungal therapy, particularly in conjunction with surgical manipulations, is being recognized. The advent of less-toxic therapies
(e.g., voriconazole and caspofungin) has made this management decision considerably easier.

1. Clinical Aspects of Invasive Aspergillosis in Solid Organ-Transplant Recipients

The genus *Aspergillus* encompasses close to 200 different species, with only a few of these being of significance in man. *Aspergillus fumigatus* is responsible for about 90% of the cases of invasive disease, with a variety of virulent factors being defined to account for this remarkable pathogenicity. *Aspergillus flavus* accounts for the majority of the remaining cases, particularly those involving the paranasal sinuses. Other species, such as *A. terreus*, *A. nidulans*, and *A. niger* occasionally produce disease, with there being some evidence that these unusual infections are increasing, particularly among lung-transplant recipients. This is an important trend to follow, as these uncommon species have a greater tendency to be drug resistant; for example, *A. terreus* is amphotericin resistant (32,40–42).

The key characteristic of invasive aspergillosis, whatever the portal of entry, is its angioinvasive behavior. This means that the three cardinal features of invasive aspergillosis are hemorrhage, infarction, and metastases. Of particular importance is the occurrence of metastatic infection, for example, to the brain. As many as half of patients with invasive aspergillosis have evidence of dissemination when initially diagnosed, which greatly decreases their chances for survival. For example, in our experience with renal, cardiac, and hepatic transplant patients, >65% of patients with a single pulmonary lesion were cured with amphotericin B therapy, whereas those with disseminated disease had a survival rate of <25%, and those with CNS disease had a survival rate that approached “zero” (1,32,40,43).

The clinical presentation of invasive aspergillosis is non-specific: fever, hemoptysis, pleurisy, cough, headache, etc. Depending on how suppressed the inflammatory response is, invasive disease can even be relatively asymptomatic. Hence, the need for early diagnosis is great, and a variety of approaches to accomplish this are becoming available:

   a. **Radiology.** Organ-transplant patients with nodules, an infarct pattern, or focal infiltrates with or without cavitation on high-resolution CT scan of the chest should be suspected as harboring invasive *Aspergillus* infection. Conventional chest radiography is not sensitive enough, as the impaired inflammatory response of these patients attenuates the findings on conventional radiographs, and a CT scan should be ordered when the clinician is evaluating respiratory symptoms, unexplained fever, symptoms of sinusitis, or other subtle signs of possible infection. In the even more susceptible HSCT patient, protocol CT scans in the absence of symptoms have been suggested (44,45); such a recommendation does not appear to be warranted with the organ-transplant patient. In HSCT patients, a halo sign (Fig. 4) has been correlated with a high probability of the presence of invasive pulmonary aspergillosis. This halo effect (a ground-glass abnormality thought to be a result of local invasion and hemorrhage that surrounds a focal lesion) is far less common in organ-transplant patients; further, it is non-specific, as we have observed halo signs in HSCT patients with such other processes as nocardiosis, recurrent esophageal cancer, and fungal infections caused by *Scedosporium* and *Fusarium* species. Air-crescent lesions (45) are late manifestations of angioinvasive infection and do not fulfill the need for early diagnosis (1,43,45,46).

   b. **Conventional mycology.** Although the isolation of *Aspergillus* species from respiratory secretions has long been a marker for the presence of significant disease
of the tracheobronchial tree (e.g., bronchiectasis and chronic bronchitis), it has not been a major part of the diagnostic approach in transplant patients. Recent observations have suggested that the isolation of these organisms from sputum is a useful part of the diagnostic approach. Thus, high-risk HSCT patients studied in Seattle who are colonized with *Aspergillus* species have a >60% risk of having or developing invasive disease; in liver-, heart-, and kidney-transplant patients, the risk is >50%, and pre-emptive antifungal therapy is advocated. In lung-transplant patients, the positive predictive value is somewhat less (presumably because of the presence of tracheobronchial disease), but pre-emptive approaches are still recommended. The bigger problem is that the sensitivity of this approach is only about 30%. In this circumstance, tissue sampling (for culture and pathology) is needed to make the appropriate diagnosis at this point in time (Fig. 5) (1,32,46,47).

c. Non-cultural diagnostics. A significant effort has been expended to develop serologic techniques for the diagnosis of invasive aspergillosis. These have been largely abandoned because of two factors: circulating antibody to this ubiquitous organism is not uncommon in the general population, making interpretation of these results difficult; conversely, in immunocompromised patients such as these, an antibody response may be either delayed or blunted. Two other approaches, however, appear promising: a double sandwich ELISA assay (Platelia® Aspergillus, Bio-Rad Laboratories, Redmond, Washington, U.S.A.) that detects *Aspergillus* cell-wall galactomannan when done serially on a biweekly schedule in HSCT patients has had a negative and positive predictive values around 90%. Thus, this assay could

![Figure 4](image-url) Pulmonary nodule with a halo sign in a hematopoietic stem-cell transplant patient. Subsequent video-assisted thoracic surgical (VATS) resection demonstrated invasive pulmonary aspergillosis. Inset shows the negative image to illustrate the subtlety of the halo sign.
allow for a pre-emptive antifungal strategy (32,48,49). Alternatively, molecular amplification techniques to detect fungal nucleic acids appear to be comparably useful. Both these approaches will permit prospective surveillance, provide an estimate of microbial load, and insight into the response to therapy (“proof of cure”) (50).

Details of drug therapy will be discussed subsequently. The points to be emphasized, however, are clear-cut: there are a variety of drugs available for the treatment of invasive aspergillosis at present, with voriconazole offering the best results as a single agent (51); combination therapy with multiple drugs, particularly in the treatment of CNS or disseminated infection, is being actively pursued and should be studied systematically. Surgical extirpation, together with antifungal therapy, is appropriate in the presence of a single lesion and no metastatic disease elsewhere. The key is to recognize that invasive aspergillosis is a medical emergency requiring the immediate initiation of therapy (1,32,46).

C. Cryptococcosis

The precise source of human cryptococcal infection in the United States remains unclear, but it is thought to stem from the inhalation of infectious propagules derived from sites contaminated with pigeon excreta. There are three potential outcomes from this inhalation: clearance of infection, development of latent infection, and acute infection with or without hematogenous dissemination. The first two of these are the usual outcome of this initial encounter with *C. neoformans*. Latent *C. neoformans* capable of being reactivated to cause clinical disease, especially after the onset of immunocompromise, is not uncommon in the general population. The pathologic response to cryptococcal invasion is quite variable: from no response (in

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**Figure 5** Brain biopsy from a transplant patient with disseminated aspergillosis. The classic behaviors of metastatic infection, angioinvasion (*thin arrow*), and tissue infarction (*thick arrow*) are illustrated. *(Source: Photomicrograph courtesy of Danny Milner, MD, Department of Pathology, Brigham & Women’s Hospital; Hematoxylin & Eosin, 100×.)*
those patients most immunocompromised) to a strong granulomatous response, which is the cornerstone of the individual’s ability to control this infection. The activated macrophage is the most prominent cell type in this attempt to limit the extent of cryptococcal infection. The capsular polysaccharide of *C. neoformans* serves to impede phagocytosis and is thought to be an important virulence factor (17).

Clinical disease caused by *C. neoformans* in the organ-transplant recipient usually occurs more than 6 months post-transplant. The major exception to this general pattern is the group of patients who come to transplant with a history of immunosuppression for their underlying disease or a previous failed transplant. It is our practice to evaluate such individuals for the possibility of cryptococcal (and such other opportunistic infections as pneumocystosis) prior to the performance of a new transplant (1).

The most common presentation of cryptococcosis post-transplant is that of an asymptomatic chest nodule discovered by chance on a chest x-ray. Diagnosis usually requires a biopsy, as antigen testing in this circumstance is often negative; in addition, evaluation of the transplant patient for other sites of cryptococcal disease (CNS, skin, prostate, and skeletal system) is recommended. For those patients with cryptococcal nodules with no evidence of other sites of infection, fluconazole therapy is prescribed for 2–4 weeks after diagnosis to protect against the development of metastatic infection, which is not uncommon after such surgical manipulation. Those with other sites of infection are treated for systemic cryptococcosis as outlined in what follows. The transplant patient presenting with an asymptomatic nodule is often a patient whose net state of immunosuppression is not very great (the patient with a good outcome from transplantation who is receiving only maintenance immunosuppression); in contrast, the patient with disseminated infection is usually the patient with a poor outcome from transplantation, whose net state of immunosuppression is greater than it should be (the previously described “chronic n’er do well”) (1,17,46).

Cryptococcal pneumonia, ranging from asymptomatic to that causing significant respiratory compromise, can also occur. Pulmonary disease in general, whether true pneumonia or just a nodule, may present with a negative serum cryptococcal antigen test, thus mandating more invasive tests (bronchoscopy and/or biopsy) for diagnosis. Patients with cryptococcal pneumonia also merit evaluation for other sites of infection. The evaluation should include brain CT, lumbar puncture, bone scan, urine culture and prostate exam, and a careful examination of the skin for evidence of cryptococcal lesions. In 20% of patients with cryptococcosis, skin findings (nodules, papules, ulcerations, etc.) are the first signs of cryptococcosis. Synchronous dual infection is not uncommon in this setting; such combinations including tuberculosis, nocardiosis, and aspergillosis with cryptococcal disease (17).

The key issue with cryptococcal infection is whether or not hematogenous dissemination occurs, and if so, is the CNS involved? Cryptococcal CNS infection is properly termed a meningoencephalitis, although the meningitis component is usually the most evident. The pathologic changes are those of a granulomatous process, both in the meninges and in the brain itself (with cerebral mass lesions occasionally being found on brain imaging). The most common presentation of CNS cryptococcosis in the transplant patient is a febrile headache of gradual onset, often over several weeks. However, other symptoms such as change in the level of consciousness, difficulty concentrating, and memory loss may be present. Less than half of the patients with cryptococcal meningitis will exhibit signs of meningeal inflammation on physical exam. A variety of ocular findings may be present in these patients, most commonly cranial nerve palsies (e.g., of the sixth cranial nerve), but also papilledema, endophthalmitis, and visual loss because of increased intracranial pressure (17).
Virtually, any bodily site can be infected by *C. neoformans*, with two of these warranting special mention here: the bones and joints and the prostate gland. Cryptococcal osteomyelitis may be present as an isolated site of infection; alternatively, it is present as part of disseminated infection involving multiple sites. The prostate gland can serve as a protected site of cryptococcal infection that is relatively resistant to antifungal therapy. Although best demonstrated among AIDS patients, active cryptococcal infection of the prostate has been demonstrated in transplant patients post-therapy. Because of this, more intensive therapy is advocated in men with evidence of prostatic infection at the time of presentation (17).

Of all the fungal infections that can occur in transplant patients, the diagnosis of cryptococcal disease is perhaps the easiest to accomplish, provided the possibility of this infection is considered. Blood cultures, particularly when the lysis centrifugation method is used, have a sensitivity around 70%. The classical cerebrospinal fluid (CSF) formula for cryptococcal meningitis is a lymphocytic pleocytosis, low sugar, and elevated protein, with increased intracranial pressure. The India ink test on CSF is positive in ~50% of transplant patients with CNS disease and is a measure of the organism burden present. The ability to measure cryptococcal capsular polysaccharide, both in serum and in CSF, has become the cornerstone of the diagnosis of cryptococcal disease and should be employed in the evaluation of any transplant patients with unexplained febrile illnesses, as well as in patients with the focal findings described. The titer of antigen present on diagnosis is a good estimate of the organism burden and prognosis, although serial measurements to monitor therapy have had mixed results (17).

### D. Zygomycosis (Mucormycosis)

These two terms, usually used interchangeably, describe a group of invasive fungal infections characterized by rapid progression, tissue necrosis, angioinvasion, and the need for aggressive surgery in addition to antifungal therapy. These infections are caused by fungi of the class Zygomycetes, which is currently subdivided into three orders: the Mucorales, the Mortierellales, and the Entomophthorales (52). The Mucorales and Mortierellales are responsible virtually for all the diseases that occur in organ-transplant patients, with a rapid course that constitutes a medical emergency; the Entomophthorales are usually found in tropical areas and cause a slowly progressive disease involving the skin and/or sinuses of relatively normal individuals. We will restrict our attention to the Mucorales, which are ubiquitous in the environment (easily isolated from the soil) and are present in particularly high numbers in decaying organic materials such as fruit and bread. Most cases of infection with these organisms are a result of inhalation of spore-laden air, although direct inoculation of damaged skin is also possible (53).

Risk factors that predispose to infection with the Mucorales include three major categories of abnormality: immunosuppression, caused by both neutropenia and post-transplant immunosuppressive therapy, especially corticosteroids; metabolic derangement such as diabetic ketoacidosis, chronic metabolic acidosis, deferoxamine therapy, and protein-calorie malnutrition; and skin and soft-tissue injury: burn wounds, skin macerated by compression dressings laden with spores, and traumatic or surgical injury. There appear to be two important stages in the evolution of these infections where host defenses are of critical importance: suppression of spore germination and the killing of hyphal elements. Hence, neutrophil number and function, as well as macrophage/monocyte number and function, are critical variables in
dealing with these organisms, with the effects of both immunosuppression and metabolic derangement likely to be exerted through their impact on neutrophil and macrophage function (53).

The association between zygomycosis (especially the rhinocerebral form) and diabetic ketoacidosis has long been recognized. In addition, chronic acidosis itself can predispose to zygomycosis. For example, we have observed necrotizing pneumonia caused by these organisms in a nondiabetic man with a failing renal transplant, whose renal failure rendered him chronically acidic. Similarly, rhinocerebral disease has been observed in patients with combined renal/pancreas transplants in which the exocrine secretions of the pancreatic allograft have been excreted through a bladder anastomosis, resulting in a bicarbonate leak, which is not compensated if kidney function deteriorates. This results in a euglycemic, formerly diabetic individual with chronic acidosis, and an increased risk of life-threatening Mucorales infection. Finally, deferoxamine therapy for iron overload, particularly if immunosuppression is also present, has emerged as a major risk factor for this infection. Iron is a critical growth factor for these (and other) organisms, and this iron chelator provides the organism with access to large amounts of the metal, thus promoting infection (1,53,54).

The clinical syndromes produced by the Mucorales are characterized by the following features: it is angioinvasive, causing necrosis and infarction, as well as hemorrhage; it is rapidly progressive and is relatively resistant to available antifungal agents. The most common syndromes are rhinocerebral, pulmonary, skin and soft tissue, and disseminated. When compared with Aspergillus infection, another angioinvasive fungus, the Mucorales, cause less disseminated disease and much more rapid spread into contiguous structures (1,53).

Rhinocerebral zygomycosis involves sequentially the nose, sinuses, eyes, and brain. Presenting symptoms include nasal congestion, epistaxis, sinus tenderness, retroorbital headache, and local swelling. As the process progresses, swelling of involved tissues becomes more noticeable and ocular symptoms (blurred vision, diplopia, proptosis, and blindness) become evident, with loss of cutaneous sensation at involved sites being caused by infarction of nerves. On physical examination, a black eschar in the nasal cavity or hard palate is characteristic. As the infection invades the cranial vault, cavernous sinus thrombosis, carotid thrombosis, and focal brain disease result. This is a medical emergency requiring urgent surgical excision (1,53).

Only slightly less dramatic is the rapidly progressive, necrotizing pneumonia that the Mucorales can cause. Not only the lungs are involved, but also contiguous spread across fascial planes to involve the great vessels, the pericardium, the chest wall, and the diaphragm is not uncommon. Again, rapid recognition and aggressive surgery are the only hope for an increasingly desperate situation (1,53).

The normal skin is remarkably resistant to invasion by the Mucorales, but skin damaged by a variety of means, including the placement of vascular access devices, becomes quite vulnerable. A particular problem in the past was the placement of pressure dressings tapes, which not only damaged the skin, but also were often laden with fungal spores, an ideal situation for producing necrotizing skin infection. Hematogenous seeding of the skin is also possible, but is relatively less common, presumably because of the rapid progression of the primary site of infection (1).

The diagnosis of zygomycosis requires pathologic assessment and/or culture. Early biopsies of sites of necrotizing infection are of critical importance. One of our rules of thumb in the evaluation of immunocompromised hosts is that no
necrotic lesion should be left without biopsy, as it may be the earliest clinical manifestation of these or other angioinvasive molds.

High-dose amphotericin B should be used as adjunctive therapy to surgical debridement in the management of this infection. Voriconazole and the echinocandins are unfortunately not active against the Mucorales, but there are promising in vitro data and initial clinical experience with the use of posaconazole in treating this infection, although it is not yet approved for this indication.

E. New and Emerging Fungi

With the licensure of a number of broad-spectrum antifungal agents (e.g., the new azoles and echinocandins) and their rapid deployment, it is not surprising that new fungal species are coming to attention. In general, these fungi are most apt to occur in HSCT and oncology patients, where they now account for ≥5% of the invasive fungal infections. For the most part, these have not been important pathogens as yet in solid organ-transplant patients, with the notable exception of lung allograft recipients. Such angioinvasive molds as Scedosporium and Fusarium have been noted to produce clinical syndromes akin to those produced by Aspergillus species: colonization being associated with increased risk of invasion; infarction, hemorrhage, and metastatic infection being the norm; and amphotericin B—as well as other drugs—resistance being common. These infections appear to be increasing in number. When infection with one of these agents is documented, every effort should be made to speciate the isolate and to test for drug susceptibility, as the incidence of primary resistance is higher with these organisms than with the usual fungal pathogens (11).

Agents of phaeohyphomycosis (the black or pigmented fungi in tissue sections) are occasionally found as causes of infections in organ-transplant recipients. These are usually cutaneous and subcutaneous infections, although they can rarely cause more invasive disease. Acquisition is usually through skin trauma or contamination of wounds. Treatment of localized disease with excision and ablation is usually preferred. Amphotericin B, itraconazole, voriconazole, and terbinafine have been used successfully for the treatment of both localized and systemic infections (55).

VI. PRINCIPLES OF ANTIFUNGAL THERAPY IN SOLID ORGAN-TRANSPLANT RECIPIENTS

A. Modes of Therapy

There are four different modes in which antimicrobial therapy may be deployed in the management of the infectious disease problems of the transplant recipient (1,4):

1. A therapeutic mode, in which antimicrobial agents are prescribed to control and, hopefully, cure clinical and microbiologically identified disease. Early diagnosis and initiation of therapy is the key to the successful therapeutic use of antimicrobial agents. This truism applies particularly to fungal infections.

2. An empiric mode, in which a pre-determined antimicrobial prescription is administered without knowledge of the specific etiologic agent or agents causing a certain syndrome. It is based on knowledge of prior epidemiology for such a syndrome and the risk of a bad outcome in a specific population. The empiric use of antibiotics in patients with fever and neutropenia
or in patients with community-acquired pneumonia is now a standardized avenue of such empiric therapy.

3. A prophylactic mode, in which everyone in a cohort receives an antimicrobial program before an event to prevent important infection. Antifungal prophylaxis has a particular role to play in the management of liver-transplant recipients who receive routine fluconazole prophylaxis to prevent perioperative Candida infections and lung-transplant recipients who are treated with amphotericin B nebulizations to prevent pulmonary infections, especially anastomotic site infections caused by Candida or Aspergillus.

4. A pre-emptive mode, in which a subgroup of patients is identified as being at particularly high risk of invasive infection on the basis of a laboratory marker and/or a clinical or epidemiologic characteristic. For example, colonization of the respiratory tract of a heart- or liver-transplant patient with Aspergillus species is correlated with a >50% risk of invasive aspergillosis. If a surgical procedure (e.g., an excisional biopsy of a nodule in the lung) reveals the presence of an endemic fungus, there is a 10% risk that hematogenous spread of the organism to the CNS, bone, or eyes occurred because of surgical manipulation of infected tissue. In both instances, pre-emptive therapy with the appropriate azole agent (e.g., fluconazole) can eliminate these problems.

It is likely that as new diagnostic tests such as polymerase chain reaction (PCR) and fungal antigen detection become available, pre-emptive therapy will become even more important in the management of these patients.

B. Drug Interactions

Antimicrobial therapy in the organ-transplant patient is associated with frequent drug interactions, particularly with the mainstays of modern immunosuppression: tacrolimus and cyclosporine. There are essentially three classes of interactions that are observed, two of which are related to effects on the hepatic cytochrome P450 enzyme isoforms that catalyze the metabolism of the two calcineurin inhibitors (1,4):

1. Upregulation of cytochrome P450 isoforms, resulting in low blood levels, inadequate levels of immunosuppression, and a high probability of allograft rejection. Although currently available antifungal agents do not have this effect, it should be remembered that antimicrobial drugs such as rifampin, isoniazid, and nafcillin do (and it is not unlikely that other drugs may have this effect as well).

2. Downregulation of cytochrome P450 isoforms, resulting in high blood levels, renal toxicity, the possibility of over immunosuppression, and an increase in the risk of opportunistic infection. The azoles have a significant effect of this type, with ketoconazole being greater than itraconazole or voriconazole, and all these greater than fluconazole. All azoles have the same effect but of differing magnitude. A potentially dangerous interaction is that of sirolimus and voriconazole because the introduction of voriconazole inhibits the CYP-2C19 causing a steep increase in sirolimus levels.

3. The third mechanism has to do with the causation of significant renal injury by the administration of an antifungal agent (usually an amphotericin B preparation) in patients with appropriate therapeutic (not toxic) blood levels of tacrolimus or cyclosporine. There are at least two forms of this interaction: (a) single doses as small as 10 mg of conventional amphotericin B (deoxycholate) have induced acute, oliguric renal failure in transplant patients, probably because of polymorphisms in
the Toll-like receptors that bind amphotericin B and make this patients uniquely susceptible to amphotericin B toxicity and (b) renal function will deteriorate much earlier in the course of amphotericin therapy (e.g., after as little as 200 mg of amphotericin B as opposed to a similar effect only after more than 500 mg of cumulative amphotericin B dose) when therapeutic levels of a calcineurin inhibitor are present, in a principle that we have called synergistic nephrotoxicity (1).

The end result is that the nature of the antimicrobial agents employed will be somewhat different. It is imperative that evaluation for direct and indirect drug interactions in organ-transplant recipients be part of the clinical assessment of any confirmed or suspected fungal infection and that treatment is tailored to minimize drug toxicities. Furthermore, transplant patients should acquire the habit of consulting with their treating teams concerning any new medication they are prescribed. This includes over-the-counter and herbal medications. We have witnessed patients who have lost their allografts or developed new infections when this basic practice was overlooked. The assessment of acute and cumulative toxicity of amphotericin B should be part of the ongoing care of these patients, and the use of lipid formulations of amphotericin B, azoles, or echinocandins should be reevaluated if any significant toxicity develops.

These considerations on the nature, dynamics, and treatment of invasive fungal infections have also led us to view antifungal therapy as having several stages: (a) induction therapy, in which the most potent regimen available is administered to gain control of the disease process as quickly as possible; toxicity and cost issues are of relatively minor consequence until the patient stabilizes; (b) consolidation therapy, in which cure of the patient is sought over a prolonged period of, when feasible, oral therapy; at this stage, toxicity and cost are major considerations; (c) maintenance therapy (also conceived as secondary prophylaxis), in which the fungal process has been brought into remission, but, because of the need for continuing immunosuppression, relapse is possible and needs to be prevented. Again, the use of non-toxic, relatively inexpensive oral therapy is emphasized. This approach, borrowed from oncology, is illustrated in Table 2 for the different forms of fungal infection seen in solid organ-transplant patients.

The remaining therapeutic general principle that merits attention is how to determine the duration of therapy in the individual patient, an area in which there is relatively little data. Indeed, as stated by the esteemed Professor Louis Weinstein, “there are only two things we don’t know in infectious disease: how much to treat with and how long to treat for.” This observation is particularly applicable to antifungal therapy for transplant patients, as changes in the immunosuppressive regimen can have profound effects on the antifungal program. The approach we advocate does not utilize fixed courses of therapy for a particular infection; rather, our plan is to treat until all signs and symptoms referable to the fungal infection are eliminated, and then add an additional buffer or consolidation period. The duration of the buffer period will depend on the seriousness of the condition, the rapidity of response, the net state of immunosuppression (if it can be improved), the consequences of relapse, and the toxicities of the therapy. For example, CNS infection merits consideration of long-term maintenance therapy, as well as a prolonged buffer period. In sum, the complexity of antifungal therapy for this population of patients requires not a standard regimen for all, but rather individualized therapy that encompasses all parts of the clinical situation and the therapeutic milieu in which the therapy is being deployed.
<table>
<thead>
<tr>
<th>Infection</th>
<th>Induction therapy</th>
<th>Consolidation therapy</th>
<th>Maintenance therapy</th>
<th>Additional interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillosis</td>
<td>Voriconazole or amphotericin B, or caspofungin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Voriconazole or caspofungin</td>
<td>Voriconazole</td>
<td>Consider excision and ablation of singleton lesions</td>
</tr>
<tr>
<td>Blastomycosis</td>
<td>Amphotericin B</td>
<td>Itraconazole</td>
<td>Itraconazole</td>
<td>Remove contaminated indwelling devices</td>
</tr>
<tr>
<td>Candidemia (and/or deep tissue infection)</td>
<td>Amphotericin B, or caspofungin, or fluconazole&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Fluconazole, or caspofungin, or amphotericin B</td>
<td>Only if vascular anastomosis at risk or prosthetic devices cannot be removed</td>
<td></td>
</tr>
<tr>
<td>Coccidioidomycosis</td>
<td>Amphotericin B</td>
<td>Fluconazole</td>
<td>Fluconazole</td>
<td>Consider secondary prophylaxis in seropositive patients undergoing solid organ transplantation</td>
</tr>
<tr>
<td>Cryptococcosis</td>
<td>Amphotericin B and flucytosine&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Fluconazole</td>
<td>Fluconazole</td>
<td>Management of intracranial pressure critical with CNS involvement</td>
</tr>
<tr>
<td>Fusariosis</td>
<td>Voriconazole or amphotericin B</td>
<td>Voriconazole or amphotericin B</td>
<td>Voriconazole</td>
<td></td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td>Amphotericin B</td>
<td>Itraconazole</td>
<td>Itraconazole</td>
<td></td>
</tr>
<tr>
<td>Paracoccidioidomycosis</td>
<td>Amphotericin B</td>
<td>Itraconazole or TMP–SMX</td>
<td>Itraconazole</td>
<td></td>
</tr>
<tr>
<td>Penicilliosis</td>
<td>Amphotericin B</td>
<td>Itraconazole</td>
<td>Itraconazole</td>
<td>Only if disseminated disease suspected</td>
</tr>
<tr>
<td>Phaeohyphomycosis</td>
<td>Local excision and itraconazole, or voriconazole, or terbinafine</td>
<td>Itraconazole, or voriconazole, or terbinafine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive Fungal Infection</td>
<td>Voriconazole</td>
<td>Voriconazole</td>
<td>Voriconazole</td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------</td>
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<td>--------------</td>
<td></td>
</tr>
<tr>
<td>S. apiospermum, S. prolificans</td>
<td>S. prolificans</td>
<td>Voriconazole and terbinafine</td>
<td>Voriconazole and terbinafine</td>
<td></td>
</tr>
<tr>
<td>Trichosporonosis</td>
<td>Fluconazole or voriconazole, and amphotericin B</td>
<td>Fluconazole or voriconazole</td>
<td>Fluconazole or voriconazole</td>
<td></td>
</tr>
<tr>
<td>Zygomycosis</td>
<td>Amphotericin B (1.25 mg/kg/day or lipid formulation equivalent)</td>
<td>Amphotericin B</td>
<td>Excision and ablation is the cornerstone of therapy. Consider expanded access posaconazole</td>
<td></td>
</tr>
</tbody>
</table>

The benefit of combination therapy is currently under investigation. Antifungal susceptibility testing and final species identification results should guide final therapy. Empiric therapy should be based on local epidemiology, bearing in mind that some Candida species are likely fluconazole resistant (C. krusei), amphotericin B resistant (C. lusitaniae), or caspofungin resistant (C. guillermondii) and some strains of C. parapsilosis. Avoid flucytosine use if there is renal dysfunction unless drug level determinations are readily available in “real clinical time.” We prefer use of a lipid formulation of amphotericin B in this situation, given preferential targeting of the reticuloendothelial system. Consider fluconazole for CNS histoplasmosis or if no other alternatives are feasible.

Abbreviations: TMP-SMX, trimethoprim–sulfamethoxazole.
C. Antifungal Agents

After decades in which amphotericin B deoxycholate was the only form of systemic antifungal therapy available, there has been a much-needed expansion in drugs approved for the treatment of invasive fungal infection. This welcome occurrence is tempered by the fact that the information base on which therapeutic decisions are made is presently incomplete; so the recommendations made in Table 2 should be regarded as subject to change, as more information is garnered and even more useful drugs become available. The following observations, however, are important adjuncts to Table 2.

1. Amphotericin B is well known for two major toxicities: acute infusion toxicity (“cytokine storm”), particularly with the first few doses, with fever, chills, hypotension being the rule, and dose-related nephrotoxicity. The lipid formulations of amphotericin B significantly attenuate the first of these and decrease, but do not eliminate, the incidence and severity of the renal injury. As far as efficacy is concerned, available information suggests that all of the amphotericin B preparations have comparable efficacy.

2. The advent of azole antifungal therapy has been a major advance. Fluconazole, as a treatment of many yeast infections, both candidal and cryptococcal, is well tolerated, pharmacologically ideal, and only limited by two factors: its antifungal spectrum does not encompass the molds; resistance is beginning to be an issue and the possibility of resistance needs to be assessed as part of the therapeutic decision making. Itraconazole is a broad-spectrum drug, including Aspergillus species, but its use has been hampered by pharmacokinetic issues: unreliable absorption orally (the new oral suspension formulation and the availability of an IV form help greatly in this area); poor penetration into respiratory secretions, the urinary tract, the CNS, and the eye; and the limited information available on efficacy in serious human disease. Voriconazole is a broad-spectrum drug, shown to be superior to amphotericin B in the treatment of invasive aspergillosis, as well as to several of the new and emerging fungi (e.g., Scedosporium apiospermum, but not S. prolificans), and although transient visual symptoms and liver dysfunction can occur, the drug is generally well tolerated.

3. The advent of caspofungin provides a new approach for the treatment of both Candida and Aspergillus infection. Its mechanism of action by glucan synthetase inhibition resulting in impairment of fungal cell-wall synthesis is of interest not only used alone, but also raises the possibility of synergistic multidrug regimens for greater efficacy (indeed, it has been suggested that echinocandins could be the “penicillins of antifungal therapy,” the cell-wall effects potentiating the entry of the other antifungal agents). Until more data become available, our policy is to consider multidrug therapy in the face of disseminated disease, particularly that which is involving the CNS and to participate in trials of systematic evaluations of antifungal combinations.

4. Antifungal susceptibility testing of most pathogenic yeasts has now been standardized (56). Although the correlation between in vitro resistance and clinical failure is not precise, routine susceptibility testing of all invasive yeast isolates will provide useful information both to refine the management of an individual patient and to provide epidemiologic information to guide future empiric therapy. We hope that this valuable information will be incorporated into routine clinical practice.

5. In addition to the choice of an appropriate antifungal agent, optimal management of these patients includes several other considerations: (a) decrease the net state of immunosuppression, primarily by decreasing the doses of the immunosuppressive
drugs being administered; (b) augment host defenses whenever possible, such as reversing neutropenia—whether disease or drug related—by use of colony stimulating factor or neutrophil transfusions in the case of HSCT recipients; (c) always consider the possibility of surgical resection of the site of infection: excision and cursive to first ablation rather and incision and drainage of infected tissues; (d) diagnosis and control of CMV and other immunomodulating viruses; (e) optimize other aspects of the patient’s care: for example, the prompt removal of vascular access devices in patients with candidemia.

VII. SUMMARY AND CONCLUSIONS

Fungal infections remain a significant problem among solid organ-transplant recipients, with the incidence of such infections being determined by the interactions of three factors: technical-anatomic abnormalities, environmental exposures, and the net state of immunosuppression. Prevention of infection remains the goal, failing which early recognition and prompt therapy remain major factors determining outcome. Early diagnosis remains challenging because of the impaired inflammatory response present in transplant patients. This renders signs and symptoms of fungal infection, as well as radiological findings in this patient population subtler, and necessitates closer follow-up and an aggressive diagnostic approach that usually includes invasive procedures and tissue sampling. Recent advances have made the possibility of detecting fungal infection earlier by means of antigen-detection approaches (e.g., the sandwich EIA assay for galactomannan) or the detection of circulating fungal DNA by PCR quite possible. These approaches, if effective, will not only permit early diagnosis, but also make possible a more targeted pre-emptive therapy and an assessment of the response to therapy (“proof of cure”), and help in establishing optimal durations of therapy. Therapy of fungal infection, both preventative and treatment related, is currently in a state of flux, at least in part because of the advent of new therapies. It is clear that voriconazole and caspofungin are significant additions to our therapeutic armamentarium, but optimal use and the utility of combination therapy remain to be determined. Drug interactions with the calcineurin inhibitors, sirolimus, and other medications remain important issues. In sum, much has been accomplished in this field, but much remains to be done.

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3

Fungal Infections in Blood and Marrow Transplant Recipients

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I. INTRODUCTION

Patients who undergo blood or marrow transplantation (BMT) are at risk for death because of relapse of the underlying condition (e.g., malignancy), as well as complications that arise from the transplant itself. Common causes of transplant-related mortality include opportunistic infections and organ toxicities resultant from receipt of cytotoxic conditioning or other immunosuppressive therapies. Over the last few decades, multiple changes in transplantation practices have occurred, including changes in stem cell sources, conditioning regimens, and supportive care strategies used. These changes have impacted the relative proportion of relapse-related vs. transplant-related mortality, and have led to a changing spectrum of opportunistic pathogens.

During the last two decades, fungi have become important pathogens in BMT patients, and the fungi of importance have changed as well, with yeasts giving way to molds as the most common fungi causing death after BMT. Infections thus remain a dominant cause of non-relapse related deaths after BMT, even late after receipt of stem cells (1,2). To optimize outcomes after BMT, efforts continue to focus on developing effective strategies to prevent and treat infections. This chapter focuses on the epidemiology of fungal infections in BMT patients. The incidence of, risks for, and outcomes of the most common fungal infections are discussed.

II. FUNGAL INFECTIONS: OVERALL INCIDENCE AND RISKS

For fungal infections to develop after BMT, microbial exposure is required, but exposure alone will not result in infection without the presence of other factors to impair host defenses. Immunologic defects that pose risks for fungal infections in BMT recipients include severe neutropenia, and defects in cell-mediated immunity, which typically last for a longer duration, especially in people who require therapy for graft vs. host disease (GVHD) or receive T-cell depleted stem cell products. Thus,
multiple host factors, transplantation variables, and microbial factors impact overall risks for infection (Fig. 1).

The magnitude of risks is not constant, but it expands and contracts during the course of BMT. Early post-transplant infection risks are largely influenced by severe neutropenia. A second high-risk period occurs later after transplantation with the onset of GVHD. In fact, severe acute GVHD may currently be the most important risk for invasive fungal infections (IFI), especially for those caused by molds. In one study, 50% of allogeneic BMT patients who developed severe (grades III–IV) acute GVHD developed an IFI, compared to 8% of patients who had minimal GVHD (3). Similarly, 57% of patients with clinically extensive chronic GVHD developed IFI, compared to 5% of those with limited chronic GVHD. Type of therapy for GVHD may also impact overall risks; this same study noted that 77% of patients who received high-dose steroids (methylprednisolone 0.25–1 g/day for 5 days) developed IFI, compared with 5% of patients who received lower doses (<2 mg/kg/day) of prednisone for GVHD therapy (3). Multiple studies have now shown that steroid containing regimens used to prevent and treat GVHD are associated with particularly high risks for infection and infection-related death (4–6). Unfortunately, transplanters are often forced to negotiate between the effects of GVHD and the overwhelming immunosuppression that may be required to suppress it.

Other transplantation variables, such as stem cell source and type of conditioning therapy, alter risk periods by impacting the duration and magnitude of immune reconstitution and other complications (e.g., gastrointestinal mucositis, GVHD). For instance, people who receive transplantation after non-myeloablative condi-

![Figure 1](image-url) **Figure 1** Multiple factors that impact risks for fungal infections after BMT. Overall risks for infection are influenced by microbial, host, and transplantation variables. The magnitude of each variable may expand and contract during the course of BMT, creating time periods that correspond to low and high risks for infection.
tioning therapy, which relies on graft versus host effects to elicit an adequate anti-tumor response, appear to develop infections later, during the period of GVHD (7–9). On the other hand, people who undergo transplantation using cord-blood as a stem cell source appear to have higher risks early after BMT, prior to stem cell engraftment (1,10,11). Typical risk periods for the most common fungal infections that occur after BMT, and corresponding host factors, are outlined in Figure 2. This time course provides a helpful guideline for understanding typical risk periods, which can be utilized to develop appropriate preventative strategies. However, it is important to appreciate that these generalizations do not account for important individual variables that may impact the risk for infection, such as severity of underlying disease and prior cytotoxic therapies, stem cell dose, and genetic differences in immunity. Incidence of, and specific risks, for the most common fungal infections are discussed in more detail in the following sections.

A. Candida Species

1. Manifestations, Incidence of Infection, and Risks

Candida species cause both mucosal infections and invasive diseases, such as candidemia and hepatosplenic candidiasis. More information on the pathogenesis of disease, clinical presentation, and diagnosis and management of candidal infections

Figure 2  Risk periods for specific fungal infections, according to risk factors and time relative to receipt of stem cells (day 0). Size of arrows represents relative risk of infection during early and late time periods. Variables listed are those that have been identified with multivariable modeling (1,64–68).
is presented in Chapter 10. This section focuses on the epidemiology of mucosal and invasive candidiasis in BMT recipients.

Oral complications are important in BMT recipients, often causing pain and debilitation, with decreased oral intake potentially leading to systemic complications. Although *Candida* species are known to be a common cause of oral mucositis, few studies have specifically examined the incidence of, or risk factors for mucosal disease in BMT recipients. This may in part be due to diagnostic uncertainties attributable to the fact that *Candida* species are common colonizing organisms, and ulcerative mucositis and plaque-like lesions are caused by multiple factors, such as cytotoxic therapy, Herpes viruses, and GVHD. Older studies performed prior to the use of prophylactic azole antifungals in BMT recipients noted that the majority of patients have oral colonization with *Candida* species during the early post-transplant period, corresponding with mucositis associated with cytotoxic and/or radiation conditioning therapy (12,13), however, the causative role of *Candida* is not clear. Infections may progress to involve the esophagus in BMT patients, however, the incidence of esophageal candidiasis in the BMT setting is not well described. A review of esophageal infections that occurred in patients who underwent BMT without prophylactic antimicrobials in the early 1980s noted that the prevalence of esophageal infection is high, however, causative organisms (viral vs. fungal) could not be identified based on symptoms, oropharyngeal culture, or X-ray (14). Also, the concept of diagnostic parsimony does not hold for mucosal infections with *Candida* species, as in several studies, yeasts were found to cause esophageal infection with cooperation of an underlying Herpes virus (HSV or CMV) and/or GVHD (14,15).

Data on clinical significance of mucosal disease may be more reliable from the prospective studies performed to evaluate the efficacy of fluconazole for prophylactic therapy. In the placebo-controlled trials of the early 1990s, the incidence of “superficial” candidal infections ranged from 10% to 30% (16,17). In these studies, fluconazole administration decreased the incidence of both symptomatic mucosal candidiasis and gastrointestinal tract colonization.

Subsequent to the use of prophylactic therapy in allogeneic BMT patients, the morbidity of mucosal candidiasis declined dramatically. In part, this is related to the fact that azole-susceptible *C. albicans* more frequently cause oropharyngeal candidiasis than azole-resistant non-*albicans Candida* species (18,19). However, it is noteworthy that fluconazole-resistant *C. albicans* has not appeared as a common cause of mucosal candidiasis in BMT patients, as it did in the AIDS population (20). This is somewhat surprising since azole-resistant *C. albicans* species have been documented to colonize the GI tract and cause invasive disease in BMT patients receiving fluconazole prophylaxis (20–22). These differences may be due to multiple factors, including the relative durations of impairment in cell-mediated immunity, mucosal factors that impact microbial colonization, or to the use of topical or empiric antifungals in BMT patients.

The epidemiology of invasive candidiasis is better documented in this patient population. Several large studies have examined the clinical significance of, and risk factors for invasive candidal infections, both before and after the use of azoles for prophylactic therapy. In one institution, prior to the use of azoles for prophylaxis, the 1-year cumulative incidence of candidemia and/or deep tissue infection following allogeneic or autologous BMT was 12.5% (23). Investigators from another institution reported that the incidence of candidemia and/or deep tissue infection increased between the years 1980 and 1986, ranging from 11.4% to 40% (24). Outcomes of
candidemia during this time period were poor; attributable mortality in these studies ranged from 32–37% (23,24).

Risks for candidemia that have been identified using multivariable modeling include the factors that increase exposure to the organism (e.g., GI tract colonization, presence of IV catheters) and factors that increase susceptibility of the host (e.g., prolonged neutropenia (Fig. 2) (23–25). Although the majority of candidemias occur during the early post-transplant period, patients who have severe GVHD have continued risks for candidemia, largely because of continued portals of entry via GI tract mucositis and indwelling catheters. Other factors that have been identified as risks for candidemia include bacteremia, CMV disease, and receipt of antibiotics (23–27). Whether these factors truly increase the risk for infection by altering microflora in the gut (28–30), or by modulating immune responses to fungi (31,32), or whether these events serve as markers of increased susceptibility is not clear.

2. Microbial Epidemiology

A change in the microbial epidemiology of candidiasis has been well described in large studies performed by networks of hospitals located throughout the world; nearly all studies performed in the latter 1990s noted a relative increase in non-
albicans Candida species as pathogens, however, C. albicans has remained an important cause of invasive disease (33–35). In BMT patients, the use of azole antifungals has been a factor contributing to the change in epidemiology; prophylaxis has decreased the incidence of both candidemia and hepatosplenic candidiasis in BMT recipients, and changed the spectrum of Candida species that cause disease (25,36). In one institution, the use of prophylactic fluconazole was associated with a decreased incidence of candidemia (11–5%) and hepatosplenic candidiasis (9–3%) (25,36). The Candida species that cause disease have changed, with fewer infections by species that are typically azole-susceptible, and more caused by azole-resistant organisms (25,36,37). In the 1980s and early 1990s, C. albicans and C. tropicalis were the most frequent causes of invasive infection, with risks largely related to GI tract colonization (23,24,37,38). One surveillance study reported that C. albicans and C. tropicalis were the most frequent colonizing organisms, detected in 43% and 10.8% of BMT patients in the 1980s (38). Fluconazole administration appears to decrease colonization with C. albicans and encourage colonization with azole-resistant non-
albicans Candida species, especially C. glabrata and C. krusei, resulting in increased risks for invasive disease with the potentially resistant organisms (25,39–41).

Case reports and series of infections caused by non-
albicans Candida species emphasize that the mode of acquisition and risks for infection are species-dependent. Detailed cases of infection with C. krusei, C. parapsilosis, C. dubliniensis, C. lusitaniae, C. lipolytica, and C. guilliermondii have been presented in the literature (25,42–52). Candidemia is most common in patients with heavy GI tract colonization, and animal studies have shown that C. albicans and C. tropicalis are particularly adept at invading GI mucosa (3,25). Several other Candida species, including C. parapsilosis, C. lusitaniae, and C. lipolytica may more frequently be acquired through an exogenous route, potentially through IV catheters or infusates (25,37,49,51). Hence, risks for infection in particular patient populations impact microbial epidemiology to a large degree. More information on the pathogenesis of candidal infections is found in Chapter 10.
B. Aspergillus Species

1. Manifestations, Incidence of Infection, and Risks

During the 1990s, invasive infections caused by molds became more common in BMT patients. Currently, Aspergillus species are a frequent cause of invasive fungal infections, and in some centers, aspergillosis has become the most common cause of infection-related death (53). Disease caused by these organisms usually involves the respiratory tract, as organisms are usually acquired through aerosolization. Sinusitis and invasive pulmonary infection are the most common manifestations, however, complications can develop from local invasion into the orbit and brain, as well as hematogenous dissemination (Fig. 3). Aspergillus species can also cause locally invasive gastrointestinal disease in BMT patients (Fig. 3) (54), as well as a primary cutaneous infection involving areas of skin breakdown (55). More information on clinical manifestations and pathogenesis of Aspergillus infections is found in Chapter 10. The following discussion is focused on the risks for, and epidemiology of pulmonary aspergillosis in BMT recipients.

Aspergillus species were recognized as an important cause of pneumonia and death in BMT patients in the late 1970s and 1980s (56,57). During this period of time, the nosocomial nature of these infections became apparent; in one hospital, Aspergillus species were recognized as the single most common agent of nosocomial pneumonia, causing 36% of episodes (58,59). Infection typically occurred early after BMT; in a large review of patients who received BMT in one institution between 1974 and 1989, 58% of infections (including aspergillosis) occurred prior to neutrophil engraftment (60). Risks identified during this early period include prolonged neutropenia and graft rejection, especially in combination with nosocomial exposure to the organism (61).

During the 1980s aspergillosis was also recognized as a common cause of infection late after BMT (4,62,63). In a survey of European BMT centers, aspergillosis was identified as one of the most common infections that occurred greater than 3 months after BMT, along with CMV disease, Pneumocystis carinii pneumonia, and invasive infection with Pseudomonas species (63). A review of infections that occurred in 549 patients who received BMT in one transplant center during the 1980s showed that the majority of Aspergillus infections in allogeneic BMT patients occurred post-engraftment, especially in association with corticosteroid receipt for GVHD (4).

The results of most recent studies emphasize that aspergillosis now occurs more frequently late after allogeneic BMT, creating two peaks of infection, or a ‘bimodal’ incidence (1,64–68). In cohorts of patients who underwent transplantation during the latter 1990s, the median day of diagnosis ranged from day 92 to day 136 after receipt of stem cells (1,65–69). In these series, only 10–14% of Aspergillus infections occurred during neutropenia (65,68,70). The reason that aspergillosis occurs more frequently as a late complication of allogeneic BMT is not well understood. Potential explanations include reporting bias, increased diagnostic certainty, changes in GVHD therapies, increased environmental exposure, and shifts in microbial resistance patterns (1,36,71).

Factors that are important in conferring risks for aspergillosis during the early or late time periods after BMT are different; in general, early aspergillosis is associated with delayed neutrophil engraftment and late aspergillosis is associated with factors that impair cell-mediated immunity (Fig. 2) (1). Host and transplant variables that extend the duration of neutropenia and factors that lead to increased
exposure to the organism increase the risk for early infection. Hence, stem cell sources and graft rejection are important factors associated with early disease (1,64); in one study, receipt of bone marrow or cord blood instead of peripheral blood as a stem cell source was associated with increased risks for early aspergillosis (1). Likewise, cohort studies have noted that patients who receive CD34-selected stem cells for either autologous or allogeneic transplantation may have relatively higher risks for infections early after BMT (72–74). Factors associated with an increased risk for post-transplant exposure to the organism, such as hospital construction and transplantation in the absence of protective isolation have also been identified as important variables in risk factor models focusing on the early time

Figure 3 Radiographic images of diseases caused by *Aspergillus* species. Panels A–D demonstrate multiple presentations of pulmonary aspergillosis. This infection can present as isolated nodular lesions (A), cavitated lesions (B), pleural-based infiltrates (C), and scattered ground-glass appearing infiltrates (D). *Aspergillus* species may cause sinus opacification (E) with thickening of membranes and potentially bone destruction. Disease can disseminate to sites distant from lungs, including brain (F), liver (G, arrow), and other organs. *Aspergillus* species can also involve the GI tract. The patient in panel H presented with abdominal distress and ileus, which were found to be caused by *Aspergillus* gastritis and enterocolitis.
period (64). In one study, season of BMT was associated with increased risks during the early period; whether this is associated with increased environmental or hospital exposure is unknown (64).

Host factors that increase the likelihood of GVHD, such as receipt of stem cells from an unrelated or HLA-mismatched donor, increase the likelihood of aspergillosis later after BMT (1,65). Multivariable modeling noted a trend to increased risks for aspergillosis in patients who received peripheral blood instead of bone marrow as a stem cell source (1), possibly related to increased severity of GVHD (75,76). Type of therapy for GVHD is important; the highest risks for infection have been noted in people with severe GVHD (acute grades 3–4 and chronic clinically extensive disease) that is treated with high doses of corticosteroids (1,4,66,67). The relative magnitude of risks in people who undergo BMT using T-cell depleted stem cell products is unclear, as few comparative studies have been performed. However, the results of cohort studies suggest that T-cell depletion increases relative risks for aspergillosis late after BMT (77,78). The finding that T cells may be important in conferring risks for aspergillosis is not entirely surprising; multiple animal studies and a small clinical study showed that CD4+ T cells are important determinants for both infection risk and outcome (79–82). Precisely how these cells function to impact *Aspergillus* host defenses is not well understood.

The results of multiple studies have also demonstrated increased risks for aspergillosis in patients who have bacterial (27) and viral (1,32) infections after BMT. An association between CMV disease and aspergillosis has been particularly well described and noted in other immunosuppressed patients (e.g., solid organ transplant) (1,32,83). Other recent studies also found that certain respiratory viruses (Parainfluenza 3 and respiratory syncytial virus) are associated with increased risks for aspergillosis in multivariable models (1). It has been proposed that some viruses may impact risks by having a direct impact on fungal host defenses (32,84–88). However, other studies have noted that CMV disease and receipt of corticosteroids are statistically linked, raising the possibility that the infection serves as a marker of generalized impaired immunity, rather than a risk in itself (66).

Underlying diseases impact risk for aspergillosis both early and late after BMT. These factors may be important for a number of reasons, including age of the host, receipt of prior cytotoxic therapies, and type of BMT typically used to treat the underlying condition. In multiple studies, the underlying diseases of aplastic anemia and acute leukemias not in remission were associated with increased risks for post-transplant aspergillosis, especially early post-transplantation (1,64). Also, patients who have myelodysplastic syndromes have been noted to have increased risks for aspergillosis, both early and late after BMT (1,65). Underlying diseases may impact risks by specific impairments in host defenses, impacting prior cytotoxic therapies, or impacting the frequency (or severity) of GVHD or organ dysfunction.

Finally, different types of conditioning therapy impact risks for aspergillosis during the early and late time periods. Recent attention has been focused on the sustained risks for aspergillosis after non-myeloablative transplantation, despite decreased cytotoxic therapy and neutropenia in these patients. In studies performed to date, risks for late aspergillosis appear to be roughly equivalent in patients who underwent BMT after non-myeloablative and myeloablative conditioning therapy, although the rate of infection is likely to be dependent on specific treatment regimens (8,9,78). Not surprisingly, specific risks during the late time period include severe GVHD and CMV disease (9).
2. Microbial Epidemiology

The vast majority (>90%) of aspergillosis cases are caused by *A. fumigatus* (53,89). This is most likely reflective of an increased virulence of this organism, as relative exposure does not appear to be increased compared to other molds (90). Other *Aspergillus* species, notably, *A. terreus*, *A. niger*, and *A. flavus*, also cause diseases, possibly with increasing frequency in most recent years (53,91). These organisms cause disease that is limited to the sinuses more frequently than *A. fumigatus*, although all species appear to be able to cause invasive and disseminated disease in particularly immunosuppressed hosts. It is becoming increasingly apparent that different transplant centers may have unique patterns in microbial epidemiology, potentially because of a geographic impact on environmental mold quantity and differences in factors that impact nosocomial exposure. For instance, one center reported an outbreak of *A. terreus* infections associated with contamination of potted plants in their hospital (91). As the reported frequency of infection is influenced by both the patient populations at risk for disease and reporting bias, it is unclear how much variation in *Aspergillus* species exists between centers. Surveillance studies currently underway to track infections in transplant centers may allow us to assess better regional differences in the epidemiology of molds.

The source of exposure to *Aspergillus* species has recently come into question. For many years, these organisms were thought to be acquired by inhalation of aerosols produced in or around areas of construction. In one study, the presence of hospital construction increased rates of *Aspergillus* recovery in BMT patients (92). Regression modeling and molecular typing studies have also documented a relationship between aspergillosis in hospitalized patients and air contamination with *Aspergillus* spores (93–95). However, other studies have not been able to establish correlations with infections and results of air sampling (92,96–98). Potential explanations include the insensitivity of air sampling for *A. fumigatus* (99,100), biodiversity of isolates (96), identification of pseudo-epidemics that result from contamination of culture media in the microbiology laboratory (101), as well as the possibility that *Aspergillus* species may be acquired through alternative sources (102,103). One suggestion is that *Aspergillus* species may be acquired from hospital water supplies. In several studies, molds were isolated in up to 70% of water sources (71,103–108). However, the frequency of recovery of molds from water supplies also appears to be variable; it is likely that mold contamination is impacted both by geography and the mechanism by which hospitals acquire, and deliver water to patient’s rooms (106,108). Finally, it is possible that water-borne and food-borne *Aspergillus* species may also allow for acquisition through the gastrointestinal tract, although the relative frequency of “primary gastrointestinal aspergillosis” is not well described (102).

Hence, microbial epidemiology and clinical syndromes observed in different patient populations are likely to be impacted by air and water distribution practices in both the hospital and the environment. Multiple mechanisms of acquisition, including aerosolization of air and water, and ingestion may contribute to high risks for disease post-transplantation.

C. Other Fungi

Other molds and yeasts cause invasive diseases in BMT recipients. Other molds that are most frequent include the Zygomycetes, *Fusarium* spp., and *Scedosporium* spp. One BMT center reported that infections caused by Zygomycetes and *Fusarium*
have increased in frequency during the latter 1990s (53). Although \textit{Scedosporium} spp. did not appear to be increasing in frequency in this center located in Seattle, Washington, centers located in different geographic regions report increasing invasive infections with these molds. The epidemiology of these infections is discussed in more detail in the following sections.

1. Infections Caused by Zygomycetes

The class Zygomycetes is split into two orders: \textit{Mucorales} and \textit{Entomophthorales}. Although invasive infections with organisms in the \textit{Entomophthorales} order occur (109–113), the vast majority of infections in humans are caused by the \textit{Mucorales}. \textit{Mucorales} is split into six families: \textit{Mucoraceae}, \textit{Cunninghamellaceae}, \textit{Thamnidiales}, \textit{Syncephalaceae}, and \textit{Mortierellaceae} (114). The most common causes of invasive infections in humans are caused by \textit{Rhizopus} spp., \textit{Mucor} spp., \textit{Absidia} spp., and \textit{Rhizomucor} spp., members of the family \textit{Mucoraceae} (53,114,115), although increasing reports of infections caused by \textit{Cunninghamella} spp. have appeared in the literature (116–119).

The majority of invasive infections caused by Zygomycetes originate in the sinciphalic tract, and can invade through local tissues or by hematogenous dissemination (53,115). In our experience, pulmonary lesions caused by these organisms tend to be large and bulky, and local invasion through the chest wall can occur (Fig. 4). Some Zygomycetes infections may also be acquired through the GI tract, causing local colonic invasion and dissemination to the liver (109–111,115). It is likely that a certain number of these infections are acquired by contaminated food products. In one report, hepatic abscesses caused by Zygomycetes in a BMT patient was traced to ingestion of contaminated herbal medicines (120).

The incidence of Zygomycetes infections in BMT centers is not well defined. In retrospective studies performed in cancer centers, the incidence of invasive disease ranged from $<1\%$ to $2.5\%$ (53,60,115), however, two recent studies reported that the frequency of disease increased in the latter 1990s (53,115). Severe neutropenia and corticosteroid therapy are important risks (53,114,115). Given the association

![Figure 4](image_url)
with GVHD and corticosteroid use, these infections may occur very late after BMT (53). There is some evidence that the median duration of survival after Zygomycetes infections is longer than infection with other molds (Aspergillus spp., Fusarium spp., and Scedosporium spp.); however, survival 1 year after infection appears equivalent and poor (20%) (53). Many of these patients die despite successful antifungal therapy with other complications of severe GVHD.

2. Infections Caused by Fusarium species

Fusarium species, common plant pathogens, also cause disease in immunocompromised patients. The majority of invasive infections are caused by F. solani, although infections with F. moniliforme and F. oxysporum occur as well (121,122). These organisms cause both cutaneous infections, such as onychomycosis and wound infections, as well as invasive infections, which can originate in the sinopulmonary tract, gastrointestinal tract, IV catheter, or through skin breakdown (114,122).

The incidence of invasive Fusarium infections is variable among centers worldwide. A large cancer center in Texas reported that the frequency of infection increased over the years spanning 1970–1995, although with approximately four cases per year, the annual incidence is low, when compared to aspergillosis (123). In contrast, European centers report few infections with these organisms (123).

Explanations for geographic clustering remain elusive. Because Fusarium spp. can be isolated from water supplies, it was suggested that these infections may be primarily nosocomial, with acquisition related to shower exposure (121). However, another study failed to find an association between water culture of Fusarium spp. and infection; instead, the organisms were more frequently recovered from environmental air (124). These investigators noted that recovery increased in rainy weather, which corresponded with the seasonal distribution of infection (122,124). As with other fungi, it is most likely that these organisms can be acquired through multiple sources.

Few risk factor analyses for fusariosis in BMT recipients have been performed, however, nearly all reports have linked infection with severe neutropenia after conditioning therapy (53,122). Prolonged neutropenia and an underlying disease of multiple myeloma have been identified as risks for fusariosis (53). Outcomes with these infections have been poor during the amphotericin B era, with few patients (≤30%) responding to therapy (53,122). Factors that improve prognosis include neutrophil recovery, limited GVHD, and local disease (53,122). More discussion on antifungal therapy of these infections is provided in Chapter 10.

3. Infections Caused by Scedosporium Species

Scedosporium prolificans and S. apiospermum (the sexual anamorph of Pseudallescheria boydii) both cause disease in BMT patients. Scedosporium apiospermum is a well-known agent of mycetomas. In immunosuppressed patients, this organism generally causes a spectrum of disease that is similar to those caused by Aspergillus species, including sinusitis, inflammatory pneumonitis, cutaneous infections, and invasive pulmonary infection (125,126). Scedosporium prolificans, although reported as a cause of invasive disease less frequently than S. apiospermum, has become an important pathogen in certain parts of the world, especially Spain, Australia, and parts of the United States (127,128). Variability in epidemiology is likely to be related to the observation that this fungus proliferates best in a Mediterranean climate (129), although nosocomial sources are also possible. In one recent
an outbreak of *S. prolificans* infections was found to be associated with contamination of the ambient air in hospital rooms (130).

Both *S. prolificans* and *S. apiospermum* infections are associated with a very poor prognosis in BMT patients (<20% survival). *Scedosporium prolificans* appears particularly resistant to current therapies, owing possibly to antifungal drug resistance and potentially, an increase in virulence of the organism (53,129–131). Correction of underlying neutropenia, the primary risk factor, is critical (127). More information on treatment of these infections is provided in Chapter 10.

4. **Infections Caused by non-Candida Yeasts and Endemic Fungi**

Patients who undergo BMT are at risk for infections with organisms that can be considered less pathogenic, “normal” flora, such as *Trichosporon* species, *Blastoschizomyces capitatus*, *Rhodotorula* spp., *Cryptococcus laurentii*, and *Malassezia furfur*, and infection in this setting is most likely to occur through IV catheters (132–137). Other relatively more pathogenic organisms that occur frequently among other immunosuppressed patients (e.g., AIDS) also occur infrequently in BMT patients. *Cryptococcus neoformans* can cause meningitis in this setting (138), however, these infections are notably rare. Agents of endemic mycoses, such as *Coccidioides immitis*, *Penicillium marneffei*, and *Histoplasma capsulatum*, also cause disease infrequently, even within endemic regions. Among 137 patients who received allogeneic BMT within a hyperendemic region for histoplasmosis, none of the patients developed invasive disease (139). Whether the infrequency of infection is associated with degree or duration of impairment in cell-mediated immunity, use of antifungals or some other factor in BMT patients is not known.

III. **CONCLUSION**

The pathogens of importance in BMT patients have evolved over the last 30 years, with emphasis shifted from gram-negative bacteria and gram-positive bacteria to Herpes viruses and fungi. Although early infections caused by Herpes simplex virus, CMV, and *C. albicans* have decreased, the late risk period has become more prominent. Recent studies showed that CMV still causes a great deal of morbidity and mortality after BMT. However, compared to in the 1980s (140), molds now are frequent pathogens during GVHD (1). These changes in epidemiology result from multiple variables, such as supportive care strategies that prevent early infection, changes in the population of patients who undergo transplantation, and changes in methods to perform the transplant itself. It is likely that new conditioning regimens, immunosuppressive drugs, and stem cell sources will continue to impact the epidemiology of infection in upcoming years. Although host variables are critically important, microbial virulence factors and microbial ecology play a role in defining the pathogens of importance. The combinatorial impact of all of these factors has led to the emergence of non-*albicans Candida* species, *Aspergillus* species, and other molds as important causes of death after BMT. Given the poor outcomes of treating documented infections, current efforts are focused on developing effective strategies to prevent these infections, particularly aspergillosis, during GVHD. We can thus expect to see more changes in epidemiology in the future, however, given the ubiquitous nature of these organisms, fungi are likely to continue to play a prominent role in transplantation-related mortality for years to come.
REFERENCES


Assessment of the Risk for Invasive Fungal Infection Among Oncology Patients

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I. INTRODUCTION

The prevalence of patients living with cancer is rising (1) particularly as the spectrum and sophistication of available anticancer treatment strategies expands. Malignant disease and treatments thereof have profound negative effects upon the ability of the system of human host defenses to successfully engage and deflect a plethora of opportunistic pathogens met in the course of daily living. Superficial and invasive fungal infections have assumed a much more prominent role in the supportive care planning for patients undergoing intensive treatment for malignant disease.

The risk of invasive fungal infection in cancer patients varies with the underlying diagnosis, the cytotoxic or immunosuppressive regimen administered to treat the underlying cancer, and the circumstances of responsiveness of the underlying neoplasm for which the treatments are administered (2). A number of categories of predictors have been identified that have been associated with the risk of fungal infection-related morbidity. These include advanced age (3,4), advanced underlying disease (5,6), myelosuppression (7–9), immunosuppression (5–10), colonization of epithelial surfaces (6,11–14), environmental exposure (15–25), and physical damage to the integumental surfaces (6,26). Table 1 lists these variables. This chapter focuses upon the epidemiology of invasive fungal infection among cancer patient populations and reviews the relevant information pertaining to risk factors and predictors for invasive fungal infections related to cancer treatment.

II. EPIDEMIOLOGY OF INVASIVE FUNGAL INFECTION OBSERVED AT AUTOPSY

The prevalence of invasive fungal infection has been rising since the earliest descriptions among autopsied patients with cancer (8,27–30). A total of 8 (0.15%) invasive
Table 1 Principal Risk Factor Categories for Superficial and Invasive Fungal Infections in Patients Receiving Antineoplastic Therapy

<table>
<thead>
<tr>
<th>Category</th>
<th>Principal risk factors</th>
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<tr>
<td>Myelosuppression</td>
<td>- Neutropenia&lt;br&gt;  ▶ ANC &lt; 0.5 × 10^9/L&lt;br&gt;  ▶ Prolonged aplasia &gt; 10–14 days&lt;br&gt;  ▶ Monocytopenia&lt;br&gt;  ▶ AMC &lt; 0.2 × 10^9/L</td>
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<td>Immunosuppression</td>
<td>- Lymphopenia&lt;br&gt;  ▶ ALC &lt; 0.7 × 10^9/L&lt;br&gt;  ▶ CD4^+ T-lymphocytopenia&lt;br&gt;  ▶ ALC CD4^+ &lt; 0.2 × 10^9/L&lt;br&gt;  ▶ CD19^+ B-lymphocytopenia:&lt;br&gt;  ▶ ALC CD19^+ &lt; 0.1 × 10^9/L&lt;br&gt;  ▶ Therapies that augment immunosuppression&lt;br&gt;  ▶ Corticosteroid therapy: Prednisone dose equivalent of 20 mg/day over 30 days or at least 700 mg cumulative dose over 30 days&lt;br&gt;  ▶ Monoclonal antibody reagents such as rituxumab and alemtuzumab&lt;br&gt;  ▶ Purine analogs including fludarabine, cladribine, and deoxycoformycin</td>
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<tr>
<td>Colonization</td>
<td>- Multiple sites&lt;br&gt;  ▶ Colonization index &gt; 0.25&lt;br&gt;  ▶ Candida spp.&lt;br&gt;  ▶ Oropharynx, nasopharynx, rectum, urine&lt;br&gt;  ▶ C. tropicalis, C. glabrata, C. krusei&lt;br&gt;  ▶ Aspergillus spp.&lt;br&gt;  ▶ Nasopharynx&lt;br&gt;  ▶ Administration of broad-spectrum antibacterial therapy&lt;br&gt;  ▶ Antianaerobic activity&lt;br&gt;  ▶ Biliary excretion&lt;br&gt;  ▶ Administration of quinolone-based antibacterial prophylaxis&lt;br&gt;  ▶ Colonization by opportunistic yeasts&lt;br&gt;  ▶ Administration of azole-based antifungal prophylaxis&lt;br&gt;  ▶ Selection for colonization by C. glabrata, C. krusei</td>
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<td>Exposure</td>
<td>- Health care worker-related transmission&lt;br&gt;  ▶ Transmission of opportunistic yeast on HCW hands&lt;br&gt;  ▶ Fomites&lt;br&gt;  ▶ Transmission of opportunistic yeast on equipment&lt;br&gt;  ▶ Environment&lt;br&gt;  ▶ Institutional demolition, construction, and maintenance&lt;br&gt;  ▶ High conidial counts per unit time&lt;br&gt;  ▶ Duration of stay in risk environments</td>
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*(Continued)*
mycoses were noted among 5530 autopsies performed between 1919 and 1936, 3 (0.1%) in 2879 performed between 1937 and 1941, 9 (0.26%), and 50 (1.2%) in 4167 performed between 1948 and 1955 (27). The prevalence of invasive mycoses at autopsy continued to rise from 1948 onwards, particularly among patients with leukemia and lymphoma in whom the rate increased from 5% in 1947–1948 to 20% in 1954–1955 (Fig. 1). Co-incident with this increase was the introduction of several new antineoplastic agents effective in these diseases including nitrogen mustard in 1945, aminopterin in 1948, prednisone in 1949, and mercaptopurine in 1952. Moreover, the introduction of penicillin in 1941, streptomycin in 1944, and the tetracyclines in 1948 provided physicians prescribing these agents with the antibacterial tools to manage the consequent, otherwise, fatal febrile neutropenic episodes.

The combination of the new antineoplastic and antibacterial agents arguably permitted leukemia and lymphoma patients to survive sufficiently long enough to become colonized and infected by opportunistic fungi. Figure 2 illustrates the continued rise in prevalence of invasive mycoses at autopsy as reported between 1948 and 1980 (8,27,29). The reduction in the incidence of invasive fungal infection noted in the international autopsy study of Bodey et al. (28) in Figure 2 corresponds

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**Table 1** Principal Risk Factor Categories for Superficial and Invasive Fungal Infections in Patients Receiving Antineoplastic Therapy (Continued)

<table>
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<tr>
<th>Category</th>
<th>Principal risk factors</th>
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<td>Transport routes</td>
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<td>High-efficiency particulate air-handling protective environments</td>
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<td>Integumental damage</td>
<td>Cyotoxic therapy</td>
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<td>▶ Local irradiation vs. systemic chemotherapy</td>
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<td>▶ Class of cytotoxic agent</td>
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<td>Antimetabolites</td>
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<td>Anthracyclines / anthraquinone</td>
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<td>Topoisomerase inhibitors</td>
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<td></td>
<td>Alkylating agents</td>
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<td></td>
<td>Anti-CD33 monoclonal antibody reagents</td>
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<tr>
<td>Mucositis</td>
<td>Infection risk and the mucositis score</td>
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<td></td>
<td>Infection risk and permeability changes</td>
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<td>Indwelling venous access devices</td>
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<tr>
<td></td>
<td>▶ Interaction with gut colonization</td>
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<tr>
<td>Urinary catheters</td>
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<tr>
<td></td>
<td>▶ Interaction with antimicrobial use and colonization</td>
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<tr>
<td>Ventilation assistance devices</td>
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<td></td>
<td>▶ Interaction with antimicrobial use and colonization</td>
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<tr>
<td>Surgical wounds</td>
<td></td>
</tr>
<tr>
<td></td>
<td>▶ Interaction with antimicrobial use and colonization</td>
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*Abbreviations:* ANC, absolute neutrophil count; AMC, absolute monocytes count; ALC, absolute lymphocyte count; ALC CD4+, absolute CD4+ T-lymphocyte count; ALC CD19+, absolute CD19+ B-lymphocyte count.
to the time during which the empirical use of amphotericin B deoxycholate for suspected but unproven fungal infection became a standard of practice (31–33).

In more recent analysis of data from 4096 patients autopsied between 1980 and 1988 from centers in Europe, Canada, and Japan demonstrated that the prevalence of invasive mycoses was highest among acute leukemia patients (25%), followed by lymphoma (12%), and solid tumor patients (5%), respectively (28). The proportionate frequency of invasive fungal infection by underlying diagnosis was 5:2.5:1 for leukemia, lymphoma, and solid tumor, respectively (28). A single center study from Germany reporting on 8124 patients autopsied between 1978 and 1992 demonstrated the highest prevalence of invasive mycoses among patients with acute leukemia (55 of 176, 31.3%), followed by patients with the human immunodeficiency virus-mediated acquired immunodeficiency syndrome (AIDS, 60 of 314, 19.1%), organ
transplantation (9 of 61, 14.8%), lymphoma (57 of 442, 12.9%), and solid tumors (37 of 2356, 1.6%) (30). In that study, a pattern of increasing prevalence over time was also observed from 2.2% in 1978–1982, 3.2% in 1983–1987, to 5.1% in 1988–1992 (30) similar to that observed previously (27–29).

Candida spp. has been the pathogen most commonly associated with invasive fungal infection found postmortem in cancer patients, accounting for 66% (range 42–78%) of cases in a large international autopsy review (28). Aspergillus spp. accounted for 34% (range 22–55%). Cryptococcosis was identified only infrequently (2%, range 0–4%). Among 57 autopsied patients with acute leukemia, invasive fungal infection was demonstrable in 32 (56%). Candida spp. accounted for 17 (29.8%), Aspergillus spp. for 7 (12.3%), Candida spp. and Aspergillus spp. for 7 (12.3%), and Mucor spp. for 1 (1.7%) (8). In this study, molds accounted for over one-quarter (26.3%) of the infections.

As in the older series, the most common pathogens observed at autopsy in the German series (30) were Candida spp. and Aspergillus spp.; however, the proportion of Candida spp. in these autopsies decreased from 77.3% to 24.6% and molds increased from 22.7% to 66.7% from 1978 to 1992. This stands in contrast to the results of the National Nosocomial Infections Surveillance System (NNISS) study in the United States, wherein Candida spp. accounted for 78% of nosocomial fungal infections and Aspergillus spp. for only 1.3% (34). While it is clear that there is a limited ability to reliably establish premortem diagnoses of invasive mold infections (35), it seems likely that the declining prevalence of Candida spp. at autopsy data may be a function of increased awareness by physicians of the possibility of invasive fungal infection in high-risk groups, and the more aggressive use of effective antifungal therapy (36–39).

### III. SUPERFICIAL AND INVASIVE CANDIDIASIS

Oropharyngeal and esophageal candidiasis are relatively common among patients receiving immunosuppressive or myelosuppressive anticancer therapy (40,41). Superficial infection with opportunistic yeasts involves tissues of the skin, genitourinary tract, and gastrointestinal tract predominantly, the oropharynx, esophagus, and bowel. Predisposing factors include protracted courses of antibacterial therapy, irradiation to the mediastinum and area of the head and neck, and immunosuppression due to the underlying cancer and its treatment. Corticosteroids have long been recognized as a factor predisposing patients to oropharyngeal candidiasis, OPC (7).

OPC is an important problem affecting 17% to 53% of patients undergoing involved-field irradiation for head and neck cancer (42–47). One of the major risk factors appears to be compromised salivary gland function, secondary to the destruction of glandular tissue, as a function of the local cytotoxic irradiation (42–44,46,48). The serous acini of the parotids are very sensitive to the cytotoxic effects of irradiation (49–54). Moreover, cytotoxic effects of irradiation on the oral mucosal epithelium may reduce the anti-candidal activity of mucosal membrane-associated carbohydrate moieties (55). While oropharyngeal colonization with Candida spp. is present in almost three-quarters of patients beginning a course of irradiation therapy, more than one in four patients undergoing a course of irradiation will develop signs and symptoms of OPC (47). The incidence of OPC has been based upon the recognition of signs and symptoms that include oral pain, and/or burning associated with oral plaques, or pseudomembranes from which budding yeasts may be recognized in 10% potassium hydroxide microscopical preparations, and/or grown in microbiological
culture (45,47). It is very difficult to ascribe pathogenetic significance to a pre-existing colonizing yeast isolate such as *C. albicans* in the presence of oropharyngeal inflammatory change with pseudomembrane formation that can be due to either cytotoxic therapy-related tissue damage or to the microorganism. For this reason, many clinicians opt to treat the microorganism with agents such as fluconazole.

Amifostine, an agent known to concentrate in the salivary glands (49), has been used as a cytoprotectant to reduce the cytotoxic effects of the irradiation (53,56–59). In one non-randomized, no treatment-controlled study, amifostine reduced the incidence of OPC by 48% (OR 0.32, 95% CI 0.09–1.06) and xerostomia by 75% (OR 0.05, 95% CI 0.04–0.063); however, the incidence of grade II to III mucositis was unaffected (OR 0.21, 95% CI 0.03–1.86) (53). Investigators from Duke University reported similar observations in a randomized, untreated-controlled trial in which 303 subjects were allocated to receive amifostine prior to each irradiation treatment for squamous cell head and neck cancer (58). Grade II or more acute xerostomia was reduced by 35% (OR 0.29, 95% CI 0.18–0.46), but mucositis was unaffected (58). Although OPC was not an end-point of this study, it seems possible that the magnitude of the amifostine-related xerostomia treatment effect could have permitted the recognition of a protective effect in this outcome as well. These studies have underscored the importance of cytotoxic therapy-related xerostomia in the pathogenesis of OPC in cancer patients (60).

OPC can be a marker for esophageal candidiasis (61), which in turn may increase the risk for invasive bloodstream infection (62,63). OPC has been studied among patients with HIV/AIDS (64–66); however, the relationship between OPC and esophageal candidiasis has not been as extensively analyzed in cancer patients other than those with head and neck carcinoma (44–47). Approximately 90% of HIV/AIDS patients will develop OPC at some point in the course of the illness; however, only approximately 10% will develop esophageal candidiasis (67). In one recent study from Crete, 21 of 22 cancer patients with OPC had endoscopic evidence of esophageal candidiasis and in 14 of 22 (64%), histological evidence of mucosal invasion was also present (61). This relationship may be stronger than heretofore thought.

The NNISS reported and increase in nosocomial invasive fungal infections from 2.0/1000 hospital discharges in 1980 to 3.8/1000 hospital discharges in 1990 (34). The American Society for Microbiology identified the threat posed by antimicrobial resistance as sufficiently important to recommend the development of on-going surveillance programs to detect emerging resistance, to monitor resistance rates, and to guide infection control and formulary intervention programs (68). Surveillance programs reporting on 5995 isolates from candidemia patients between 1992 and 2001 have provided a rank order of species distribution: *C. albicans* (45–58%), *C. glabrata* (12–24%), *C. parapsilosis* (7–21%), *C. tropicalis* (10–12%), *C. krusei* (0–4%), other non- *albicans* Candida spp. (1–4%) (69–83). There has been an increase in the proportion of candidemia due to non- *albicans* Candida spp., in particular *C. glabrata* that now ranks second in frequency at approximately 20% overall in the United States (71,75,77–80). Of note, centers in South America have reported a predominance of *C. parapsilosis* over *C. glabrata* (Fig. 3) (74). Wingard reported a rise in the prevalence of invasive infection in cancer patients due to non- *albicans* Candida spp., particularly *C. glabrata* (from 6.4% to 14.5%), and *C. krusei* (from 1.6% to 13%), since the introduction of fluconazole in the early 1990s (84). This observation has been made by others (85,86) and has important implications for the treatment of suspected invasive fungal infection due to *Candida* spp.
The incidence of *C. glabrata* bloodstream infection appears to be higher among older patients (70,80) who are more likely to have cancer.

Invasive fungal infection is observed more often among patients with hematological malignancies such as leukemia or lymphoma than among patients with solid tissue malignancies; however, the Invasive Fungal Infection Group (IFIG), of the European Organization for Research and Treatment of Cancer (EORTC), demonstrated species differences among candidemic patients according to whether the diagnosis was a hematological malignancy or a solid tissue malignancy (Fig. 4). Previous studies have demonstrated the gastrointestinal tract including the esophagus, stomach, and bowel as the likely sources for invasive candidiasis (30,87). *Candida albicans* accounted for 70% and 36% of the candidemias among patients with solid tissue and hematological malignancies, respectively (4). In contrast, *C. glabrata* and *C. tropicalis* accounted for only 4% and 7% of solid tumor patients as compared

![Figure 3](image3.png)  
*Figure 3* Percentage of Candidal isolates by species from 22 centers in the United States, six centers in Canada, and six centers in South America.

![Figure 4](image4.png)  
*Figure 4* Percentage of Candidal isolates by species and underlying diagnosis.
to 13% and 14% of hematological malignancy patients, respectively (4). Others have made similar observations (88).

The major risk factors that have been identified among cancer patients for fungemia include neutropenia, hematopoietic stem cell transplantation, corticosteroid therapy, broad-spectrum antibacterial therapy (12), receipt of mucosal toxic chemotherapy regimens containing high-dose cytarabine (26) or etoposide (89,90), and indwelling venous access devices (85,91). Non-albicans Candida spp. have predominated among candidemic patients with hematological malignancies in Europe, whereas C. albicans has predominated among patients with solid tissue malignancies (4).

One of the non-albicans Candida spp., C. parapsilosis, has a characteristic epidemiology. The incidence of bloodstream infection because of this species has increased in the United States, Europe, Latin America, and South America (74,92–94). This is in exogenously acquired opportunistic yeast that has the propensity to colonize indwelling venous access devices, particularly in cancer patients (85,95–97), and grow in the presence of high concentrations of glucose (98). The lower attributable mortality of C. parapsilosis compared to C. albicans has been linked to its overall lower virulence (4,84,92,98,99) and is susceptibility to fluconazole.

Candida krusei has emerged as an important pathogen in neutropenic leukemia patients largely because of its intrinsic resistance to fluconazole (85,100,101). Compared to patients with C. albicans bloodstream infections, patients with C. krusei are more likely to have an underlying hematological malignancy, severe neutropenia (ANC < 0.5 × 10^9/L), had a hematopoietic stem cell transplant, and received fluconazole prophylaxis (85,102). In one study, only 22% of patients with C. krusei fungemia compared to 79% of patients with C. albicans fungemia treated with fluconazole responded (102); however, these observations were not controlled for the presence or severity of neutropenia. Others have demonstrated a reduced susceptibility of C. krusei to polyenes, thus, accounting for the observation of breakthrough fungemia due to this species among patients receiving amphotericin B deoxycholate (69,102). Invasive C. glabrata infections have been associated with higher mortality rates in cancer patients (4). It has been argued that invasive C. tropicalis infections have a more virulent course because of more prolonged fungemia (88), higher APACHE II physiological scores (85), and longer critical care unit stays (84,85,103).

Fluconazole has been recommended as the agent of choice for the prevention of susceptible Candida spp. infections in hematopoietic stem cell transplant recipients (104,105). Others have shown a prophylactic treatment effect among other groups including acute myeloid leukemia patients undergoing primary or salvage remission-induction therapy (106) and those with prolonged (> 14 days) severe neutropenia (absolute neutrophils < 0.5 × 10^9/L) (107). Such patients, however, are at risk of invasive bloodstream infections due to opportunistic fungi less susceptible to azole-based antifungal prophylaxis (107,108); that is, the problem of breakthrough candidemia.

Breakthrough candidemia has been defined as candidemia observed during the administration of systemic antifungal therapy for prophylaxis, empirical treatment of prolonged fever during persistent severe neutropenia, or for treatment of possible, probable, or proven invasive fungal infection for more than 3 days prior to the index blood culture (109–111). The occurrence of candidemia among patients receiving systemic antifungal therapy with amphotericin B deoxycholate (32,112) or fluconazole (112) was previously unusual. The incidence appears to be on the rise (113). Blumberg and Reboli (69) reported 11 neutropenic cancer patients with candidemia among whom five (45%; Candida albicans, two, and C. krusei, three) occurred during
therapy with amphotericin B deoxycholate at doses ≥0.6 mg/kg/day. The presence of a hematological malignancy (109,110,113), mucositis (114), inadequate antifungal agent serum levels (115), indwelling central venous access devices (114,116), steroid therapy (more than 20 mg of prednisone equivalent daily for more than 30 days or a cumulative dose of 700 mg within 30 days) (109,111), broad-spectrum antibacterial therapy (109,111), prophylaxis with oral fluoroquinolones (114), and prolonged neutropenia (111,114) have been reported as risk factors for breakthrough candidemia. Whereas, the aetiology of de novo candidemia has been predominantly \textit{C. albicans}, \textit{C. tropicalis}, and \textit{C. parapsilosis}, the isolates in breakthrough candidemia have been \textit{C. albicans}, \textit{C. glabrata}, and \textit{C. krusei}, particularly among azole recipients (109,110,114). Other non-\textit{Candida} spp. also have been reported including \textit{Trichosporon} spp. and \textit{Fusarium} spp. (114). A multivariate analysis of the risk factors for breakthrough candidemia identified the presence of neutropenia at the time of the index blood culture (OR 5.35, 95% CI 2.01–14.23), being in a critical care unit at the time of the index blood culture (OR 2.60, 95% CI 1.28–5.29), duration of neutropenia before the index blood culture (OR 1.02, 95% CI 1.00–1.04), and previous corticosteroid therapy (OR 2.50, 95% CI 1.23–5.15) as independent predictors (109).

IV. INVASIVE FILAMENTOUS FUNGAL INFECTION

The first case of disseminated aspergillosis was described in 1953 in a 45-year old window cleaner treated with multiple antibacterial agents, including chloramphenicol, who subsequently developed agranulocytosis (117). Filamentous fungi such as \textit{Aspergillus} spp. are ubiquitous in the environment and have been isolated from the air, dust, furniture, soil of potted plants, ground coffee, spices, powdered milk, water condensation from refrigerators, and from moisture around bathtubs and sinks (118–122).

Acquisition is primarily through the inhalation of conidia; accordingly, it is not surprising that the commonest sites of infection involve the sinopulmonary tree (27). The small particle size (2.5–3.5 μm) of \textit{Aspergillus} spp. conidia is conducive to transportation through the air currents to the distal Airways in the lung (123). The respiratory mucosal surfaces with the alveolar macrophages constitute the first line of host defense against the inhaled conidia, and following germination into the hyphal phase, the circulating neutrophil provides the most important line of defense against dissemination from the primary pulmonary or paranasal site of inoculation (124–126) Angioinvasion by hyphae is the mechanism by which hematogenous dissemination occurs to distant sites such as the brain, skin, kidneys, or liver.

The accurate identification of the offending fungus from tissue is problematic. Fungi recognized in histological examination are not isolated in microbiological cultures in 40–60% of cases (127–130). It is nearly impossible to differentiate microscopically the hyphae of \textit{Aspergillus} spp. from those of \textit{Scedosporium} spp., \textit{Fusarium} spp., or \textit{Penicillium} spp. (131). In the absence of culture-based identification, investigators have classified hyphae that are small (3–6 μm wide), uniform, dichotomously branching, and regularly septate as \textit{Aspergillus} spp. and hyphae that are broad (5–25 μm wide), thin-walled, irregularly shaped, and infrequently septate as \textit{Zygomycetes} (27,30,132). Accordingly, the true prevalence and incidence of specific pathogens may be overestimated. In rank order, the majority of filamentous fungal infections are caused by \textit{Aspergillus} spp. [including in rank order in invasive aspergillosis \textit{A. fumigatus} (67%), \textit{A. flavus} (16%), \textit{A. niger} (5%), \textit{A. terreus} (3%), and \textit{A. nidulans}...
Fusarium spp. (including \textit{F. solani}, \textit{F. oxysporum}, and \textit{F. moniliforme}), \textit{Scedosporium} spp. (including \textit{S. prolificans} and \textit{S. apiospermum}, the asexual phase of \textit{Pseudallescheria boydii}), the Zygomycetes (including \textit{Rhizopus} spp., \textit{Mucor} spp., \textit{Rhizomucor} spp., \textit{Absidia} spp., and \textit{Cunninghamella bertholletiae}), and the dematiaceous molds (including \textit{Alternaria} spp., \textit{Bipolaris} spp., \textit{Curvularia} spp., and \textit{Wangiella} spp.) (135). A simplified approach to classification of clinically important molds has been described by Segal et al. (136). Molds may be categorized as \textit{Aspergillus} spp. vs. non-\textit{Aspergillus} spp. wherein \textit{Aspergillus} spp. may be subdivided as \textit{A. fumigatus} or non-\textit{fumigatus} \textit{Aspergillus} spp., and non-\textit{Aspergillus} spp. may be subdivided into \textit{Fusarium} spp., the Zygomycetes, \textit{Scedosporium} spp., and the dematiaceous fungi.

The prevalence of invasive mold infection observed at autopsy is increasing (30,137). In a study of 8124 autopsies in Germany, there was an increase in the prevalence of invasive molds from 0.5% in 1978–1982 to 3.4% in 1988–1992, a proportional increase of 580% (30). Invasive aspergillosis is much more often associated with neoplastic (47.1–69.8%) than with non-neoplastic conditions such as solid organ transplant (4.2–10.3%), AIDS (9.2–31.1%), or other immunodeficiency states (10.7–17.6%) (30,138,139). Among neoplastic diseases, the hematological malignancies [acute leukemia (48.5%), chronic leukemia (19.1%), lymphoma (14.1%), myelodysplasia (7%), multiple myeloma (3.8%), and fanconi’s syndrome (2%)] have accounted for 96.1% of cases compared to solid tumors (3.8%) (139). The incidence in patients with acute myeloid leukemia is 2-fold higher than patients with acute lymphoblastic leukemia (139). In addition, the incidence of invasive aspergillosis among allogeneic hematopoietic stem cell transplant recipients has increased between 1990 and 1998 from approximately 5% to approximately 12% (5,140). Of note, there was an increase among autologous hematopoietic stem cell transplant recipients over this period from 1.1% to 5.3%, a phenomenon conceivably linked to low doses of CD34$^+$ stem cells (141).

The incidence of invasive mold infection, particularly with aspergillosis, is associated with significant ramifications with regard to health care resources. Fraser predicted a 2-fold increase in the number of aspergillosis-related hospitalizations between 1970 and 1976 (137). In the 20 year period since that report, the number of hospitalizations for this diagnosis have increased nearly 8-fold (142). A report based upon the National Hospital Discharge Survey from 1980 to 1994 demonstrated an annual increase of 5.7% in the incidence of hospitalization for fungal disease in the United States (143). Patients with cancer or leukemia who develop secondary aspergillosis utilized 3.7 times the number of hospital days, incurred 6.7 times the cost, and was associated with 3.3 times the mortality rate than patients with similar diagnoses, but without aspergillosis (142).

These observations provide ample rationale for the development of effective strategies of prevention and treatment.

The understanding of the pathogenesis of aspergillosis has provided some insight into the factors that predispose susceptible patients to this infection. Outbreaks of aspergillosis have been linked to high concentrations of conidia in the environment where patients with hematological malignancies are under treatment (5,16,19,20,22,144–149). The relationship between increased ambient conidial concentrations and invasive mycoses has been difficult to demonstrate (23,150–153). However, the institution of infection control measures that reduce patient exposure to airborne conidia have been associated with reductions in the incidence of invasive aspergillosis (19,20,22,25,149,154). Recent studies have implicated stairwells as a
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means of conveyance of airborne conidia (24). A recent study from the International Bone Marrow Transplant Registry was able to demonstrate a treatment effect high-efficiency particulate air filtration (HEPA) with or without laminar airflow (LAF) on reducing transplant-related mortality and increasing overall survival (155). The probability of fungal pneumonia among patients undergoing allogeneic unrelated or mismatched donor hematopoietic stem cell transplant was 6% among those managed in HEPA/LAF facilities as compared to 23% among patients managed in conventional isolation, thus, a 74% risk reduction (155). Others have also been able to demonstrate a protective effect of HEPA + LAF (156). In a retrospective cohort study, Hahn et al. (149) reported the development of invasive aspergillosis due to A. flavus in 9 of 35 (26%) of patients (of whom 7 of 9, 78%, died) managed in a non-HEPA/LAF unit as compared to 1 of 20 patients (5%, OR 6.58, 95% CI 0.77–56.41) managed in a HEPA/LAF unit. The non-HEPA/LAF unit was associated with high Aspergillus spp. conidia counts, >150 colony-forming units, CFU/m³ compared to < 4 CFU/m³ in the HEPA/LAF unit (149). These observations have formed the basis of recommendations by the American Society of Blood and Marrow Transplantation, the Infectious Diseases Society of America, and the Centers for Disease Control and Prevention to use HEPA/LAF for the management of hematopoietic stem cell transplant in patients at highest risk for aspergillosis (104,105). Evidence suggests that other patients such as those undergoing intensive cytotoxic therapy for acute myeloid leukemia may also benefit from HEPA-based strategies (149).

In addition, increases in ambient concentrations of conidia may be generated from construction and demolition activities outside the hospital environment (157). Conidia may enter the hospital, particularly if the ambient air pressure is negative relative to the outside and be transported on air currents associated with stairwells and elevator shafts (24). Measures such as the use of N95 masks, wet buffing, and closing stairwells can reduce the risk to patients of encountering airborne conidia (24).

V. COLONIZATION

Colonization of the intestinal mucosal surfaces is thought to be the initial step in the pathogenesis of invasive infections due to opportunistic yeasts (26,87,158,159). Surveillance cultures have been used to examine colonization profiles in the study of the epidemiology of infection and the impact of infection prevention strategies in high-risk patient population (160). Although surveillance cultures have had limited value in predicting the etiology of invasive infection (161), low yeast colonization indices (162) of less than 0.25 have a high negative predictive value for invasive fungal infection (13). The risk of invasive infection increases with the number of anatomic sites colonized (162,163) and with the particular fungal species (11,163,164). Serial surveillance cultures of the nasopharynx, oropharynx, and rectum may be useful in high-risk cancer patients undergoing intensive cytotoxic therapy for acute myeloid leukemia or hematopoietic stem cell transplant receiving azole-based antifungal prophylaxis (106) to monitor the emergence of azole-resistant yeasts (165). Such a program should only be engaged after appropriate communication and agreement with the clinical microbiology laboratory (160).

Gut decontamination using oral absorbable or non-absorbable antimicrobial agents has been studied as a strategy to prevent invasive infection among neutropenic
cancer patients. Manipulation of the gut microflora through use of antibacterial agents, particularly those with activity against the normal anaerobic “colonization resistance” microflora (166), has increased the likelihood of colonization of the gut by resistant microorganisms, particularly with the use of antibacterial agents with significant antianaerobic activity (167,168). A survey of antimicrobial use in a tertiary care hospital found that 58% of patients beginning a course of antibacterial therapy received agents that were inappropriate, and in 59% of the patients in this group the unnecessary agents included those with significant antianaerobic activity (169). Febrile neutropenic cancer patients who receive empirical antibacterial regimens that suppresses both the normal aerobic and anaerobic intestinal microflora have a significantly higher risk of colonization by opportunistic yeasts and invasive fungal infection (OR 3.56, 95% CI 1.24–10.23) (170). A similar quantitative study from the MD Anderson Cancer Center correlated yeast colonization with use of antibacterial regimens with antianaerobic activity, such as ticarcillin-clavulanate, or high biliary excretion rates, such as ceftriaxone (171). Augmentation of fluoroquinolone-based antibacterial prophylaxis in neutropaenic cancer patients with the administration of rifampin resulted in increased yeast colonization of the gut (172). Another prophylaxis study from Essen, Germany, sought to reduce the incidence of acute graft-vs.-host disease (GVHD) among allogeneic hematopoietic stem cell transplant recipients through suppression of the anaerobic gut microflora by the co-administration of metronidazole with prophylactic ciprofloxacin plus fluconazole (173). In comparison to the control patients receiving ciprofloxacin plus fluconazole, the study group had a 50% reduction in grades II–IV GVHD, particularly involving the liver or intestine. However, there was also a 58% increase in intestinal colonization by yeasts in the study group (odds ratio 1.94, 95% CI 1.46–2.58) and, despite the relatively limited sample size, there was an associated increase in the incidence of candidemia from 1.5% to 4.4% (OR 3.1, 95% CI 0.3–30.5) also noted (174).

The administration of multiple antibiotics has been long recognized as being associated from a 1.7- to 25.1- fold risk for colonization and infection by opportunistic fungi (91,175,176). Many agents used for the empirical management of fever in the neutropenic patient have significant suppressive effects upon the normal enteric gut microflora. Such agents, including the carbapenems (imipenem/cilastatin and meropenem), cefipime, and piperacillin/tazobactam or ticarcillin/clavulanate have been associated with suppression of the anaerobic intestinal microflora and increase in colonization by opportunistic yeasts. These examples illustrate that the choice of antibacterial agents has a significant impact upon the risk for fungal colonization and, perhaps, the risk of invasive fungal infection in neutropaenic cancer patients.

VI. MYELOSUPPRESSION AND IMMUNOSUPPRESSION

The potential anticancer therapeutic benefit of cytotoxic therapy dose-intensification, defined by the amount of drug administered per unit time (177), has been limited by myelotoxicity primarily affecting the absolute neutrophil count and the platelet count. The inverse relationship between the absolute neutrophil count and the risk of pyogenic bacterial infection has been well established (178,179). Improvements in the management of the febrile neutropenic patient (33) and the availability of platelet transfusions have ameliorated some of the dose-limiting problems. The addition of
hematopoietic growth factors (granulocyte colony stimulating factor or granulocyte/macrophage colony stimulating factor) have also been important in reducing the duration of cytotoxic therapy-induced neutropenia [relative risk (RR), 0.64, 95% CI 0.55–0.75], the incidence of febrile neutropenic episodes (RR 0.74, 95% CI 0.62–0.89), and documented infection (RR 0.74, 95% CI 0.64–0.85) (180); however, there has been no observable treatment effect with regards to the prescription of antibacterial therapy (RR 0.82, 95% CI 0.57–1.18), infection-related mortality (RR 2.07, 95% CI 0.81–5.34), tumor response (RR 1.06, 95% CI 0.96–1.16), freedom from treatment failure (hazard ratio 1.22, 95% CI 0.83–1.80), or overall survival (hazard ratio 0.98, 95% CI 0.81–1.18) (180).

The use of peripheral blood as the source of stem cells for hematopoietic reconstitution has been associated with earlier neutrophil and platelet engraftment (181). Moreover, the dose of CD34+ stem cells of $3 \times 10^6$/kg or more recipient body weight has been associated with faster engraftment of neutrophils, lymphocytes, monocytes, and platelets (141) and this has been associated with a reduced risk of invasive fungal infection from 26.3% to 12.2% (a 54% reduction, hazard ratio 0.41, 95% CI 0.21–0.79, $P = 0.008$) (141).

The absolute monocyte count (AMC) has also been studied as a useful predictor of infection in cancer patients receiving cytotoxic therapy. Previous studies have examined the relationship of monocytopenia (AMC < 0.2 $\times 10^9$/L) and the risk of bacterial infection, predominantly in pediatric patient populations (182–188). One study in patients with aplastic anemia addressed the correlation between monocytopenia and invasive fungal infection (189); however, the analysis was confounded by the effects of concomitant neutropenia. Among cancer patients receiving myelosuppressive cytotoxic therapy resulting in pancytopenia, the ability to discriminate the relative contributions of monocytopenia and neutropenia to infection risk is very difficult. This question is better addressed among patients with normal absolute neutrophil counts and monocytopenia. More recently, Storek (190) reported an inverse relationship between persistent B-lymphocytopenia or monocytopenia and the risk of fungal infection following engraftment among allogeneic hematopoietic stem cell transplant recipients that appeared to be independent of the influence of neutropenia. These observations are consistent with the known role of mononuclear phagocytes in host defense against inhaled conidia and with the observed therapeutic effects of hematopoietic growth factors that affect the mononuclear phagocyte in patients with invasive fungal infection (191–194). Accordingly, monocytopenia in the absence of significant neutropenia (ANC < 0.5 $\times 10^9$/L) may be more important as a risk factor for invasive infection than heretofore thought.

The inverse relationship between the circulating CD4+ T-lymphocyte count and opportunistic infections, such as those due to Candida spp. and Aspergillus spp. in HIV/AIDS patients has been well established (10,66,195,196). Little attention has been paid to cancer chemotherapy-induced reduction of the absolute lymphocyte count (ALC) and its related subsets with regard to infection risk (198). Reports published in the early 1980s drew attention to the relative lymphopenia produced in women receiving adjuvant chemotherapy with chlorambucil, methotrexate, and fluorouracil for breast cancer (198). Moreover, the late 1980s and early 1990s brought reports of lymphoma patients receiving intensive multiagent chemotherapy, and breast cancer patients receiving adjuvant chemotherapy following breast conserving lumpectomy and irradiation developing pulmonary pneumocystosis associated with profound CD4+ lymphopenia (199,200). Kontoyiannis and colleagues (201) at the MD Anderson Cancer Center noted that the majority (61%) of cancer patients...
with invasive cryptococcosis were significantly lymphopenic with absolute lymphocyte counts of less than $0.5 \times 10^9/L$ whereas only 16% were neutropenic (absolute neutrophil count $< 0.5 \times 10^9/L$).

The recognition of early lymphopenia may be helpful in predicting those recipients of cytotoxic therapy who are at highest risk of a febrile neutropenic episode. Blay and colleagues (202) observed a correlation between an ALC of less than $0.7 \times 10^9/L$ on day 5 of cytotoxic therapy, approximately 4 days before the ANC had fallen, and subsequent infection (OR 7.17, 95% CI 2.52–20.35). Moreover, the administration of high-dose chemotherapy (defined by the receipt of at least one of the following regimens: doxorubicin, $> 90 \text{mg/m}^2$; cisplatin, $> 100 \text{mg/m}^2$; cyclophosphamide, $> 1000 \text{mg/m}^2$; ifosfamide, $> 9000 \text{mg/m}^2$; etoposide, $> 500 \text{mg/m}^2$; or cytarabine, $> 1000 \text{mg/m}^2$) also proved to be a useful predictor independent of other variables including patient age, performance status, tumour extension, bone marrow involvement, number of previous chemotherapy sites, and corticosteroid therapy. The odds ratio for a febrile neutropenic episode for patients with both predictors, high-dose chemotherapy and a day 5 ALC of $< 0.7 \times 10^9/L$, was 48.4 (95% CI 10.7–219.7) (202). The day 1 ALC of $< 0.7 \times 10^9/L$ has also been of value as a predictor of severe thrombocytopenia (203) and severe anaemia requiring red cell transfusion (204).

While it seems clear that suppression of the circulating absolute neutrophil count, the absolute monocyte count, and the absolute lymphocyte count correlate with the risk for infection in general, it is less clear as to how this influences the risk for fungal infection in cancer patient populations. Among 35,252 HIV-infected subjects contained in a national HIV surveillance database, the incidence of invasive aspergillosis was 3.5 cases (95% CI 3.0–4.0) per 1000 patient-years. However, as the CD4$^+$ T-lymphocyte count fell, the incidence increased significantly from 1.0 case/1000 patient-years (95% CI 0.6–1.4) associated with a CD4$^+$ ALC of $\geq 0.2 \times 10^9/L$, to 1.0 (95% CI 0.2–1.7) with a CD4$^+$ ALC of 0.1–0.199 $\times 10^9/L$, 5.1 (95% CI 2.8–7.3) with a CD4$^+$ ALC of 0.05–0.099 $\times 10^9/L$, and 10.2 (95% CI 8.0–12.2) with a CD4$^+$ ALC of 0–0.049 $\times 10^9/L$ (10).

Irinotecan, a topoisomerase I inhibitor is in wide use alone and in combination for metastatic colorectal malignancy. The major toxicities have been associated with neutropenia and diarrhea. More recently, significant reductions in circulating CD19$^+$ B-lymphocytes, CD4$^+$ T-lymphocytes and monocytes have been reported (205). Till date, no unexpectedly high incidence of superficial or invasive fungal infection has been reported with the use of this agent.

Purine analog therapy has increased in the treatment of lymphoreticular malignancies (206–209), acute myeloid leukemia (210) and in conditioning therapy of non-myeloablative allogeneic hematopoietic stem cell transplantation (211). These agents include fludarabine, cladribine (2-chlorodeoxyadenosine or CaD), and pentostatin (2$^\text{d}$-deoxyxoforomycin or DCF). The infectious complications associated with the use of these agents have been reviewed extensively elsewhere (207,208). There are predictable immunological sequelae of the use of this class of agents including a reduction in the total absolute lymphocyte counts, prolonged suppression of circulating CD19$^+$ and CD20$^+$ B-lymphocyte counts and CD4$^+$ T-lymphocyte counts, less prolonged suppression of CD8$^+$ T-lymphocytes, transient monocytopenia and CD16$^+$ NK lymphocyte counts, and variable effects upon immunoglobulin levels. As a consequence of these effects, it is not surprising that since the early 1990s an increased incidence of opportunistic infections due to *Listeria monocytogenes*, *Herpes zoster*, and fungi including *Pneumocystis jiroveci* (formerly carinii), *Candida*
spp., *Aspergillus* spp., and *Cryptococcus neoformans* reminiscent of those observed in the acquired immunodeficiency syndrome (212,213) has been reported among CLL patients receiving these agents (207,214,215).

CD4⁺ T-cell numbers fall to levels similar to those observed in advanced HIV infection (207). CD4⁺ and CD8⁺ T-cell counts among recipients of fludarabine administered for untreated chronic lymphocytic leukemia (CLL) decreased over three courses of treatment from medians of $1.562 \times 10^9/L$ and $0.510 \times 10^9/L$, respectively, to $0.172 \times 10^9/L$ and $0.138 \times 10^9/L$, an 89% and 73% reduction, respectively (216). Despite this, no association between CD4⁺ T-cell count at the end of fludarabine therapy and infection was observed (216). The CD4⁺ T-lymphopenic effects of fludarabine among CLL patients may last a year or more (217). In a recent randomized study of 518 previously untreated CLL patients, the overall infection risk among fludarabine monotherapy recipients was higher than that among chlorambucil monotherapy recipients (9% vs. 16%, OR 1.91, 95% CI 0.99–3.69); however, recipients of the combination of fludarabine and alkylating agent had an increased risk than that of fludarabine monotherapy (16% vs. 28%, OR 2.05, 95% CI 1.17–3.60) (218). Infections due to *P. jiroveci* were seen during that study in only 0.9% of 329 fludarabine recipients (219). No cases of invasive aspergillosis infection were observed despite expectations otherwise (217,220,221) The incidence of major candidal infections (defined by the need for hospitalization for treatment) was 0%, 2%, and 5% for the chlorambucil, fludarabine, and chlorambucil plus fludarabine groups, respectively ($P = 0.01$) (219). While the incidence of invasive fungal infection among CLL patients appears relatively low, combination therapy with fludarabine in addition to an alkylating agent does increase the risk.

The incidence of invasive fungal infection among purine analog recipients has been difficult to glean from the brief descriptions available in published reports (207). Among the 2213 subjects enrolled in the 60 studies of purine therapy in patients with hematological malignancies reported by Cheson, only 51 (2.3%) examples of invasive fungal infection were sufficiently delineated to estimate the rate including invasive candidiasis ($n = 22$), pulmonary pneumocystosis ($n = 10$), invasive aspergillosis ($n = 17$), and cryptococcosis ($n = 2$). The invasive fungal infection rates seemed to be higher among CdA recipients (3.9% of 823 subjects) as compared to fludarabine (1.6% of 739 subjects, OR 2.45, 95% CI 1.25–4.79) or DCF (1.1% of 644 subjects, OR 3.72, 95% CI 1.63–8.49) recipients (207). These observations are consistent with subsequent reports wherein among CdA recipients fungal pneumonias were observed in 4% of 184 pretreated subjects and 7% of 194 untreated subjects (222).

CdA produces a predictable acute monocytopenia, the nadir of which occurs by day 7 from beginning treatment (223,224) with recovery to baseline levels by day 17 (223). Moreover, there is also significant lymphopenia, initially more pronounced for CD8⁺ T-lymphocytes and CD20⁺ B-lymphocytes than for CD4⁺ T-lymphocytes and CD16⁺ NK lymphocytes (225). Prolonged monocytopenia and CD19⁺ B-lymphocytopenia have been linked to the risk of invasive fungal infection among hematopoietic stem cell transplant recipients (190), particularly wherein bone marrow was the source of stem cell reconstitution (226). The effect of CdA on monocytes and B-lymphocytes may independent of its effects upon the absolute neutrophil count and have a pathogenetic relationship to the greater risk for invasive fungal infection observed among the very heterogeneous population of CdA recipients reported by Cheson (207).
Alemtuzumab (Campath-IH) is an anti-CD52 monoclonal antibody with activity in lymphoreticular malignancies such as chronic lymphocytic leukemia (227–230) and for the purposes of T-cell depletion in peripheral blood stem cell transplantation (231,232). The use of this product has been associated with profound reductions in CD19 B-lymphocytes, CD4 and CD8 T-lymphocytes, and CD16 natural killer cells (227,228,230). Many reports of infectious morbidity associated with the use of alemtuzumab have focused upon DNA viral infections (231,233–238). Reports of fungal infection-related morbidity have been relatively few and vague (219,239–241). Among 24 subjects with advanced CLL progressing after fludarabine therapy, there was almost 100% reduction in circulating CD19 B-lymphocytes that lasted beyond 28 weeks of the study. In this group, 10 (42%) experienced opportunistic infections of which eight (80%) were pneumonias and seven (70%) were fungal (P. jiroveci, four, Candida spp., one, Aspergillus spp. with or without Candida spp. two) (230).

Rituximab is a humanized chimeric anti-CD20 monoclonal antibody product with activity in CD20 B-cell lymphoreticular malignancies such as diffuse large cell lymphoma (242–245). Administration of this product leads to circulating B-lymphocyte depletion lasting 6–9 months (246). However, this does not result in a significant reduction in circulating immunoglobulin levels or an increase in opportunistic infections. There is, however, an effect upon primary and secondary immune responsiveness. Van der Kolk and colleagues (247) challenged patients with progressive low-grade non-Hodgkin’s lymphoma with two primary antigens and two recall antigens before and after rituximab treatment (375 mg/m²/week intravenously for 4 weeks). All subjects had a depletion of the circulating B cells within 73 hr of administration but the quantitative immunoglobulin levels (IgG, IgA, and IgM) remained stable throughout the course of treatment. The response to recall antigens was significantly lower after rituximab therapy compared to baseline suggesting a depletion in memory B cells. However, none of the subjects developed a primary response, either before or after rituximab therapy, suggesting that the underlying disease status may have played a role in the unresponsiveness. Some investigators have observed a higher risk for late onset opportunistic viral infections due to CMV and JC papovavirus among CD34-selected peripheral blood stem cell autograft recipients of peritransplant rituximab-mediated residual lymphoma disease reduction (OR 38, 95% CI 2–729) in association with delays in CD4 T-cell recovery (248,249). To date, there has been very little to implicate a relationship between rituximab and an increased risk for invasive fungal infection.

Paclitaxel, an enhancer of tubulin polymerization leading to mitotic arrest used for the treatment of non-small cell lung cancer (NSCLC), has been associated with dose-dependent leukopenia and neutropenia when used as monotherapy in daily doses of 135–175 mg/m² (2,250,251). Lower doses, 50–86 mg/m², administered in association with involved field irradiation has been associated with lowered CD4, CD8, NK cell, and CD19 lymphocyte counts (252). Such changes have been linked to the development of a syndrome of interstitial pneumonitis (252) among recipients of paclitaxel and involved field irradiation for NSCLC. This syndrome differs from the more commonly observed occurring outside the irradiation field in that the latter is a T-cell-mediated hypersensitivity, which is bilateral and associated with normal or elevated absolute lymphocyte counts (253).
VII. CYTOTOXIC THERAPY-INDUCED MUCOSAL INJURY AND INVASIVE FUNGAL INFECTION

Modern cytotoxic anticancer treatments, whether chemical or ionizing irradiation, exert their effects on tissues, either directly or indirectly, through the activation of cellular apoptotic pathways. The groups of cells most susceptible to the effects of these agents are those associated with cell renewal and differentiation: including hair, gonadal tissues, hematopoietic stem cells, and the committed epithelial progenitor cells. The clinical manifestation of damage to the latter group of progenitors is mucositis. Elaborate systems of scoring the severity of mucositis have been developed and direct correlations between mucositis scores and infection risk have been observed (254,255). Pseudomembranous candidiasis co-exists with mucositis in over half of patients receiving cytotoxic therapy for solid tumors (256). Mucosal colonization with Candida spp. in the presence of severe mucositis after high-dose cytarabine therapy has been linked to an increased risk of invasive candidiasis (26,257), The choice of cytotoxic regimen and its relative cytotoxic intensity are the most important determinants of mucosal damage (2,26,258).

A series of phases of mucosal damage have been recognized and described (259). An early inflammatory and vascular phase consists of nonspecific injury to mucosal basal cells or intestinal crypt cells in association with the release of interleukin 1, increased blood flow with consequent superficial erythema. Thereafter, an epithelial phase is recognized, wherein cytotoxic agents such as cytarabine induce apoptosis in the intestinal crypts leading to a reduction in crypt length and mitotic index, loss of villus area and reduced enterocyte height, and increased opening of intestinal mucosal tight junctions (259,261). This leads to mucosal thinning and atrophy detectable by day 4 or 5 from the start of the cytotoxic regimen. This is followed at day 7 to 10 by an ulcerative phase due to functional trauma, the cellular debris from which is recognized clinically as pseudomembrane formation (259). Micro-organisms such as opportunistic yeasts colonizing these damaged surfaces and their associated metabolic and structural lipopolysaccharide-like products may then translocate via the now incompetent cellular tight juctions. They may interact with submucosal host defences such as mononuclear phagocytes resulting in a cascade of inflammatory cytokine production that becomes associated with tissue damage. This process can be sequentially measured over time using molecular probes e.g., monosaccharides such as manitol or D-xylose, disaccharides such as lactulose, or radiolabelled products such as ethylenediamine tetraacetic acid (EDTA). Such studies have demonstrated that the time of maximum cytotoxic therapy-induced damage to the intestinal occurs at approximately 14 days following the beginning of the cytotoxic regimen, and at the same time as the neutrophil nadir (262–264). The pathogenesis of hepatosplenic fungal infection in acute leukemia patients is thought to be related to translocation of colonizing yeasts from the gut to the portal circulation and then to the liver (129,130,159,265–268). Despite the fact that hepatosplenic candidiasis in acute leukemia patients is most often diagnosed after neutrophil recovery late in the fourth week of therapy (267–269), the development of this infectious complication has been shown to correlate with malabsorption of D-xylose in week 2 of cytotoxic therapy at the time when most invasive bloodstream infections are observed (263). While difficult to demonstrate in patients, these observations support a pathogenetic model wherein candidemia develops in week 2 in following translocation across maximally damaged epithelial mucosal surfaces with seeding
of viscera such as the liver and spleen. This process can only be recognized with the recovery of the host inflammatory response with neutrophil recovery (270,271).

Chemotherapeutic regimens based on agents such as irinotecan, paclitaxel, doxorubicin, etoposide, cytarabine, high-dose melphelan, or busulfan are more likely to be associated with more severe mucositis (26,256,258). Careful selection of regimen components based on an understanding of the associated mucosal toxicity can be helpful in identifying patients at risk of invasive fungal infection and for targeted application of antifungal prophylaxis regimens (107).

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Invasive Fungal Infection


Epidemiology of Fungal Infection in Patients with Human Immunodeficiency Virus

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I. INTRODUCTION

With the advent of highly active antiretroviral therapy (HAART), the incidence of HIV-associated opportunistic infections (OIs) has decreased substantially (Fig. 1). This trend has continued and has been reported from other countries where HAART therapy is available (1,2). Mortality associated with the acquired immunodeficiency syndrome (AIDS) has dropped precipitously and OI morbidity has declined to a rate of ~20% of that seen before the introduction of HAART (1,3–8). The decline is attributable to the efficacy of HAART, use of prophylaxis for OIs, and better all-around care of the HIV-infected individual.

Likewise, the incidence of opportunistic fungal infections is approximately 20–25% of that seen in the mid-1990s. Pneumocystis jiroveci pneumonia (PCP), candidal esophagitis, and cryptococcosis remain the most common opportunistic fungal infections, although fungal infections associated with advanced AIDS, such as azole-resistant candidiasis and aspergillus, are rarely seen (1,7). These findings suggest that the spectrum of disease and the relative frequencies of opportunistic fungal infections have not changed appreciably since the early years of the HIV epidemic. Despite the decline of OI incidence, opportunistic fungal infections still occur in patients who are nonadherent, who have not previously been under medical care, have never received antiretroviral therapy, or have received suboptimal therapy (1,9).

In this chapter, we review the epidemiology and risk factors for opportunistic fungal infections in HIV patients in the era of HAART. Risk factors, endemic areas, and clinical manifestations for these infections are summarized in Table 1.

II. SPECIFIC FUNGAL INFECTIONS

A. Pneumocystis jiroveci

PCP accounted for 36% of cases of the AIDS-defining OIs in the United States during 1992–1997 and continues to be a significant cause of morbidity and mortality.
despite the availability of effective prophylaxis and HAART (10). It has consistently been shown to be the most common pulmonary disease among HIV-infected patients requiring critical care (11–13). *Pneumocystis jiroveci* was previously thought to be a protozoan until 1988 when DNA analysis demonstrated it to be a fungus (14,15). Additional DNA analysis showed that *Pneumocystis* organisms in different mammals are different, and the organism that causes human PCP is now named *Pneumocystis jiroveci* (16). Although the reported incidence of PCP in the developed world has decreased significantly, the reported incidence of PCP in the developing world, especially in Africa, is increasing (17). It is not known whether the trend toward increase denotes a true increase in the prevalence of PCP, or whether the early reports from developing countries underestimated the actual prevalence. This determination is made more difficult by the lack of standardization of inclusion criteria from early studies from developing countries. On the basis of one 1997 U.S. study of patients with CD4 counts of <100 cells/mm³, the incidence of PCP at nine HIV clinic in eight cities is estimated to be 3.7 cases per 100 patient-years (3). Other epidemiological studies indicate the incidence rate to be 46 cases per 1000 patient-years according to Centers for Disease Control and Prevention surveillance in 1996 (4) and 0.22 episodes per 100 patient-years according to a 1995–1997 Swiss Study (18).

Considerable evidence supports the concept that the CD4 cell count is an accurate indicator of susceptibility to PCP even in patients who have received HAART or interleukin-2 (IL-2) (19–22). However, the nadir of the CD4 cell count prior to the institution of HAART or IL-2 does not influence the predictive value of counts substantially (22). The HIV viral load is also an independent predictor of AIDS-defining events (23,24), but it is still unclear as to how this factor is to be used as an indicator for initiating PCP prophylaxis. Clinical markers, including the wasting syndrome, the occurrence of a previous episode of pneumonia of any type, and the occurrence of previous AIDS-defining events are also independent risk factors for PCP (25). It is estimated that 15% of patients presenting with PCP have CD4 cell counts higher than 200 cells/mm³, and a substantial fraction of these patients have had episodes

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**Figure 1** Trend of OIs in HIV-infected adults and adolescents from 1992 to 1998. *Source:* From Ref. 1.
<table>
<thead>
<tr>
<th>Mycosis</th>
<th>Areas of endemicity</th>
<th>Risk factors</th>
<th>Clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumocystis jiroveci</td>
<td>Ubiquitous</td>
<td>CD4 &lt; 200 cells/mm³, clinical markers,&lt;sup&gt;a&lt;/sup&gt; the occurrence of previous</td>
<td>Pulmonary disease,&lt;sup&gt;c&lt;/sup&gt; dissemination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pneumonia, and AIDS-defining illnesses</td>
<td></td>
</tr>
<tr>
<td>Cryptococcosis</td>
<td>Ubiquitous</td>
<td>CD4 &lt; 100 cell/mm³, black race, injection drug use, cigarette smoking</td>
<td>Meningitis, pulmonary disease,&lt;sup&gt;c&lt;/sup&gt; skin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lesions, endophthalmitis dissemination</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>Ubiquitous</td>
<td>Immunosuppression, high level of HIV-1 RNA, previous colonization with Candida</td>
<td>Oral thrush, esophagitis</td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td>North American river valleys, Europe, Africa, Southeast</td>
<td>Age, underlying immunosuppression</td>
<td>Pulmonary diseases,&lt;sup&gt;c&lt;/sup&gt; CNS disease,&lt;sup&gt;d&lt;/sup&gt; skin lesions, dissemination</td>
</tr>
<tr>
<td></td>
<td>Asia, Caribbean, Central and South Americas</td>
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<tr>
<td>Coccidioidomycosis</td>
<td>Argentina, Central America, Southwestern United States,</td>
<td>CD4 &lt; 250 cells/mm³, clinical diagnosis of AIDS</td>
<td>Pulmonary diseases,&lt;sup&gt;c&lt;/sup&gt; meningitis, skin</td>
</tr>
<tr>
<td></td>
<td>Northwestern Mexico</td>
<td></td>
<td>lesions, dissemination</td>
</tr>
<tr>
<td>Penicilliosis</td>
<td>Southern China, Hong Kong, Thailand, Vietnam</td>
<td>Exposure to environmental reservoirs,&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Pulmonary diseases,&lt;sup&gt;c&lt;/sup&gt; skin diseases,&lt;sup&gt;c&lt;/sup&gt; dissemination</td>
</tr>
<tr>
<td>Blastomycosis</td>
<td>North American river valleys, Quebec, Ontario, Manitoba</td>
<td>Advanced AIDS</td>
<td>Pulmonary diseases,&lt;sup&gt;c&lt;/sup&gt; CNS diseases,&lt;sup&gt;e&lt;/sup&gt; dissemination</td>
</tr>
<tr>
<td>Aspergillosis</td>
<td>Ubiquitous</td>
<td>Advanced AIDS, neutropenia, malignancy, CVC</td>
<td>Pulmonary diseases,&lt;sup&gt;c&lt;/sup&gt; sinusitis, otomastoiditis, renal aspergilloma</td>
</tr>
</tbody>
</table>

<sup>a</sup>Including wasting syndrome, the occurrence of a previous episode of pneumonia of any type, the occurrence of previous AIDS-defining events.

<sup>b</sup>Occupational or other exposure to soil in northern Thailand.

<sup>c</sup>Focal and diffuse.

<sup>d</sup>Encephalopathy, meningitis, focal brain lesions.

<sup>e</sup>Meningitis, cerebral abscess.

*Abbreviation: CVC, central venous catheter.

*Source: Adapted from Ref. 88.*
of unexplained fever or oropharyngeal candidiasis (OPC) (25,26). Therefore, it is reasonable to use these clinical indicators to initiate PCP prophylaxis before the CD4 cell count falls <200 cells/mm³. Despite the efficacy of HAART and prophylaxis regimens, patients continue to develop PCP. Risk factors for PCP in patients who received chemoprophylaxis include the use of agents other than trimethoprim–sulfamethoxazole (TMP–SMX), history of prior PCP, and a CD4 cell count of <50 cells/mm³ (27).

B. Cryptococcosis

Almost all cryptococcal infections in AIDS patients are caused by Cryptococcus neoformans var. neoformans (serotype A and D) (7,28,29). Advanced-stage AIDS patients who develop this infection usually have widely disseminated diseases with fungemia (30,31). Cryptococcal meningitis is the most frequent manifestation and is the most common life-threatening yeast infection, occurring in 5–10% of patients with AIDS in developed countries prior to the introduction of HAART (30,31). The incidence of cryptococcal meningitis in AIDS is much higher in developing countries, especially in sub-Saharan Africa, where its incidence is approaching 30% (29–31). Geographic distribution variation has not been established for cryptococcosis (32,33). The male/female ratio among AIDS patients is essentially 1:1 (32). Cryptococcosis in children with ADDS is less common, with a prevalence of ~1.4% (34).

The incidence of invasive cryptococcosis has declined in HIV-infected patients during the years since 1990 (32). However, this infection remains a significant problem in patients with limited access to health care (35). The annual incidence of invasive cryptococcosis was 1700–6600 per 100,000 persons in patients with AIDS in New York City in 1991, two per 1000 persons in the Houston area in 1994, and seven per 1000 persons in the Atlanta area in 2000 (35,36).

The only factor clearly identified to alter the risk of cryptococcal meningitis, besides low CD4 count, is fluconazole use (37). Fluconazole has been shown to be an effective means of primary prophylaxis for cryptococcal infections in several studies (38–43). However, because prophylaxis has not been linked with survival benefit, the United States Public Health Service (USPHS) and Infectious Diseases Society of America (IDSA) do not endorse the routine use of primary antifungal prophylaxis in the prevention of cryptococcosis. Other risk factors that have been suggested for the development of cryptococcosis include black race, injection drug use, and cigarette smoking (32). With improvement in the treatment of HIV and increasing use of azole antifungal drugs, the survival rate of cryptococcosis in HIV-infected patients is now at 70–78% (44,45).

C. Candidiasis

Candida infection in HIV patients is almost exclusively mucosal; systemic invasion is considered rare and usually occurs as a late event (29). Mucocutaneous candidiasis occurs in three forms: OPC, esophageal, and vulvovaginal diseases. Up to 93% of persons with advanced untreated HIV develop OPC, with 60% having at least one episode per year with frequent recurrences (50–60%) (46,47). Esophageal and vulvovaginal diseases occur in 10–20% of patients and 30–60% of women, respectively. Rarely, invasive disease can manifest as candidemia, especially related to use of central venous catheters in patients with advanced ADDS. Candidemia has been described as a nosocomially acquired infection in patients with late-stage AIDS, with
an attributable mortality rate of 31% (48). There have been few studies, mostly restricted to case series or anecdotal reports, describing the incidence and prevalence of non-esophageal invasive candidiasis in HIV-infected patients, but its incidence is probably <1% (48,49).

The widespread use of antifungal azole agents and HAART has resulted in a significant decline in the incidence of mucocutaneous candidiasis (50,51). Several factors are important in the development of mucocutaneous candidiasis including immunosuppression, high level of HIV-1 RNA, and colonization with Candida (46). It is notable that the relationship between the level of immunosuppression and vaginal candidiasis may not be strong. In one cross-sectional study of 833 HIV-infected and 427 HIV-uninfected women, the annual incidence of vaginal candidiasis was similar in the two groups (9%) (52).

The most common cause of mucosal candidiasis is Candida albicans accounting for at least 80% of clinical infections, whereas the proportion of non-C. albicans species has increased over time up to 20% before the advent of HAART (2,53). The most notable predisposing factor for non-C. albicans species is prior exposure to azole antifungals (2,53). In a multicenter prospective study of 832 persons with advanced HIV infection, fluconazole-resistant mucosal candidiasis (FRMC) occurred in 4.3% with an incidence of 4.2 per 100 patient-years follow-up (53). Prior use of TMP–SMX, prolonged high-dose fluconazole, or prior candidiasis was significantly associated with the development of FRMC. In the HAART era, studies have suggested that azole-resistant Candida species has been reduced to <10% (50,54,55). It is possible to hypothesize that the significant decrease in the use of azoles has caused a reduction in the selection pressure of antifungals that has contributed to the decreased percentage of resistant strains. It is estimated that at least 80% of patients with uncomplicated mucocutaneous candidiasis respond to standard treatment (topical or systemic therapy) (2). Severe recurrent infections, however, especially with azole resistance, may require short courses of amphotericin B. Candidiasis becomes more difficult to be treated with progressive loss of CD4 cells. Therefore, HAART has emerged as the most effective mode of therapy for OIs including candidiasis.

D. Histoplasmosis

Histoplasma capsulatum is a dimorphic fungus that generally causes mild and self-limited infection in immunocompetent patients, but prolonged and severe disease in immunocompromised hosts, especially those with AIDS. The mycelial form of H. capsulatum is found in the soil and is particularly associated with bird roosts and caves (29). Histoplasmosis is an important OI among HIV patients, which occurs in 2–5% of patients with AIDS from endemic areas and up to 25% from selected cities in the United States (Kansas City, Indianapolis, Nashville, and Memphis) (56–58). The endemic areas of histoplasma are shown in Table 1. Most cases in the endemic area are caused by exogenous exposure but less commonly resulted from reactivation of an old infection (56). Histoplasmosis occurs in <1% of patients from nonendemic areas, where reactivation of latent infection is implicated as a cause of infection (59).

Approximately 90% of cases of disseminated histoplasmosis have occurred in patients with CD4 counts <200 cells/mm³ (median CD4 cell count <30 cells/mm³), but localized pulmonary disease may be seen in those with high CD4 cell counts, typically >300 cells/mm³ (56,60,61). Disseminated disease was identified at autopsy
in 8% of patients with AIDS from Brazil and 44% from Venezuela whereas cutaneous involvement was more common in South America (56,62). 5 to 10% of patients with disseminated histoplasmosis have an acute septic-shock-like syndrome that includes hypotension and evidence of disseminated coagulopathy; this presentation carries a poor prognosis (29). Histoplasmosis also has been reported in Europe, Africa, and Southeast Asia (56). Infection with *H. capsulatum* var. *duboisii* has been reported in patients with AIDS in Africa (63,64).

Most knowledge about the epidemiology of histoplasmosis has been derived from outbreak investigations. As exemplified by the large outbreak in AIDS patients in Indiana between 1988 and 1993 (57), these patients serve as a sentinel markers for histoplasmosis in these areas. A review of histoplasmosis in AIDS patients in Indiana reported it to be the only OI in 22% and the first OI in 7% (57). Histoplasmosis is often the first and/or the only OI in AIDS patients. In Houston in the 1980s, it was the first OI in 75% of patients with AIDS (57). After the introduction of HAART, the incidence of histoplasmosis has declined in recent years (65). Risk factors for histoplasmosis included age and underlying immunosuppressions, especially with AIDS (66). Sex and race were not found to be associated with increased risk of disease among HIV-infected patients (66). In general, immunocompromised persons with histoplasmosis have a higher mortality rate than those who are not immunocompromised (66). In one study, the mortality rate for immunocompromised patients was 33% compared to 17% of nonimmunocompromised persons (67). If left untreated, disseminated histoplasmosis in AIDS is progressively fatal. Two multicenter studies suggested the effectiveness of itraconazole, as primary and secondary prophylaxis, in preventing histoplasmosis in patients with HIV/AIDS (61,68). This data support the use of itraconazole prophylaxis for histoplasmosis in the endemic area.

### E. Coccidioidomycosis

*Coccidioides immitis* was included in the surveillance case definition for AIDS in 1987 (69). It is a thermal dimorphic fungus found only in the Western hemisphere in area that marked by low annual rainfall, sandy saline soil, and periodic dust storms. The areas of highest endemicity are southern San Joaquin Valley in California and the regions encompassing Phoenix and Tucson in southern Arizona (70–73). Other areas of endemicity are shown in Table 1.

The association between HIV disease and coccidioidomycosis is not entirely clear (74). The first case series of coccidioidomycosis occurring in HIV-infected persons suggested that seven out of 27 patients with AIDS living in southern Arizona developed symptomatic infecton with *C. immitis* (75). Six out of seven patients had diffuse nodular pulmonary infiltrates, and five had detectable anticoxidoidal antibodies in their sera; all died within 14 months of the diagnosis of coccidioidomycosis. The impact of coccidioidomycosis on patients infected with HIV is not geographically uniform, but it predominantly affects HIV-infected persons living in Arizona. In Arizona, 8.2% of all AIDS patients reporting to CDC had concomitant coccidioidomycosis compared to only 0.3% nationwide (74). The impact of HIV infection on the development of active coccidioidomycosis appears to be significantly greater in the coccidoidal endemic area within the state. Data from the California State Health Department suggested that 3.5% of patients living in Kern County (coccidoidal endemic area) were reported to have coccidioidomycosis as their AIDS-defining diagnosis compared to only 0.3% for the entire state (76). One prospective study in Arizona suggested that
13 out of 170 HIV-infected persons developed coccidioidomycosis after 41 months follow-up, yielding an estimated cumulative incidence of nearly 25% (77).

Two factors associated with the development of active coccidioidomycosis were CD4 counts <250 cells/mm³ and the clinical diagnosis of AIDS. Length of stay in the endemic area, history of prior diagnosis of coccidioidomycosis, and a positive coccidioidal skin test were not risk factors for active disease. These data suggest that most clinical disease is due to primary infection, as opposed to reactivation of latent infection for cases outside the endemic area (70). Similar to other OIs, the incidence of coccidioidomycosis among HIV-infected persons has declined in the era of HAART (1). Retrospective study of cases of coccidioidomycosis among HIV-infected patients in the era of HAART in Arizona suggested that the number of cases of active coccidioidomycosis declined from 77 in 1995 to 61 in 1996 and 15 in 1997 (71,73,76).

F. Penicilliosis

A dimorphic fungus, *Penicillium marneffei*, has been recognized as a significant pathogen in Southeast Asia, Hong Kong, and southern China among patients in advanced stages of AIDS (78–80). In northern Thailand, this fungal infection is the third most common OI, accounting for 15–20% of all AIDS-related illnesses, a frequency rivaling tuberculosis and cryptococcal meningoencephalitis (81). Because disseminated penicilliosis is usually diagnosed in patients with CD4 cell count <100 cells/mm³, and since it is often shortly followed by the diagnosis of more common OIs, several authors have suggested that disseminated penicilliosis should be included in the diagnostic criteria for AIDS (79–82). Infection with this organism is regarded as an AIDS-defining illness. With the further spread of HIV in Asia, disseminated *P. marneffei* infection is likely to increase in importance.

*Penicillium marneffei* was first isolated from Vietnamese bamboo rats in 1956, and subsequent animal and human cases have been reported from southern China, Hong Kong, Thailand, and Vietnam (83–87). In such cases, patients have either lived in or traveled to these endemic areas (88). A case–control study of risk factors of *P. marneffei* infection in HIV patients in northern Thailand suggests that a recent history of occupational or other exposure to soil in the rainy season is a significant risk factor (87). Although *P. marneffei* has been identified in bamboo, bamboo rats, their feces, and from soil obtained from their burrows, the actual reservoir and portal of entry have not been determined (88). Patients infected with *P. marneffei* have a poor prognosis without treatment (80). The mortality rate from disseminated *P. marneffei* infection is about 20% with a relapse rate of 50% after discontinuation of successful initial therapy (82,89). Two randomized controlled trials in northern Thailand suggested that itraconazole was safe, effective, and well tolerated as primary and secondary prophylaxis in penicilliosis and cryptococcosis in patients with advanced AIDS (82,90).

G. Blastomycosis

Blastomycosis is caused by the dimorphic fungus *Blastomyces dermatitidis*. It has been found in humid areas from soil with high organic content, high animal-waste component, and acid pH (91). Inhalation of conidia is the route of infection. The areas of endemicity are areas bordering the Mississippi, Missouri, and Ohio rivers and extending northward to Quebec, Ontario and Manitoba. Blastomycosis has been rarely reported in patients with AIDS and appears to occur in the later stages of HIV infections, generally with CD4 cell count <200 cells/mm³ (29,88,92,93). In
immunocompromised hosts, blastomycosis is associated with pulmonary fibrosis, pulmonary nodules, or skin infection; however, in patients with AIDS, widely disseminated infection usually occurs (94). Blastomycosis in AIDS is strongly associated with central nervous system involvement (~46%), with mortality rate of 90% (93,94).

H. Mycoses Caused by Molds

The clinical manifestations of selected mold infections in patients with AIDS are summarized in Table 2. Infection caused by molds is the least commonly encountered mycosis in AIDS (95,96). The incidence of mold infection has also decreased since the introduction of HAART (94). Although uncommon, infections caused by molds are important causes of invasive mycoses in patients with AIDS. Early recognition of these infections allows prompt diagnosis and early institution of antifungal therapy and possible surgical intervention. Their clinical manifestations can range from localized soft-tissue infections or sinusitis to widely disseminated disease. Most often, patients with mold infections have advanced AIDS with CD4 counts <200 cells/mm³, causing widely disseminated infection (94). Other risk factors include neutropenia, use of corticosteroids, cytomegalovirus (CMV) infection, and chemotherapy (88,94). Because relapse is frequent, long-term suppressive therapy is often required. The most common routes of infection are through inhalation of conidia for dimorphic fungi, Aspergillus species, and various Zygomyces, intravenous injection of conidia via injection drug use for Zygomyces and Aspergillus species, and percutaneous inoculation for Sporothrix schenckii (94).

Table 2  Clinical Manifestations of Selected Mold Infections in Patients with AIDS

<table>
<thead>
<tr>
<th>Organism</th>
<th>Clinical manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agents of hyalohyphomycosis</td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus</em> spp.</td>
<td>Pulmonary, sinustitis, cutaneous, focal abscess, disseminated</td>
</tr>
<tr>
<td><em>Pseudallescheria</em> spp.</td>
<td>Pneumonia, sinustitis, endocarditis, disseminated disease, meningitis</td>
</tr>
<tr>
<td><em>Fusarium</em> spp.</td>
<td>Fungemia, endocarditis, disseminated infection</td>
</tr>
<tr>
<td><em>Chrysosporium</em> spp.</td>
<td>Osteomyelitis</td>
</tr>
<tr>
<td><em>Trichosporon</em> spp.</td>
<td>Catheter-related fungemia</td>
</tr>
<tr>
<td><em>Geotrichum</em> spp.</td>
<td>Esophageal ulcer</td>
</tr>
<tr>
<td><em>Pennicillium decumbens</em></td>
<td>Disseminated infection</td>
</tr>
<tr>
<td>Agents of Phaeohyphomycosis</td>
<td></td>
</tr>
<tr>
<td><em>Alternaria</em> spp.</td>
<td>Nasal soft-tissue infection, sinusitis</td>
</tr>
<tr>
<td><em>Exophiala</em> spp.</td>
<td>Esophagitis, soft-tissue infection</td>
</tr>
<tr>
<td><em>Hormonema</em> spp.</td>
<td>Liver abscess</td>
</tr>
<tr>
<td><em>Cladophialophora</em> spp.</td>
<td>Brain abscess, pulmonary</td>
</tr>
<tr>
<td><em>Phialophora</em> spp.</td>
<td>Disseminated infection</td>
</tr>
<tr>
<td><em>Bipolaris</em> spp.</td>
<td>Endophthalmitis</td>
</tr>
<tr>
<td><em>Scedosporium prolificans</em></td>
<td>Disseminated infection</td>
</tr>
<tr>
<td>Agents of Zygomyces</td>
<td></td>
</tr>
<tr>
<td><em>Rhizopus arrhizus</em></td>
<td>Orbit, soft tissue, sinusitis</td>
</tr>
<tr>
<td><em>Absidia corymbifera</em></td>
<td>Renal abscesses, pharyngeal, pulmonary</td>
</tr>
<tr>
<td><em>Cunninghamella bertholletiae</em></td>
<td>Soft-tissue abscess</td>
</tr>
<tr>
<td><em>Muco</em> spp.</td>
<td>Sinusitis</td>
</tr>
</tbody>
</table>

Source: Adapted with permission from Ref. 94.
I. Dimorphic Mycoses

The dimorphic mycoses are caused by endemic mycoses, histoplasmosis, coccidioidomycosis, paracoccidioidomycosis, blastomycosis, penicilliosis, and sporotrichosis. With the exception of sporotrichosis and paracoccidioidomycosis, the epidemiology of other dimorphic fungi has been discussed in previous sections. *Sporothrix schenckii* does not occur in a specific geographic zone but occurs in the specific setting of exposure to contaminated rosebush thorns or moss (94). Disseminated *S. schenckii* had been reported in AIDS patients acquired from percutaneous inoculation (97). Paracoccidioidomyces is endemic in South America, but infection in patients with AIDS is rare (98). In contrast to immunocompetent subjects, rapid progressive paracoccidioidomyces with dissemination are usually seen in patients with AIDS and resemble disseminated histoplasmosis (94). If left untreated, these mycoses are commonly progressive and fatal.

J. Agents of Hyalohyphomycosis (*Aspergillus, Fusarium, Pseudallescheria, Chrysosporium*)

Hyalohyphomycoses is a group of lightly pigmented (hyaline) molds. These organisms have been reported occasionally in patients with advanced AIDS and uncontrolled HIV replication (99). Since the advent of HAART, these organisms have become rare in HIV patients but have become ever more common in patients with leukemia/lymphoma (100). These organisms appear similar, morphologically, to each other and require culture to confirm the specific agent (94). Risk factors for these infections include advanced AIDS with CD4 cell counts <50 cells/mm³ and other alterations in host defenses, such as neutropenia, malignancy, indwelling catheters, and comorbid conditions such as diabetes mellitus (88).

Invasive aspergillosis is not currently defined as an OI associated with AIDS. Although the incidence of these infections was increasing for some years, they have once again become uncommon. Clinical presentations may vary from sinusitis to widely disseminated infection (94,100). The course of disease may be indolent or fulminant (94). *Aspergillus* spp. colonization has been reported in ~5% of AIDS patients with the rate of proven invasive disease being <1% (101). *Aspergillus fumigatus* and *A. flavus* are the most commonly isolated species in patients with or without AIDS (88). *Aspergillus* species can be detected in soil, water, and decaying vegetation, and has been cultured from the air, dust, and environmental surfaces in hospitals (88). Outbreaks involving HIV-infected patients have not been described. Other agents of hyalohyphomycosis that have been reported to cause invasive mycoses in patients with AIDS include *Fusarium* spp., *Pseudallescheria boydii, Chrysosporium* species, *Geotrichum, Trichosporon beigelii*, and *Penicillium* spp. (Table 2).

K. Agents of Phaeohyphomycosis (*Cladophialophora, Alternaria, Exophiala*)

Phaeohyphomycoses comprise a group of opportunistic molds that are dematiaceous or darkly pigmented. Although rarely occurring, these emerging pathogens cause disease in both HIV and non-HIV persons. These fungi may occur at any stage of AIDS, but a more rapid course is commonly seen with late-stage AIDS (94). Neutropenia and diabetes are risk factors for infection, whereas gardening with traumatic inoculation is a major mode of infection.
L. Zygozymes  (*Rhizopus Species, Absidia, Mucor, Rhizomucor, Cunninghamamella*)

These organisms have been reported uncommonly in patients with AIDS but may cause localized deep tissue abscesses in various organs including the kidney, liver, spleen, and stomach (102). Disseminated infection may occur and carry a poor outcome (mortality ~80%), but some of these infections progress slowly in patients with AIDS and respond well to surgery and standard antifungal therapy (103–105). Risk factors for infection include injection drug use, neutropenia, corticosteroid use, and diabetes mellitus. Contamination of injected drugs is presumed to be the most common route of infection, though inhalation of conidia is also possible (94).

III. SPECIAL CONSIDERATIONS IN HIV-INFECTED PATIENTS

A. Impact of HAART on the Clinical Presentation and Prophylaxis of OIs

Successful therapy with HAART leads to a rise in CD4 cell counts, suppression of viral replication to below detectable limits, and restoration of immune function (106–108). The increase in CD4 cell counts often occurs in two phases. The first phase (usually 2–3 months after the initiation of antiretroviral therapy) is associated with a rapid increase in CD4+ and CD8+ T cells, mainly of the memory phenotype. This phase appears to represent redistribution and expansion of pre-existing cells and may not represent true immune recovery. The second phase (starting 2–3 months after therapy) involves a more gradual rise in CD4+ T cells, mainly naïve T cells, and probably represents gradual recovery of the immune system. This may be maintained for several years and is usually adequate to protect individuals against OIs. Immune restoration not only results in significant improvement in morbidity and survival (1,3), but also results in the change in approach to prophylaxis of opportunistic mycoses.

B. Immune Reconstitution Illness

Immune reconstitution is thought to represent an inflammatory state induced by the newly restored immune system against pathogens that have previously infected the HIV-positive host. It usually occurs within the first 3 months after starting antiretroviral therapy and has been well described with *Mycobacterium avium* complex (MAC) infection, tuberculosis, CMV retinitis, Hepatitis C and Hepatitis B virus infections, fungal infections (cryptococcosis, histoplasmosis, and PCP), varicella zoster, progressive multifocal leukoencephalopathy (PML), and even malignant and noninfectious disorders (109–118). This syndrome presents with typical clinical features such as local MAC lymphadenitis without bacteremia, vitreal and extraocular disease with CMV, and PML with contrast enhancement on MRI. Fatalities have been reported in some of these cases (109–118). Along the same line, opportunistic fungal infections can be manifested with highly unusual localizations, accompanied by an intriguing spectrum of clinical findings. Five case descriptions have been reported in reference to immune reconstitution exacerbating or unveiling cryptococcal disease in 12 patients (112–114,117,118). The clinical presentations occurred from as early as 8 days to as late as 15 months after starting HAART. Remarkably, four of these patients had lymphadenitis as the primary mode of presentation (cervical
and mediastinal). In these four patients, the diagnosis was made by histology showing cryptococcal yeast cells, and all had positive serum cryptococcal antigen at high titers but negative cerebrospinal fluid (CSF) antigen and cultures (blood, tissue, CSF). These patients were managed diversely, with continuation of antifungals or administration of corticosteroids or NSAIDS. The common denominator in all cases was the apparent enhancement of cell-mediated immunity, which paradoxically worsened the clinical outcome in response to a heavy burden of latent infections. Few cases of this novel syndrome have been reported with PCP and histoplasmosis (115,117). Because this syndrome can present with atypical clinical features, a high index of suspicion is necessary for early diagnosis. Table 3 summarizes the clinical presentations of specific OIs associated with immune reconstitution.

C. Discontinuation of Prophylactic Medications

Evidence for restoration of clinically relevant immune function has come from data on successful discontinuation of primary and secondary prophylaxis for most major IOs, when the CD4 cell count rises above a certain threshold. This has been well demonstrated for PCP, CMV retinitis, and candidiasis (119–128). There are also sufficient cohort studies to recommend appropriate prophylaxis discontinuation for toxoplasmosis, MAC, and cryptococcal meningitis (129–136). Indeed, it is probably true for all opportunistic pathogens, where the immune system is known to be capable of controlling infection. The exception is *C. immitis* infection, where cure is very difficult even in the normal host. Therefore, it is possible that specific antimicrobial prophylaxis can be stopped if viral suppression occurs and the CD4 cell counts rises above the threshold levels. Currently, the USPHS and IDSA recommend discontinuing primary prophylaxis for PCP, MAC, and toxoplasmosis in patients with sustained increases (≥3 months) in CD4 cell counts above thresholds (137). Secondary prophylaxis should be discontinued for PCP, MAC, toxoplasmosis, CMV retinitis, and cryptococcosis among patients with a sustained increase in CD4 cell counts (≥3–6 months) above the thresholds (137).

<table>
<thead>
<tr>
<th>Opportunistic infection</th>
<th>Clinical presentation associated with immune reconstitution illness</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycobacterium avium</em> complex</td>
<td>Focal lymphadenitis; bacteremia rare</td>
</tr>
<tr>
<td>CMV infection</td>
<td>Vitritis, retinitis, extraocular disease</td>
</tr>
<tr>
<td>Cryptococcosis</td>
<td>Pulmonary nodule, cavitary pneumonia, aseptic meningitis with elevated intracranial pressure, CSF leukocytosis, focal and necrotizing lymphadenitis, intrathoracic lymphadenopathy with hypercalcemia, supraclavicular abscess</td>
</tr>
<tr>
<td><em>Pneumocystis jiroveci</em></td>
<td>Granulomatous pneumonia</td>
</tr>
<tr>
<td>PML</td>
<td>Neurologic deficits, hypodensities with peripheral enhancement finding from magnetic resonance image</td>
</tr>
<tr>
<td>Varicella zoster</td>
<td>Mild, uncomplicated presentation</td>
</tr>
<tr>
<td>Hepatitis C virus (HCV) infection</td>
<td>Overt hepatitis, cirrhosis, HCV-associated disorders (e.g., cryoglobulinemia)</td>
</tr>
</tbody>
</table>
There are some reports describing breakthrough OI in patients despite a rise in CD4 cell counts (138,139). These cases are rare and represent the exception rather than the rule and suggest that immune responses to specific pathogens in some patients may not be restored satisfactorily. These cases also indicate that immune recovery may be incomplete in some patients and that close monitoring of patients is warranted. Relapse can be seen if the antiretroviral treatment fails and immunodeficiency resumes (140). Therefore, secondary prophylaxis should be restarted if the CD4 cell count falls below threshold levels.

IV. CONCLUSION

The incidence of opportunistic mycoses has decreased significantly in developed countries as a consequence of improved treatment of HIV and prophylaxis for opportunistic mycoses. Nevertheless, HIV treatment is not optimal because of multiple factors such as adherence issues, side effects of medications, or viral resistance. Opportunistic fungal infections still occur in the developing world, where HIV treatment is still suboptimal, and in developed countries in patients who are nonadherent, who have not previously been under medical care, have never received antiretroviral therapy, or have received suboptimal therapy. Current optimism regarding the future depends on the continuing success of antiviral treatment. If treatment fails, we are likely to see the return of all OIs. Physicians who care for HIV patients in the era of HAART need to be aware of the change in clinical presentations of OIs in HIV patients and the possibility that prophylaxis medications may be discontinued in selected patients to minimize side effects and drug interactions. It appears that the future of HIV-associated mycoses is linked to the future of effective treatment of HIV itself.

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Candida Infections in the Intensive Care Unit

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I. INTRODUCTION

There have been substantial changes in notions of prevention and management of Candida infection syndromes seen in critical care settings. Prospective randomized trials have been reported in the area of prophylactic therapy, and, although controversial, have legitimized the expansion of prophylactic therapy. Similarly, considerable information has come from therapeutic trials of new agents including the first echinocandin, caspofungin and an expanded spectrum imidazole, voriconazole. The availability of imidazoles and echinocandins warrant close review of the utility, if any, of continued use of amphotericin B and its derivatives in Candida infection. This review will focus on these particular issues.

II. PATHOGENICITY AND VIRULENCE FACTORS FOR CANDIDA

Four virulence factors have been demonstrated for Candida spp.: adherence to epithelial and endothelial cells, secretion of proteinases that degrade connective tissue proteins and facilitate invasion, production of cell-surface and shed mannans, which serve to attach the yeast to host tissues and suppress host response, and resistance to oxidative killing by neutrophils (1–5).

A. Candida albicans

C. albicans appears to be more virulent than other Candida species, and has been most closely studied. The conversion from yeast to filamentous growth is a critical step in the subsequent expression of several virulence mechanisms for C. albicans (2). The yeast-to-hyphal transition of C. albicans can be is triggered by a wide variety of factors, suggesting that hyphal growth is a response to nutrient deprivation, especially low nitrogen and that filamentous growth enables the fungus to forage for nutrients more effectively (6).
B. Adhesion, Proteases, and Mannans as Virulence Factors

*C. albicans* is the most common fungal pathogen of humans and has developed an extensive repertoire of virulence mechanisms that allows successful colonization and infection of the host under suitable predisposing conditions. Yeast forms adhere to various host cells and matrix elements primarily through the protein and carbohydrate elements of mannoproteins. Adherence is achieved by a combination of specific (ligand–receptor interactions) and non-specific (electrostatic charge, van der Waals forces) mechanisms which allow the yeast to attach to a wide range of tissue types and inanimate surfaces (7). Adherence for filamentous forms of *C. albicans* is mediated in part by an integrin analogue that shares antigenic, structural, and functional homologies with the β2-integrin subunits αM and αX (8). Antibodies to the integrin α5β1 (the fibronectin receptor on various human cell types), and antibodies specific for the integrin β1 sub unit recognized a *C. tropicalis* membrane protein (9,10). *C. albicans* and *C. tropicalis* recognize distinct RGD ligands present at the surface of the epithelial cell (11–14).

This information supports a two-stage model for adherence of *C. albicans* to host tissues. In this model, conventional adhesins composed of a mixture of agglutinin-like cell wall proteins initiate binding to host cells. This is followed by formation of germ tubes. The signaling that results in a switch to filamentous forms is, therefore a critical element and becomes a potential target for therapeutic intervention.

The secreted aspartic proteinases of *C. albicans* are major factors in virulence. SAP proteins fulfill a number of specialized functions during the infective process, which include the simple role of digesting molecules for nutrients, altering host cell membranes to facilitate adhesion and tissue invasion, and digesting cells and molecules of the host immune system to avoid or resist antimicrobial attack by the host (15). There are at least seven different genes that encode for secreted aspartic proteinase (16–19). SAP expression depends on the type of infection, with different SAP isogenes being activated during systemic disease as compared with mucosal infection. In addition, the activation of individual SAP genes depends on the progress of the infection; some members of the gene family being induced immediately after contact with the host whereas others are expressed only after dissemination into deep organs.

An additional element in fungal pathogenesis is cell surface mannans. These are important both for *albicans* and non-*albicans* species (20). Mannan consists of a large number of various hypermannosylated proteins that are deposited mainly at the outside of the cell wall, thereby protecting internal regions of the cell wall from large molecules like proteases. Many critical aspects of the interaction between the fungus and the host, such as adhesion, immunosurveillance, and immunomodulation, are mediated by host recognition and interaction with this mannan-rich surface of the cell wall (21).

III. THE MICROBIOLOGY, INCIDENCE, MORBIDITY, AND MORTALITY OF *CANDIDA* INFECTION

The incidence of fungal infections, particularly with *Candida* species increased substantially through the 1980s, but has since leveled off (22,23) (Fig. 1A). At many medical centers, *Candida* species remain the fourth leading cause of nosocomial bloodstream infection, preceded only by coagulase-negative staphylococci, *Staphylococcus aureus*, and enterococci (24). The prevalence of *Candida* in a particular ICU is
dependent on the type of patients seen, their acuity, and other local factors such as fluconazole usage.

A. The Changing Microbiological Picture of Candida Infections

Although there are more than 100 described species of Candida, only four are commonly associated with infection: C. albicans, C. tropicalis, C. parapsilosis, and C. glabrata. Of these, C. albicans has long been the most common (>60% of infections). The other three major species are seen at rates varying from 5% to 20%.
C. tropicalis is a virulent organism and mucosal colonization by this organism frequently leads to invasive infection.

An evolution of the epidemiology of candidiasis has been recently described with a reduction in the incidence of C. albicans in favor of the non-albicans species, in particular C. glabrata and C. krusei (Fig. 1B) (25–27). This appears to have occurred because of wide usage of fluconazole, and is important because several strains of C. glabrata have reduced susceptibility to fluconazole. C. krusei is highly resistant to all triazoles.

A recent study by the NNIS group evaluated the trends in species distribution and susceptibility to fluconazole among 1579 bloodstream isolates of Candida spp. over a 7-year period (1992–1998) from more than 50 U.S. hospitals (26). C. albicans accounted for 52% of isolates, followed by C. glabrata 18%, C. parapsilosis 15%, C. tropicalis 11%, and C. krusei 2%. Since 1995, C. glabrata has become more prevalent than C. parapsilosis. The susceptibility of all Candida species to fluconazole remained stable, and, particularly, there was no increased level of resistance in C. albicans. This decrease in C. albicans and increase in C. glabrata has not been observed in all centers where fluconazole usage has increased (28). It is important for individual centers to monitor their experience, as a guide to utility of fluconazole as empiric therapy.

### B. Mortality of Candida Infection

Observational clinical studies, either retrospective or prospective, have consistently identified a crude mortality rate of 30–60% for patients with candidemia (29–31). This is illustrated in Figure 2, where the findings of two sequential case-matched studies from the same institution are displayed (29,32). A much larger observational study involving 1447 adults at tertiary centers has recently been reported and this provides a broader survey (33). In this study, overall mortality 3 months after the

![Figure 2](image_url)

**Figure 2** Attributable mortality from two case controlled retrospective studies of candidemia at the University of Iowa. Source: From Refs. 29,32.
initial positive blood culture result was 40%, and cause-specific mortality was 12%. Conversely, prospective therapeutic trials have found a substantially lower mortality rate, partly because of selection bias of clinical trials, which typically exclude patients with organ failure and other risk factors for mortality. As an example, a recent prospective study examining different amphotericin dosing regimens based on duration of candidemia excluded patients with renal failure (34).

C. How Does Candida Infection Cause Mortality?

Mortality from infectious disease occurs most commonly as a consequence of the host physiologic response to the organism. The measured severity of infection, based on acute cardiovascular, respiratory, organ function scoring, and limited blood tests, is a reliable and reproducible measure of mortality risk, and is generally regardless of the infecting microorganism, if effective antimicrobial therapy is available. The physiologic disturbance is most commonly quantified using the APACHE II or APACHE III system (35,36).

To what extent the particular infecting organism independently affects mortality resulting from specific virulence mechanisms is obvious with toxin-secreting bacteria (e.g., Group A streptococcal or clostridial infections). As Candida infection syndromes were being defined pathologically, death commonly resulted from untreated metastatic infection with associated organ failure. This was certainly because of organism-specific virulence factors. However, since the recognition of the virulence of these organisms, the development of less toxic antifungal agents, and more aggressive treatment of Candida infections, these syndromes of disseminated micro-abscesses are now rarely seen in patients without neutropenia. As this condition was obviated, outcome became primarily dependent on acute physiologic shifts and background disease.

In case-controlled studies, the controls do not experience the physiologic derangements that the candidemic patients suffer. More suitable controls might be patients with bacteremia. This has been examined in Pappas et al.’s (33) observational study (Fig. 3A), with the finding that species did not affect mortality at different severity levels. An APACHE-based analysis system was used to demonstrate outcome differences between two antifungal regimens, where a therapeutic effect appeared most notable in moderately ill patients (Fig. 3B).

IV. CLINICAL ASPECTS OF CANDIDA INFECTION

A. Sources of Candida in the Critical Care Patient

In humans, as well as in animals, the GI tract is considered to be an important portal of entry for microorganisms, including yeasts, into the bloodstream. The passage of endogenous fungi across the mucosal barrier is referred to as fungal translocation (by analogy with bacterial translocation).

Although yeast cells have no intrinsic motility, they are able to translocate across the intestinal mucosa within a few hours of ingestion, if present in high enough concentration. That Candida spp. can translocate from the gut into the bloodstream in humans was demonstrated in a study, where signs and symptoms of sepsis developed in a healthy volunteer 2 hr after ingestion of a suspension containing $10^{12}$ C. albicans organisms, and blood cultures taken 3 and 6 hr after ingestion were positive for Candida (37). That this mechanism is important in clinical disease
is attested by the finding of GI tract involvement and submucosal invasion in an autopsy study of patients with hematogenous candidiasis (38).

Microbial translocation has been demonstrated in several animal models, using a range of stresses. These include, most notably, malnutrition, parenteral nutrition, endotoxemia, bacterial overgrowth, and burn injury (39–42). It is enhanced by fasting (which induces complex changes in host defenses), and protein deficiency (which results in intestinal microbial overgrowth, shortened villi, increased intestinal absorption of intact proteins, and decreased intracellular killing of bacteria and fungi) (43,44).
Translocation is thought to occur as a result of flattening of the intestinal villi that results from the administration of total parenteral nutrition (45). In another animal model, bile duct ligation resulted in bacterial translocation (46). In a study involving *C. albicans*, volatile fatty acids and secondary bile salts reduced fungal adhesion to the mucosa, colonization, and dissemination in mice by causing the formation of a dense layer of bacteria in the mucosal biofilm. These bacteria successfully competed with the yeast cells for the same adhesion sites and produced various substances that inhibited fungal growth (47). Similar results were obtained in guinea pigs and rats that had burns covering 50% of their total body surface area. After the yeast cells penetrated the lamina propria, the cells were found either free in the lymphatic vessels and in the blood vessels or engulfed by macrophages (48).

Microbial translocation is relevant in human disease. Surveillance cultures from patients with leukemia have demonstrated an association between the bacterial biotype or serotype that was most prevalent in the patient’s feces, and the bacterial biotype or serotype that subsequently caused septicemia (49). Significant microbial translocation has been demonstrated in patients undergoing operation for colorectal carcinoma, intestinal obstruction, and Crohn’s disease (50–52).

**B. Immunosuppression of the Host Leading to Dissemination**

Host defenses against *Candida* infections include T-cell immunity, important at the mucosal level in preventing colonization and superficial invasion, and phagocytic immunity. Professional phagocytes, including macrophages and neutrophils, serve to prevent deeper tissue invasion and hematogenous dissemination. Suppression of any of these arms of the immune system puts the patient at increased risk of *Candida* infection. If phagocyte function is suppressed, these cells may actually serve to transport microbes out of the intestine and liberate them in extraintestinal sites (53). Such settings include hematological malignancy, bone marrow, and/or organ transplantation, and immunosuppressive therapy including cancer chemotherapy, corticosteroid therapy, and others. It is also worth noting that major sepsis and major injury, including burns, results in numerous defects in phagocytic function and in T-cell function (54,55). These are likely to be participatory in the emergence of *Candida* in these patient populations.

Total parenteral nutrition is a significant risk factor for *Candida* infection. This is likely to be multifactorial, including host immunosuppression and alterations in the GI tract related to absence of enteral feeding (56). In animal models, total parenteral nutrition induced macrophage suppression with decreased peritoneal macrophage superoxide production and *Candida* phagocytosis, associated with bacterial translocation to mesenteric lymph nodes (57).

**C. Colonization as a Major Risk Factor for Subsequent *Candida* Infection**

An important implication of this work on translocation from the intestinal tract is the potential utility of prophylaxis. The notion of an intestinal reservoir has led to a broader notion of colonization at intestinal and various extraintestinal sites as a prelude to infection. This is a central notion, suggesting points for interruption of this sequence. Clinical studies of antifungal prophylaxis in neutropenic cancer patients have shown that antifungal regimens effectively prevent hematogenous infection when they can eliminate or reduce colonization by *Candida* (58,59).
In critically ill patients, colonization with Candida spp. precedes and leads to infection. If multiple body sites are colonized, there will be an increased risk of severe infection in high-risk patients and the chance of invasion can be predicted by the extent of pre-existing colonization.

This notion is supported by studies demonstrating that 95% of neutropenic patients and 84% of non-neutropenic patients with documented fungal infection were infected with the same strains that had previously colonized them (60,61). Patients who were not colonized were significantly less likely to develop infection. In a study investigating the sequence of colonization and candidemia in non-neutropenic patients, Voss et al. (61) found that in patients with disseminated candidiasis, the strains recovered from the initial colonized or infected site and from the bloodstream were identical. Furthermore, nearly every patient was infected with a distinct or unique Candida strain. In another study involving 111 patients on bone marrow transplant and hematologic malignancy services, positive surveillance cultures were found to be highly predictive of systemic infection for tropicalis but not for albicans whereas negative surveillance cultures correlated with a low risk of candidal dissemination (62).

The density of colonization appears to be predictive of the risk of hematogenous candidiasis. In two large series of neutropenic cancer patients, hematogenous candidiasis almost never developed among noncolonized patients, compared to more than 30% infection among patients with multiple colonized sites (63,64). In another study by Richet et al. (65) among patients with acute lymphocytic leukemia, a relatively high concentration of Candida organisms in the stools was found to be a significant risk factor for hematogenous candidiasis. This also has been found in neonatal populations. In a study of 40 infants with very low birth weight, a value of $8 \times 10^6$ Candida colony-forming units (CFU)/g of stool was established as a threshold, beyond which GI symptoms (attributed to Candida) developed in 50% of the infants and a systemic septic response in 28.5% in the course of 1–3 weeks of heavy colonization (66,67). Fluconazole prophylaxis in this group is efficacious (68).

In general ICU populations, colonization is also a central risk factor for subsequent fungal infection. Pittet et al. (69) and colleagues performed a 6-month prospective cohort study among patients admitted to surgical and neonatal intensive care units in a 1600-bed university medical center. Routine microbiologic surveillance cultures at different body sites were performed. A Candida colonization index was determined daily, as the ratio of the number of distinct body sites (dbs) colonized with identical strains over the total number of dbs tested; a mean of 5.3 dbs per patient was obtained. All isolates ($n = 322$) recovered were characterized by genotyping using contour-clamped homogeneous electrical field gel electrophoresis that allowed strain delineation among these Candida species. Twenty-nine patients met the criteria for colonization; 11 patients (38%) developed severe infections (8 candidemia); the remaining 18 patients were heavily colonized but never required IV antifungal therapy. Among the potential risk factors for Candida infection, three discriminated the colonized from the infected patients—length of previous antibiotic therapy ($P < 0.02$), severity of illness assessed by APACHE II score ($P < 0.01$), and the intensity of Candida spp. colonization ($P < 0.01$). By logistic regression analysis, the latter two were the independent factors that predicted subsequent candidal infection.

Candida colonization always preceded infection with genotypically identical Candida spp. The proposed colonization indeces reached threshold values, a mean of 6 days before Candida infection, and demonstrated high positive predictive values.
The intensity of *Candida* colonization assessed by systematic screening helps predicting subsequent infections with identical strains in critically ill patients. Nolla, Leon, Roda, and colleagues performed a prospective observational study between May 1998 and January 1999 encompassing 73 ICUs in Spain. Patients were required to have stayed in the ICU for 7 or more days prior to entry. Surveillance cultures were performed weekly from tracheal aspirates, urine, and gut (oropharynx and gastric aspirates). The patients were considered *colonized* (appearance or persistence of *Candida* in surveillance cultures) or to have *invasive infection* (candidemia, endophthalmitis, peritonitis, or positive histology from an organ biopsy). *Multisite colonization* was defined as ≥2 sites positive from surveillance cultures. *Persistence* was defined as cultures of the same site revealing yeast for one or more weeks. 1766 consecutive patients were analyzed: 916 (58%) were in the colonized group, 158 with persistent multisite colonization, 359 with nonpersistent multisite colonization, 399 with single site colonization, and 107 invasive infection. Ninety-three of the 107 patients with invasive infection (87%) were previously colonized. Univariate analysis identified solid neoplasm, radiotherapy, parenteral nutrition, and hemodialysis, longer duration of ICU stay, non-*albicans*, and multisite colonization as significant risks for invasive infection. By stepwise logistic regression, persistent multisite colonization ([odds ratio (OR) 2.4, 95% confidence interval (CI) 1.3–4.2), radiotherapy OR 3.6, CI 1.1–12.3], hemodialysis (OR 2.7, CI 1.6–4.5), and colonization with non-*albicans* yeast (OR 1.8, CI 1.2–2.9) independently predicted invasive *Candida* infection.

While it is commonly stated that antibiotic administration itself is a major independent risk factor, in fact, this is not the case. In a case-controlled study, antibiotic administration was shown to be only marginally associated with candidemia and substantially less important than prior *Candida* colonization (70). There are many other factors that result in changes in the gastrointestinal flora. These include ileus, antacid therapy, and contamination with a hospital flora. The particular concern is that appropriate anti-infective therapy for a bacterial infection should not be stopped because *Candida* is identified at one or more sites. In intra-abdominal infections, mixed flora infections involving *Candida* and bacteria are the norm, rather than the exception.

V. WHO SHOULD RECEIVE ANTIFUNGAL PROPHYLAXIS IN THE ICU?

Recently, two prospective studies have been reported that add considerable clarity to this issue. Pelz and colleagues in the surgical ICU at Johns Hopkins Hospital conducted a prospective, randomized, placebo-controlled trial that entered 260 surgical patients with an anticipated length of ICU stay of at least 3 days (143). The single criteria for entry was the anticipation of 3 or more days in the ICU. The absence of any other entry criterion means that the results of the study cannot be transported to other critical care units. This unit had an extraordinarily high background incidence of *Candida* colonization and infection.

Patients were randomly assigned to receive either enteral fluconazole 400 mg/day or placebo during their stay in the ICU. The primary end point was the time of occurrence of fungal infection during the surgical ICU stay, with planned secondary analysis of patients “on-therapy” and alternate definitions of fungal infections. The risk of candidal infection in patients receiving fluconazole was significantly less than the risk in patients receiving placebo. After adjusting for potentially confounding
effects of the Acute Physiology and Chronic Health Evaluation (APACHE) III score, days to first dose, and fungal colonization at enrollment, the risk of fungal infection was reduced by 55% in the fluconazole group. No difference in death rate was observed between patients receiving fluconazole and those receiving placebo.

The key data elements from these studies are presented in Tables 1 and 2. It is apparent that colonization was far and away the most significant risk factor for candidemia, with a 10-fold higher likelihood of infection in colonized vs. non colonized patients. This means that of the 20 infected patients in the placebo group, only one or two were not colonized. Review of the conditions presented reveals that the main differences were in colonized catheters, a condition not believed to warrant therapy in the absence of candidemia, and in peritonitis. The benefits of antifungal prophylaxis in patients operated upon for postoperative infection have been previously identified (95).

Garbino et al. (71) performed a well-designed study to assess the effectiveness of adding fluconazole to a selective digestive decontamination regimen to prevent candidal infections. This study was a prospective, randomized, double blind, and placebo-controlled trial among medical and surgical intensive care unit patients at a large university hospital. All adult patients mechanically ventilated for at least 48 hr with an expectation to remain so for at least an additional 72 hr, and receiving selective decontamination of the digestive tract were eligible for entry. Patients were randomly assigned fluconazole 100 mg daily \((n = 103)\) or placebo \((n = 101)\). Candida infections occurred less frequently in the fluconazole group \(5.8\%\) than in the placebo group \(16\%;\) rate ratio 0.35; 95% CI 0.11–0.94) (Tables 3 and 4). Almost all candidemia episodes occurred in the placebo group (rate ratio for fluconazole use 0.10; 95% CI 0.02–0.74). The rate of treatment failure, development of candidal

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Results of a Prospective Randomized Trial of Prophylaxis with Fluconazole for Candida Infection in the ICU (151)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Fluconazole ((n = 130))</td>
</tr>
<tr>
<td>Candida infection</td>
<td>11 (8.5%)</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>3</td>
</tr>
<tr>
<td>Candidemia</td>
<td>1</td>
</tr>
<tr>
<td>Catheter</td>
<td>1</td>
</tr>
<tr>
<td>(C. albicans)</td>
<td>5 (45%)</td>
</tr>
<tr>
<td>(C. glabrata)</td>
<td>3 (27%)</td>
</tr>
<tr>
<td>Mortality</td>
<td>14 (11%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Multivariate Analysis of Predictors of Failure in a Randomized Trial of Fluconazole 400 mg q.d. Enterally vs. Placebo (151)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk ratio</td>
<td>95% Confidence interval</td>
</tr>
<tr>
<td>Randomization to fluconazole</td>
<td>0.45</td>
</tr>
<tr>
<td>Fungal colonization</td>
<td>10.64</td>
</tr>
<tr>
<td>APACHE III</td>
<td>1.02</td>
</tr>
<tr>
<td>Days to first dose of study drug</td>
<td>1.34</td>
</tr>
</tbody>
</table>
infection, or increased colonization was 30% in the fluconazole group and 66% in the placebo group \((P < 0.001)\). Crude in-hospital mortality was similar in the two groups (39% fluconazole vs. 41% placebo) (Fig. 4). The authors concluded that prophylactic use of fluconazole in a selected group of mechanically ventilated patients at high risk for infection reduces the incidence of \textit{Candida} infections, in particular, candidemia.

An important feature of this study is the use of low-dose fluconazole, a 200-mg loading dose and then 100 mg/day. This lower dose (vs. that used in the Pelz study) was justified by the high incidence of \textit{C. albicans}, 80% of identified colonizing isolates prior to study entry. Those patients who acquired yeast colonization on this dose maintained a similar species pattern, as did the infected patients. Conversely, only 45% of infecting isolates were \textit{C. albicans} in the fluconazole-treated patients in the Pelz study, vs. 60% in the placebo-treated patients. The lower dose is preferred because of its significantly lower cost, and its clearly demonstrated efficacy; it would also appear to have less drastic effects on the pattern of colonizing and infecting isolates.

Taken together, the results of these two studies would argue that patients known to be at high risk of colonization should be placed on lower-dose fluconazole prophylaxis. The authors of the Garbino study have settled on 200 mg/day as a standard prophylaxis dose. In the case of ICU patients, it may be more cost effective to perform surveillance cultures as done in the EPCAN study on a weekly basis. These cultures are done on yeast-selective media and extensive genus and species workup are not needed.

<table>
<thead>
<tr>
<th>Characteristics of 204 Patients Receiving Mechanical Ventilation and Selective Digestive Decontamination with Antibacterial Agents, Randomized to 100 mg Fluconazole Enterally or Placebo</th>
<th>Fluconazole ((n = 103))</th>
<th>Placebo ((n = 101))</th>
</tr>
</thead>
<tbody>
<tr>
<td>APACHE II</td>
<td>21.9</td>
<td>21.3</td>
</tr>
<tr>
<td>Colonized at entry</td>
<td>48 (47%)</td>
<td>50 (40%)</td>
</tr>
<tr>
<td>Newly colonized after study entry</td>
<td>29/55 (53%)</td>
<td>40/51 (78%)</td>
</tr>
<tr>
<td>Colonization index</td>
<td>0.56 ± 0.25</td>
<td>0.53 ± 0.21</td>
</tr>
<tr>
<td>Parenteral nutrition</td>
<td>28 (27%)</td>
<td>30 (30%)</td>
</tr>
</tbody>
</table>

\textit{Source: From Ref. 71.}

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Outcome Results from the Study by Garbino and Colleagues (70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients colonized at study entry</td>
<td>48 (47%)</td>
</tr>
<tr>
<td>Number of patients newly colonized after study entry</td>
<td>29/55 (53%)</td>
</tr>
<tr>
<td>\textit{Severe infections}</td>
<td></td>
</tr>
<tr>
<td>Candidemia</td>
<td>1</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>1</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>2</td>
</tr>
</tbody>
</table>
VI. SPECIAL POPULATIONS AT RISK OF CANDIDA INFECTIONS AND POSSIBLY MERITING PROPHYLAXIS

A. Liver Transplantation

The incidence and mechanism of microbial entry vary in different groups of transplant recipients, depending on the organ transplanted, the donor source, the type of surgical procedure performed, and the recipient’s age and general condition at the time of the procedure. Other influential factors are the conditioning regimen, the type and duration of immunosuppressive therapy, and the presence or absence of organ rejection, and graft vs. host disease. In heart transplant recipients, for example, *Aspergillus* infection is a major problem (72), whereas in other organ transplant recipients, most fungal infections are attributable to *Candida* (73). The infection is usually located at the site of the operation; an intra-abdominal abscess in liver or pancreas transplantation, the mediastinum or the lungs in heart or heart–lung transplantation, and the urinary tract in kidney transplantation; however, dissemination from the primary site is common (73–75).

Several studies have documented the efficacy of amphotericin B, liposomal amphotericin B, and fluconazole in preventing *Candida* infection (76–79). The incidence of *Candida* infection in patients not receiving prophylaxis varies between 10% and 20%, and prophylaxis is cost effective.

The results of a recently reported randomized trial illustrate the benefits of prophylaxis (80). Fungal colonization increased in patients who received placebo (from 60% to 90%) but decreased in patients who received fluconazole (from 70% to 28%). Proven fungal infection occurred in 45 of 104 placebo recipients (43%), but in only 10 of 108 fluconazole recipients (9%) \(P < 0.001\). Fluconazole prevented both superficial and invasive infection. It also prevented infection by most *Candida* species, except *C. glabrata*. Patients receiving fluconazole had higher serum cyclosporine levels and more adverse neurological events (headaches, tremors, or seizures in 13 fluconazole recipients compared to 3 placebo recipients; \(P = 0.01\)). Although the overall mortality rate was similar in both groups, [12 of 108 (11%) in the fluconazole group compared with
15 of 104 (14%) in the placebo group; $P > 0.2$, fewer deaths related to invasive fungal infection were seen in the fluconazole group [2 of 108 (2%) patients] than in the placebo group [13 of 104 (13%) patients] ($P = 0.003$).

Five recent studies of antifungal prophylaxis provided additional support for the use of antifungal agents to prevent serious fungal infections in patients undergoing liver transplantation (81–85).

B. Acute Necrotizing Pancreatitis

There is an increasing appreciation for the role of *Candida* in infections following acute pancreatitis (86–88). A large series of patients undergoing operation for infected pancreatic necrosis found *Candida* present in approximately 10% of the patients at their initial operation for infection (89). These patients had received prophylaxis with amoxicillin/clavulanate, a factor that might explain intestinal overgrowth and translocation of *Candida*. This is a particular issue because of the interest in the use of broad-spectrum antibiotics, especially imipenem/cilastatin, as prophylaxis for patients with necrotizing pancreatitis. Current experimental evidence favors the use of prophylactic antibiotics in severe acute pancreatitis. The results of contemporary randomized clinical trials, restricted to patients with prognostically severe acute pancreatitis, have demonstrated improvement in outcome associated with antibiotic treatment (90–92).

The incidence of fungal infection in antibiotic treated patients was demonstrated in a study of 250 consecutive patients treated with amoxicillin/clavulanate with severe acute pancreatitis treated from January 1986 to December 1998 (89). Overall mortality was 38.8% (97 patients). 182 patients (72.8%) suffered from infected necrosis. Among these patients, local *Candida* infection was observed in 31 patients whereas 23 patients (74%) suffered from local fungal infection detected at first operation. During the course of disease, 12 patients (39%) also experienced fungemia. Local *Candida* infection as compared to no *Candida* infection was associated with an increased mortality rate (84% vs. 32% $P = 0.0001$). Multivariate logistic regression analysis identified APACHE II score ($P < 0.0001$), age of the patient ($P < 0.003$), extent of pancreatic necrosis ($P < 0.002$), and local bacterial ($P < 0.04$) and fungal infection ($P < 0.004$) as independent factors significantly contributing to mortality.

It is believed that the appropriate strategy for such patients, a group similar to the population included in the Garbino study of patients receiving selective digestive decontamination, is to provide low-dose fluconazole enterally.

C. Postoperative Intra-Abdominal Infection

Because of the reported high incidence of *Candida* infection in patients with postoperative intra-abdominal infection (93,94), a randomized trial was undertaken in 49 surgical patients with recurrent gastrointestinal perforations or anastomotic leakages (95). Prophylaxis with IV fluconazole (400 mg/day) or placebo continued until resolution of the underlying surgical condition. Among patients who were not colonized at study entry, *Candida* was isolated from surveillance cultures during prophylaxis in 15% of the patients in the fluconazole group and in 62% of the patients in the placebo group (relative risk, 0.25; 95% CI 0.07–0.96; $P = 0.04$). *Candida* peritonitis occurred in 1 of 23 patients (4%) who received fluconazole, and in 7 of 20 patients (35%) who received placebo (relative risk, 0.12; 95% CI 0.02–0.93;
Fluconazole prophylaxis prevents colonization and invasive intra-abdominal Candida infections in high-risk surgical patients.

VII. MANAGEMENT OF SPECIFIC INFECTIONS

A. Candidemia

Candidemia is defined as the isolation of any pathogenic species of Candida from at least one blood culture specimen. The recovery of Candida species from the bloodstream is a significant observation, especially if the patient is debilitated, uremic, or receiving immunosuppressive therapy.

There is a general consensus that all episodes of candidemia require therapy (96). The particular concern is the ability of at least C. albicans to form vegetations on previously normal heart valves and to establish metastatic abscesses. While the invasiveness of other species is lessened, it seems wisest to treat all episodes.

B. Is Fluconazole Sufficient for Empiric Antifungal Therapy?

Fluconazole is now considered the primary treatment of choice for candidemia, particularly, if caused by C. albicans. This recommendation is based on several recent comparative studies of amphotericin B and fluconazole (97–100). In each of these trials, efficacy was similar, and the incidence of dose-limiting toxicities was significantly lower in persons treated with fluconazole. Therefore, fluconazole has supplanted amphotericin B as the primary treatment for uncomplicated candidemia.

The primary concern with the use of fluconazole for empiric therapy is the possibility that a resistant strain may be present, and the belief that amphotericin B, as a cidal agent, may be more efficacious in patients with shock or other evidence of a severe physiologic response to infection (96). One approach that has been recently studied in a prospective randomized trial is use of combination fluconazole/amphotericin therapy (101). This multicenter trial was conducted to compare fluconazole (800 mg/day) plus placebo with fluconazole plus amphotericin B (AmB) deoxycholate (0.7 mg/kg/day, with the placebo/AmB component given only for the first 5–6 days) as therapy for candidemia resulting from species other than Candida krusei in adults without neutropenia. This study addressed, inter alia, the adequacy of this combination regimen as empiric therapy. A total of 219 patients met criteria for a modified intent-to-treat analysis. The groups were similar except that those who were treated with fluconazole plus placebo had a higher mean (±standard error) APACHE II score (16.8 ± 0.6 vs. 15.0 ± 0.7; P = 0.039). Success rates on study day 30 by Kaplan–Meier time-to-failure analysis were 57% for fluconazole plus placebo and 69% for fluconazole plus AmB (P = 0.08). Overall success rates were 56% (60 of 107 patients) and 69% (77 of 112 patients; P = 0.043), respectively; the bloodstream infection failed to clear in 17% and 6% of subjects, respectively (P = 0.02). In non-neutropenic subjects, the combination of fluconazole plus AmB was not antagonistic compared to fluconazole alone, and the combination tended toward improved success and more rapid clearance from the bloodstream (101). No statistically significant difference was found by the prespecified analysis of time to failure between the two arms of the study. However, in the fluconazole plus placebo group, 44% of 107 infections had failed to respond to therapy by 30 days after the initiation of treatment, significantly more than the 31% of 112 patients who received fluconazole plus AmB. Moreover, positive blood cultures were
obtained after treatment in 17% of the fluconazole plus placebo patients vs. 6% of those provided fluconazole plus AmB ($P = 0.02$). The data suggest that coadministration of fluconazole plus AmB resulted in a slightly better outcome with the combination. It is unlikely that a study will be done comparing fluconazole to fluconazole plus an echinocandin. Since echinocandins are fungicidal, it may be particularly useful to use these agents in patients with evidence of hemodynamic compromise. Patients who have previously received fluconazole should receive an echinocandin or a polyene (amphotericin B or its lipid formulations).

As more experience has accumulated with fluconazole therapy, particularly recognition that it is efficacious for use in *Candida* infection, the issue of its adequacy as empiric therapy is raised. The answer to this depends primarily on whether or not the patient has received prior antifungal therapy (102,103). *C. albicans* has remained susceptible (26,102,103). There appears to have been a shift toward more frequent isolation of *Candida glabrata*, although this appears highly center dependent. It is also not clear that this is because of widened usage of fluconazole (104). In one large prospective observational study performed at four teaching centers, 13% of candidemias occurred in patients who were already receiving systemic antifungal agents. Candidemias developing while receiving antifungal therapy were more likely caused by non-*C. albicans* species than by *C. albicans* species ($P = 0.0005$). *C. parapsilosis* and *C. krusei* were more commonly seen with prior fluconazole therapy whereas *C. glabrata* was more commonly seen with prior amphotericin B therapy. *Candida* species isolated during episodes of breakthrough candidemia exhibited a significantly higher MIC to the antifungal agent being administered ($P < 0.001$) (105).

**C. Duration of Therapy**

A further issue is the notion of at least two forms of candidemia. One early study identified a group of patients with transient candidemia who had good outcomes despite receiving no antifungal therapy (106). Patients in this group had no underlying disease, had <1 day of documented candidemia, and had central IV catheters removed. A high percentage of these patients were found to be receiving parenteral nutrition, as compared to those patients with prolonged candidemia (66% vs. 24%; $P = 0.002$, $\chi^2$ test). In contrast, patients with documented sustained candidemia (>2 days duration) had a mortality rate of 74%.

The authors of this study then performed a prospective trial of management based on the duration of candidemia. Patients in the transient candidemia group received a total of 200 mg of amphotericin B administered over a 5- to 7-day interval. Patients with persisting candidemia received longer-term therapy to 500 mg total dosing. There were no relapses in either group. One patient in the short course group discontinued amphotericin B prematurely because of an adverse event. Complications of amphotericin B therapy were more common in the prolonged therapy group (68% vs. 38%; $P = 0.02$, $\chi^2$ test). Hypomagnesemia (<2.0 mg/dL) was more common in the prolonged therapy group (55% vs. 7%; $P < 0.001$, $\chi^2$ test). There was a trend toward a higher incidence of elevated creatinine levels (>50% rise from baseline) during treatment with amphotericin B in the prolonged therapy vs. short course group (34% vs. 17%; $P = 0.1$, $\chi^2$ test). The 30-day mortality rate for patients with complicated candidemia (invasive disease and/or a significant underlying condition) who were not eligible for the study was 50%, vs. 19% in the two study groups combined ($P < 0.01$, $\chi^2$ test) (34).
Duration of therapy depends on the extent and seriousness of the infection. Therapy can be limited to 7–10 days for patients with catheter-related and low-grade fungemia, without evidence of organ involvement or hemodynamic instability. On the other hand, patients with high-grade fungemia, evidence of organ involvement or hemodynamic instability need to receive antifungal therapy for 10–14 days after resolution of all signs and symptoms of infection.

VIII. CATHETER MANAGEMENT IN CANDIDEMIC PATIENTS

Candidemias seen in the intensive care unit may be catheter related. This is defined as candidemia occurring in a patient with an intravascular catheter and no other obvious site of origin of infection after careful clinical and laboratory evaluation. Several procedures have been developed to aid in the diagnosis of catheter-associated candidemia. If the catheter is removed, a quantitative culture of the tip should recover at least 15 CFU of the same *Candida* species as that found in blood culture by the roll-plate technique, or at least 100 CFU of the same *Candida* species as that found in blood culture by the sonication technique (107,108). If the catheter is not removed, a quantitative blood culture collected through a central catheter should contain at least a 10-fold greater concentration of *Candida* species than a simultaneously collected quantitative peripheral blood culture. Routine catheter tip cultures are of no value (109).

The role of central venous catheters as a factor predicting outcome in candidemia has been evaluated in recent reports. Nguyen et al. (110) reported that catheter-related candidemia had a more favorable prognosis compared to candidemia from other sources, but that prognosis was worse in patients whose catheters were retained. This was supported by a study for duration of candidemia as function of catheter removal in patients entered in a clinical trial of fluconazole vs. amphotericin B (111). A study of candidemia in Brazilian referral hospitals similarly identified only advanced age and catheter retention as significant associates of death (31).

An analysis of 363 patients who had a central venous catheter in place and received antifungal therapy revealed that catheter exchange was associated with improved outcome (80% vs. 54%, \( P < 0.001 \)). However, the no-exchange group of patients had a higher APACHE III scores and were more likely to be neutropenic. By multivariate analysis, catheter retention was not found to significantly affect outcome.

These data suggest that catheters may play a role in perpetuating infection in non-neutropenic patients. On the other hand, the primary source of candidemia is usually the gastrointestinal tract and not the IV catheter in the setting of immunosuppression (neutropenia, corticosteroids, or other immunosuppressants); other factors such as severity of disease, visceral dissemination and recovery of neutrophils, and other immune parameters appear to have more impact on the outcome of candidemia in immunosuppressed patients.

The formation of a biofilm in these infections is likely to be an important determinant of the need to remove the catheter. Histologically, biofilms consist of a dense network of yeasts, germ tubes, pseudohyphae, and hyphae (112). The ability of a species of *Candida* to form biofilm in vitro correlates with its pathogenicity in vivo. Antifungal therapy is not highly effective in removing biofilm from catheters (113). This is particularly important in patients infected with *Candida parapsilosis* (which is more likely to be catheter-related than infection with other *Candida* species) (114).
The decision to remove the vascular catheter in patients with candidemia must be individualized and depends partly upon the difficulty of inserting a catheter at a new site (Fig. 5).

The use of thrombolytic therapy for infections of surgically implanted catheter has been reported with variable success, particularly, in pediatric hematology patients.

**IX. DESCRIPTIONS OF SPECIFIC AGENTS**

**A. Imidazoles**

1. **Fluconazole**

The mechanism of action of fluconazole is preferential inhibition of cytochrome P450 enzymes in fungal organisms. Fluconazole is active against several *Candida* species, including *C. tropicalis*. *C. krusei*, however, is highly resistant to this agent (25,26,115–117). Fluconazole is available in either an oral form or an IV form, both of which are rapidly and almost completely absorbed from the GI tract. The serum concentrations after oral administration are almost identical to those achieved when the drug is administered intravenously. A major advantage of fluconazole over ketoconazole is its high degree of GI absorption, which is not affected by gastric acidity or the presence of food. Steady-state serum concentrations of fluconazole are obtained within 5–10 days. An initial loading dose that is twice the usual daily dose is recommended. Fluconazole is distributed evenly in body tissues, penetrates into the vitreous humor and the aqueous humor of the eye, and crosses the blood–brain barrier. The drug is excreted largely unchanged in the urine, with only minimal liver metabolism (118–120). Consequently, dosage schedules must be adjusted in patients with renal impairment. Hemodialysis significantly reduces the serum concentrations, and the drug also appears to be removed by peritoneal dialysis. A standard dose should be given after each course of dialysis.
The toxicities of fluconazole are similar to those of other azoles and include nausea and vomiting in about 2% of patients, headache, fatigue, abdominal pain, and diarrhea; exfoliative dermatitis also occurs, but very rarely (121). Transient abnormalities of liver function have been observed in 3% of patients receiving fluconazole. In addition, fatal hepatic necrosis developed in two patients who were receiving fluconazole, but it was unclear whether the agent played a causal role in this event. No significant hormonal abnormalities have been reported after administration of fluconazole.

2. Itraconazole

Itraconazole is highly lipophilic and is tightly bound to blood cells and plasma proteins, primarily albumin, leaving only 0.2% unbound (122). It is metabolized primarily in the liver, and the single dose pharmacokinetics is not affected by renal dysfunction. The erratic absorption and reduced bioavailability of the capsule form has been overcome with the introduction of a liquid formulation in cyclodextrin (123).

Itraconazole’s main metabolite, hydroxyitraconazole, reaches higher plasma concentrations than the parent compound and has in vitro antifungal activity similar to that of itraconazole (122).

Itraconazole, and to a lesser extent fluconazole (in high doses) are inhibitors of CYP3A4. Therefore, certain agents that are substrates of this enzyme, such as some of the new generation of H1-antihistamines, several HMG-CoA reductase inhibitors, and certain benzodiazepines, are contraindicated. Other drugs like cyclosporine and quinidine need careful monitoring if administered concurrently with these triazoles. Because fluconazole interacts with warfarin, phenytoin, and cyclosporine when given in a daily dose of 200 mg or more, serum concentrations of these agents should be monitored.

B. Voriconazole

Voriconazole is a second-generation azole antifungal agent that shows excellent in vitro activity against a wide variety of yeasts and molds. Voriconazole is a derivative of fluconazole that demonstrates enhanced in vitro activity against existing and emerging fungal pathogens. It can be given by either the IV or the oral route; the oral formulation has excellent bioavailability.

It has proven efficacy for treating Candida infections and invasive aspergillosis as well as other mould infections, such as those caused by Fusarium and Scedosporium spp. (124). Voriconazole has three important side effects that the clinician must consider: liver abnormalities, skin abnormalities, and visual disturbances. Liver abnormalities, in particular, should be monitored very carefully. The drug interaction profile of voriconazole also warrants a careful evaluation of the concomitant medications because of its inhibition of cytochrome P450. It should be noted that safety considerations related to the cyclodextrin carriers limit the IV formulation of itraconazole to administration for no longer than 14 days, and that the comparable formulation of voriconazole should not be administered to patients with a creatinine clearance of \(<50\) mL/min (package inserts).

The side effect profile of voriconazole is unique in that transient visual disturbances that do not threaten eyesight occur in \(~30\)% of patients given the drug. Rashes (which can manifest as photosensitivity) and hepatitis also occur. The potential for
drug-drug interactions is high and requires that careful attention be given to dosage regimens, monitoring of serum levels, and effects of interacting drugs (125).

Voriconazole has been approved for the treatment of invasive aspergillosis and refractory infections with *Pseudallescheria/Scedosporium* and *Fusarium* species and will likely become the drug of choice for treatment of serious infections with those filamentous fungi.

The in vitro activities of voriconazole, posaconazole, ravuconazole, and micafungin were compared with those of fluconazole, itraconazole, ketoconazole, flucytosine, and amphotericin B against 164 candidemia isolates recovered from cancer patients in two Canadian centers (126). The MIC (50) for ravuconazole, voriconazole, posaconazole, and micafungin were 0.01, 0.03, 0.12, and 0.25 mg/L, respectively. The new antifungal agents showed substantial activity against isolates demonstrating in vitro resistance to fluconazole and itraconazole. These results suggest that the newer antifungal agents possess promising activity against invasive *Candida* isolates, particularly against those with reduced susceptibility to fluconazole and itraconazole.

**C. Amphotericin B**

Amphotericin B is structurally similar to membrane sterols, and its major mechanism of action is believed to be through interaction with membrane sterols and creation of pores in the fungal outer membrane (115,127). The clinical usefulness of amphotericin B is attributable to the greater affinity of amphotericin B for ergosterol (found in fungal cell membranes) than for cholesterol (the principal sterol found in mammalian cell membranes). Oxidation-dependent amphotericin B-induced stimulation of macrophages is another proposed mechanism of action (128,129). Most species of fungi that cause human infections are susceptible to amphotericin B.

Amphotericin B (AmB) is active against most systemic fungal infections. It is supplied as an AmB–deoxycholate complex suitable for IV administration. The association between AmB and deoxycholate is relatively weak; therefore, dissociation occurs in the blood. The drug itself interacts with both mammalian and fungal cell membranes to damage cells, but the greater susceptibility of fungal cells to its effects forms the basis for its clinical usefulness.

The use of AmB is associated with frequent and potentially severe side effects, including infusion-related events such as fever, rigors, and hypotension, as well as metabolic derangements such as hypokalemia and nephrotoxicity (130). The frequency of occurrence of such events may be as high as 80%. Infusion-related toxicities (e.g., fever, chills, and rigors) are likely due to AmB stimulation of cytokine and prostaglandin synthesis (131,132). Nephrotoxicity, the primary non-infusion-related toxicity, is likely to result from the nonselective cytotoxic interaction between AmB and cholesterol-containing mammalian cells (133). An acute infusion-related reaction, consisting of fever, hypotension, and tachycardia, occurs in about 20% of patients (134). Premedication regimes with, for example, acetaminophen, or hydrocortisone, are of little if any value. Meperidine (25–50 mg IV) will alleviate fevers and chills if given after they occur (135,136). Hypotension, hypertension, hypothermia, and bradycardia are other reported infusion-related toxic effects of amphotericin B deoxycholate. Ventricular arrhythmias have been associated with administration of amphotericin B deoxycholate in patients with severe hypokalemia, renal failure, or those in whom the infusion was rapidly given. Amphotericin B suppresses production of red blood cells and causes a normocytic, normochromic
anemia. This is because of the inhibition of erythropoietin production is secondary to nephrotoxicity.

Common practice has been to give a 1-mg test dose and observe the patient for 1 hr in the hope of identifying patients at risk for severe acute reactions. The full dose of the drug (0.6–1 mg/kg/day) is then infused over a period of 4–6 hr, although recent data suggest that much shorter infusion times (e.g., 1 hr in patients with adequate cardiopulmonary and renal function) may be acceptable (137). The total dose depends on the extent of the infection and the patient’s condition. Patients must be monitored carefully during the first day of therapy. The infusion should be discontinued if the patient becomes hemodynamically unstable.

Renal toxicity and hypokalemia are the primary toxicities of amphotericin B (138–140). Amphotericin B-induced nephrotoxicity may be glomerular (decrease in glomerular filtration rate and renal blood flow) or tubular (urinary cast, hypokalemia, hypomagnesemia, renal tubular acidosis, and nephrocalcinosis). All these abnormalities occur to varying degrees in almost all patients receiving the drug. Renal dysfunction gradually resolves after discontinuation of therapy in most patients.

Amphotericin B nephrotoxicity may be minimized by avoiding other agents with synergistic nephrotoxicity (e.g., aminoglycosides, vancomycin, cisplatin, and cyclosporine), and the administration of sodium supplementation. The latter approach consists of the IV infusion of 500 ml of 0.9% saline solution 30 min before the administration of amphotericin B and a second infusion of the same amount of saline after the amphotericin B infusion is completed (141,142).

The combined use of amphotericin B deoxycholate and other nephrotoxic agents (cyclosporine, aminoglycosides, fosfomycin, and others) may result in synergistic nephrotoxicity.

D. Lipid Formulations of Amphotericin B

Newer therapeutic options have now become available with the advent of the lipid-associated formulations of amphotericin B, which are less nephrotoxic than the parent compound (143,144). So far, three lipid products of amphotericin B have been marketed in Europe and in the United States: Abelcet (amphotericin B lipid complex), Amphocil (amphotericin B colloidal dispersion), and AmBisome (liposomal amphotericin B). A prospective randomized trial has shown that Abelcet was as efficacious as conventional amphotericin B in hematogenous candidiasis (145).

Lipid formulations of amphotericin B are less nephrotoxic. These preparations differ in the amount of amphotericin B and the type of lipid used as well as in the physical form, pharmacokinetics and toxicities. Studies comparing lipid formulations of Amphotericin to the parent compound have shown a reduction in nephrotoxicity. In addition, AmBisome appears to reduce the incidence of acute infusion-related adverse events and hypokalemia, and to be better tolerated than amphotericin B lipid complex. However, given the great cost of these formulations and incomplete avoidance of amphotericin B toxicity, their use should be restricted to patients with significant renal impairment or patients failing on AmB therapy who cannot be treated with newer imidazoles or echinocandins.
E. The Echinocandins

The echinocandins are large lipopeptide molecules that are inhibitors of beta-(1,3)-glucan synthesis, an action that results in disruption of the fungal cell wall, in turn resulting in osmotic stress, lysis, and death of the microorganism (126,146). Two other echinocandins, anidulafungin and micafungin, are near FDA approval (146,147). In vitro and in vivo, the echinocandins are rapidly fungicidal against most Candida spp. and fungistatic against Aspergillus spp. No drug target is present in mammalian cells. The first of the class to be licensed was caspofungin, for refractory invasive aspergillosis (about 40% response rate). Adverse events are generally mild, including (for caspofungin) local phlebitis, fever, abnormal liver function tests, and mild hemolysis (148,149). Poor absorption after oral administration limits use to the IV route. Dosing is once daily and drug interactions are few. The echinocandins are widely distributed in the body and are metabolized by the liver.

Results of studies of caspofungin in candidemia and invasive candidiasis demonstrate equivalent efficacy to amphotericin B, with substantially fewer toxic effects (150). The results of a trial comparing caspofungin to amphotericin B are detailed in Table 5. This study randomizes patients with invasive candidiasis (83% had candidemia and 10% had peritonitis) to receive either caspofungin (70 mg on day 1, then 50 mg q.d.) or conventional amphotericin B (0.6–1.0 mg/kg q.d.) for 14 days (149). 46% of infections were caused by C. albicans, 19% by C. parapsilosis, 16% by C. tropicalis, and 11% by C. glabrata; C. krusei, and C. guilliermondii accounted for the rest. Success was defined as both symptom resolution and microbiological clearance. By a modified intent-to-treat analysis of the 224 eligible randomized patients who received at least a single dose of their assigned therapy, success was observed in 73% of caspofungin recipients and in 62% of amphotericin B recipients (P = 0.09). Among patients who received ≥5 days of therapy, success was achieved in 81% of caspofungin recipients and in 65% of amphotericin B recipients (P = 0.03).

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Results from a Randomized Trial of Caspofungin Vs. Amphotericin B in the Management of Invasive Candida Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caspofungin 70/50 mg (n = 109)</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
</tr>
<tr>
<td>Failure (end of Rx)</td>
<td>29 (26.6)</td>
</tr>
<tr>
<td>Persistently (+) cultures</td>
<td>9 (8.3)</td>
</tr>
<tr>
<td>Persistent signs/symptoms</td>
<td>6 (5.5)</td>
</tr>
<tr>
<td>New lesions at distant sites</td>
<td>4 (3.7)</td>
</tr>
<tr>
<td>Toxicity requiring additional Rx*</td>
<td>3 (2.7)</td>
</tr>
<tr>
<td>Withdrawal ≤ 4 days/indeterminate</td>
<td>7 (6.4)</td>
</tr>
<tr>
<td>Relapse (6–8 weeks post-Rx)</td>
<td>7 (6.4)</td>
</tr>
<tr>
<td>Recurrent candidemia</td>
<td>3 (2.7)</td>
</tr>
<tr>
<td>Nonblood Candida infection</td>
<td>2 (1.8)</td>
</tr>
<tr>
<td>Received systemic antifungal Rx</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>Abscess (no culture and no Rx)</td>
<td>1 (0.9)</td>
</tr>
</tbody>
</table>

Source: From Ref. 150.

*P = 0.028.
Significantly, adverse events occurred more frequently among amphotericin B recipients than among caspofungin recipients; discontinuation of therapy as a result of drug-related adverse events was observed in 23% and 3% of recipients, respectively.

X. RECOMMENDATIONS FOR THERAPY—A CARE PATH

Figure 6 is an algorithm that has been developed for use at the University of Cincinnati. This algorithm is provided to aid the development of care paths for management of *Candida* infections. Therapy is divided into prophylaxis, empiric, and directed. Indications for prophylaxis in the general ICU population center upon

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**Figure 6** A care path for antifungal therapy in the ICU.
the presence of colonization. There are, nonetheless, specific conditions that mandate prophylaxis with fluconazole in the absence of colonization. These include status post-intervention for a post-operative infection (95), necrotizing pancreatitis, particularly if parenteral prophylaxis with broad-spectrum antibacterial agents is administered (89), and units with a high (>10%) incidence of yeast infections (70A). For these patients, prophylaxis with fluconazole is directed at \textit{C. albicans}, absent evidence that fluconazole prophylaxis will decrease infections with other \textit{Candida} species. With the high level of fluconazole activity against \textit{C. albicans}, dosing with 400 mg load and 200 mg/day enterally with adjustments for renal failure is recommended (71). Prophylaxis is continued until the risk of infection is diminished, especially until transfer out of ICU. Prophylaxis will decrease infection by 50–70%.

The indications for empiric therapy in non-neutropenic patients center primarily on patients with sudden onset of signs of infection and documented colonization, particularly, in the absence of prior colonization. All patients with candidemia should receive antifungal therapy. If such patients have not previously received prophylaxis or therapy with fluconazole, then, therapy with fluconazole (800 mg loading dose with 400 mg daily) is indicated until speciation is available. Despite evidence that combination therapy with amphotericin B may improve outcomes, acute responses do not seem to be altered by this strategy.

If the patient has received fluconazole treatment, initial therapy should be with caspofungin or amphotericin B (or its lipid formulations). Voriconazole may be a suitable alternative but sufficient data to support this recommendation are not available.

With the availability of highly active agents, including fluconazole and caspofungin, there is now little role for amphotericin B. In the ICU, the toxicity of amphotericin B, when added to the severity of illness of patients with \textit{Candida} infections, becomes a significant risk for increased morbidity and mortality.

**XI. OTHER SITES OF \textit{CANDIDA} INFECTIONS**

**A. Candida Endophthalmitis**

The diagnosis of candidal endophthalmitis usually implies hematogenous spread to multiple organs and thus systemic antifungal therapy is warranted. Patients with chorioretinitis alone respond better to drug therapy than those with vitreal involvement because of the lower drug penetration in the vitreous body than in the other ocular compartments (152). Patients with vitreal involvement will require early vitrectomy in addition to antifungal therapy. Fluconazole is currently the drug of choice because of its proven efficacy and its better ocular tissue concentration including in the vitreous body (20–70% of corresponding plasma level) (153,154). A daily fluconazole dose of 800 mg is recommended until a major response has been observed at which time a reduction of the dose to 400 mg/day may be possible. While endophthalmitis due to \textit{C. albicans} is commonly seen, more recent series have reported on the importance of endophthalmitis by non-\textit{C. albicans} spp. If the infecting organism is potentially resistant to fluconazole (especially \textit{C. krusei}), initiation of therapy with IV amphotericin (0.7–1.0 mg/kg/day IV) preferably in conjunction with flucytosine is recommended. Intravitreal injection of amphotericin B is also recommended in the presence of vitreal infection.

The optimal duration of therapy for endogenous endophthalmitis is not defined, but it is recommend that it be given for at least 10–14 days after complete
resolution of all signs and symptoms of infection. Ophthalmologic consultation is critical in establishing the diagnosis, assessing the patient’s response to therapy, detecting complications, and determining whether early vitrectomy is indicated to prevent loss of sight (155).

B. Suppurative Thrombophlebitis

A rare but serious consequence of hematogenous candidemia is suppurative thrombophlebitis, which results from infection of a vessel traumatized by prolonged catheterization. Endothelial disruption exposes the basement membrane and leads to thrombus formation and propagation. Suppurative thrombophlebitis is particularly serious because intravascular infection results in a persistent high-density fungemia. Management of this disease consists of high-dose antifungal therapy, removal of the central venous catheter, and excision of the infected vein, when possible (156,157). Typically, blood cultures remain positive for several days; sometimes, they remain positive for as long as 3–4 weeks despite appropriate antifungal therapy, if the infected vein is not excised.

C. Superficial Mucosal Infection

Oral candidiasis (thrush) appears as a whitish, patchy pseudomembrane covering an inflamed oropharynx and commonly involves the tongue, the hard and soft palates, and the tonsillar pillars. Controlled trials have documented the efficacy of nystatin suspension, clotrimazole troches, oral ketoconazole, fluconazole, or itraconazole in eradicating the clinical symptoms of oral candidiasis (158,159).

Nystatin should be given as a 10- to 30-ml suspension five times daily, and the patient should be instructed to swish it around the mouth before swallowing; alternatively, the patient may take one or two troches five times daily. Clotrimazole troches are given five times daily as a 10-mg troche that should be held in the mouth until dissolved. In surgical patients at risk for hematogenous infection, systemic therapy with ketoconazole (200–400 mg once daily), oral itraconazole solution (100–200 mg/day), or fluconazole (100 mg once daily) is preferred. Antifungal therapy should be administered for 1 week.

D. Peritonitis and Intra-Abdominal Abscess

A controversial aspect of Candida infectious syndromes in surgical patients is whether systemic therapy is required to eradicate Candida found within intra-abdominal abscesses, peritoneal fluid, or fistula drainage. Candida is frequently cultured from intra-abdominal infectious foci but should be considered a serious threat only in specific patient groups. Four risk factors for intra-abdominal Candida infection have been identified including failed treatment for intra-abdominal infection, anastomotic leakage following elective or urgent operation, surgery for acute pancreatitis, and splenectomy (93,94,160).

Systemic antifungal therapy should be provided for those patients found to have Candida at the site of recurrent of intra-abdominal infection or previous operation, including patients with extensive areas of communication between the abdominal cavity and the external environment via either fistulas or drain tracts. Antibacterial therapy should be provided if bacteria are identified either by Gram stain or culture. Most of these patients will have polymicrobial infection.
Occasionally, *Candida* species may cause acalculous cholecystitis or cholangitis (161,162). This problem is increasingly found in patients with percutaneously placed drainage catheters for malignancy (163). Such patients must be given systemic therapy for clinical evidence of infection, including candidemia, and the drainage catheter must be changed.

Because fluconazole is very safe and is capable of reaching high concentrations in peritoneal fluid, it is likely to be useful in the management of candidal peritonitis (164,165). Fluconazole should be given at a dosage of 100–200 mg/day orally for 2–6 weeks. Immediate removal of the peritoneal catheter has been recommended. In one study, however, seven of nine patients treated with oral flucytosine responded to therapy without catheter removal (166).

### E. Urinary Tract Infection

The recovery of *Candida* species from the urinary tract most commonly results from contamination from the perirectal or the genital area. Bladder colonization is usually seen in patients who have undergone prolonged catheterization, or have diabetes mellitus, or any other disease that leads to incomplete bladder emptying. In addition, *Candida* species usually colonize in ileal conduits. Persistent candiduria in the surgical intensive care unit may, however, be an early marker of disseminated infection in critically ill high-risk patients (167). Alkalization of the urine with oral potassium–sodium hydrogen citrate is a simple and an effective method of treating candiduria in-patients with an indwelling catheter. Replacing or removing the bladder catheter is preferable. If *Candida* colonization persists, particularly, if the patient has a risk factor for cystitis (e.g., diabetes mellitus or a disease that leads to incomplete bladder emptying) or for hematogenous dissemination (e.g., immunosuppression or manipulation of the genitourinary system), antifungal therapy should be considered. Amphotericin B bladder irrigation only provides temporary clearance of funguria and systemic agents (single-dose IV amphotericin B or a 5-day course of oral fluconazole) are usually needed. Recently, a large multicenter prospective study has evaluated fluconazole vs. amphotericin B bladder irrigations for this condition (168). This study included very few ICU patients and thus was unable to specifically address the issue of progression to candidemia. Candiduria cleared by day 14 in 79 (50%) of 159 receiving fluconazole and 46 (29%) of 157 receiving placebo ($P < 0.001$). Fluconazole initially produced high eradication rates, but cultures at 2 weeks revealed similar candiduria rates among treated and untreated patients. Oral fluconazole was safe and effective for short-term eradication of candiduria, especially following catheter removal. Long-term eradication rates were disappointing and not associated with clinical benefit.

Flucytosine, 200 mg once daily, is a more attractive approach because of the convenience, cost, and very high drug concentrations achieved in the urine (169). Flucytosine is excreted in the urine in high concentrations and may be particularly useful against *C. glabrata* infection.

### F. Pneumonia

Pulmonary infection remains a common and important complication of ventilator therapy. There is clear evidence that quantitative microbiology obtained by protected specimen techniques provides accurate identification of patients who have a worse prognosis than patients with negative cultures. Patients with positive
cultures also appear to benefit from therapy directed specifically at the organisms identified by such techniques.

There is little information, however, regarding the significance of Candida species from such cultures. Until recently, Candida pneumonitis was considered a complication of neutropenia, with infection arising from embolism to bronchial vessels. However, this notion has more recently been challenged by data supporting the occurrence of Candida pneumonia in non-neutropenic patients, arising from aspiration of oropharyngeal contents. In one study involving immediate postmortem study of lung tissue in ventilated critically ill patients, 8% of such otherwise unse-lected patients demonstrated histologic Candida infection (170,171). These authors concluded, from a careful histologic study, that Candida colonization is uniform throughout the different lung regions, and that the presence of Candida in respiratory samples, independently of quantitative cultures, is not a good marker of pneumonia in critically ill, non-neutropenic, non-AIDS patients.

Nonetheless, there is little evidence of clinical or histological basis to support or recommend therapy for Candida obtained by tracheal or endotracheal aspirate or alveolar lavage. We believe that these techniques only document the presence of the organism in the oropharynx. While such cultures may support the use of fluconazole for antifungal prophylaxis, such cultures do not warrant therapeutic intervention.

XII. CONCLUSIONS

Continued progress in supportive care, including the development of antibiotics with increasingly broad spectra of activity has resulted in an increasing frequency of fungal infections, particularly candidiasis. Because of the inadequacy of the available knowledge base, we do not fully understand the pathophysiology of these infections in surgical patients, nor can we always be certain as to precisely when prophylaxis and therapy should be administered.

Despite these limitations, there is now sufficient information available to justify an aggressive therapeutic approach to suspected Candida infections. Now that less toxic agents are available (the newer triazoles, particularly fluconazole, and the lipid formulations of amphotericin B), the clinical approach to presumed fungal infections has been made far simpler.

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Candida Infections in the Intensive Care Unit


Fungal Infections in Immunocompromised Hosts: Host Defenses, Risks, and Epidemiology in Special Patient Groups—Pediatrics

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I. INTRODUCTION

While the basic principles with regard to identification and management of fungal infections in immunocompromised children are similar to those encountered in adult patients, there are additional considerations for pediatric patients at risk for fungal infections. Notably, the developmental immaturity of host responses to infection encountered during the first few months of life, place young infants at particularly high risk for a wide range of infectious complications, especially with fungal pathogens (1). Both term and preterm infants may be viewed as immunocompromised hosts and are at high risk for neonatal fungal infections, especially with candidiasis (2–6). However, the consequences are particularly severe in very low birth weight infants (7).

In the absence of a specific predisposing factor, the new diagnosis of an invasive or persistent fungal infection in a child should raise the possibility of an underlying defect in host defense. Indeed, most primary immunodeficiencies, such as chronic granulomatous disease (CGD) and severe combined immunodeficiency (SCID) can present in the first years of life, but are often missed until the second or third serious infection (8,9). Syndromes such as congenital cutaneous candidiasis and disseminated histoplasmosis of infancy may also be present in otherwise healthy infants (10,11). Despite significant advances in reducing the rate of perinatal HIV transmission through the use of antiretroviral therapy, previously undiagnosed HIV infection in the young child can be present with opportunistic fungal infections, such as candidiasis or in rare circumstances, cryptococcosis (12).
The clinical implications of developmental maturation of the immune system separate the child from the adult. In this regard, young children are at a high risk for primary infection with fungal pathogens, especially if there is an underlying immune deficit. At birth, the antibody repertoire is comprised of maternal IgG, which crosses the placenta easily, unlike IgM (13). Since immunoglobulin is transported across the placenta beginning in the eighth week of gestation, antibody levels in the infant reach 50% of maternal levels at 30 weeks gestation and by term are at, or above maternal levels (14). Passively acquired maternal immunoglobulin levels decline over the first months of life and reach a physiologic nadir at approximately 3–4 months of life. In term infants, the immunoglobulin levels at the nadir are frequently in the range of 400 mg/dL while in the very low birth weight infant levels may be less than 100 mg/dL (14,15). Although cell-mediated immunity in the term infant is relatively intact, infants are immunologically naïve because of a lack of exposure to complex pathogens (1). Immaturity of response to complex polysaccharide antigens (often critical components of encapsulated bacterial pathogens) is also notable in the first months of life (16). In the neonate, B-cells respond poorly to polysaccharides, and granulocytes are impaired in adhesion and in migration (17–19). Decreased granulocyte marrow reserves and mobilization are evident during severe infection or stress, rendering infants neutropenic. Together, these host factors make young infants, especially premature infants, more vulnerable to fungal colonization and also invasive disease.

Specific types of infections provide important clues to deficits in immune function. Fungal infections are more likely to occur in children with deficits of T-cell function or phagocytic defects; fungal infection in the child with a deficit in B-cell function is less common (8,9). In fact, the original descriptions of primary immune deficiencies have been partially based on the diagnosis of opportunistic infections, such as with fungal pathogens. The development of severe invasive fungal infection in childhood is distinctly rare and almost always associated with an underlying deficit in immune function. In fact, the diagnosis of an invasive mycosis should prompt a thorough examination of the immune function. Since most primary immune deficiencies are present in childhood, even the diagnosis of an atypical persistence of mucosal candidiasis should result in consideration for screening for immune function.

CD4+ T-cell lymphocyte counts vary by age; normal CD4+ T-lymphocyte counts are higher in infants and young children than in adults (20,21). Knowledge of these differences are particularly relevant when using CD4+ cell counts to evaluate an HIV-infected child’s risk for opportunistic infection (Table 1) (22). Unlike adults, children with HIV infection may experience opportunistic infections at higher CD4+ levels. In evaluating a young child for evidence of immune suppression, therefore, the extent to which CD4+ counts deviate from age-specific norms should be taken into consideration. The Centers for Disease Control and Prevention (CDC)

<table>
<thead>
<tr>
<th>Age</th>
<th>&lt; 12 months</th>
<th>1–5 years</th>
<th>6–12 years</th>
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<tr>
<td>No evidence</td>
<td>&gt; 1500</td>
<td>&gt; 1000</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>Moderate</td>
<td>750–1499</td>
<td>500–999</td>
<td>200–499</td>
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<tr>
<td>Severe</td>
<td>&lt; 750</td>
<td>&lt; 500</td>
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**Table 1** CD4+ T-lymphocyte Counts, Age and Risk for Opportunistic Infection in Pediatric HIV
1994 Revised Classification System uses age-specific norms to categorize HIV-infected children based on the degree of immunosuppression (23). The accompanying table (Table 1) summarizes the degree of immune suppression associated with age-specific CD4+ T-lymphocyte counts and percentages.

For instance, infection with *Pneumocystis carinii* arises in children with abnormal T-cell function, such as SCIDS or HIV infection (8). The spectrum of T-cell abnormalities in chromosome 22q11.2 deletion syndromes including DiGeorge syndrome are more varied than in SCIDS, but on occasions, PCP has been reported in patients with DiGeorge syndrome (24). Patients with a primary defect in phagocytic function, such as CGD are also at high risk for life-threatening infections with bacteria (*Staphylococci*, enteric-gram negatives such as *Burkholderia cepacia* and *Serratia marcescens*) and fungal (*Aspergillus* spp.) pathogens (9). Prophylaxis with trimethoprim–sulfamethoxazole and interferon gamma may reduce mortality from bacterial infection in this population by as much as 70%, but prophylaxis against fungal infection is more challenging (25,26). Recent data suggest, however, that prophylaxis with the oral antifungal agent itraconazole is well-tolerated and is effective in preventing both superficial and serious life-threatening fungal infections (27). Patients with other neutrophil disorders, such as myeloperoxidase deficiency, specific granule deficiency and leukocyte adhesion deficiency have been reported (28).

II. SPECIFIC Fungal INFECTIONS

A. *Candida* Species

1. Mucocutaneous Candidiasis

In immunocompetent patients, *Candida* is a commensal organism, and colonization of the skin or mucosal membranes is ubiquitous. Newborns are typically colonized during passage through the vagina (29). In healthy newborn infants, mild mucocutaneous infection is common and does not indicate the presence of a defect in host defenses. Oral candidiasis or thrush can be treated with oral nystatin and in older children with clotrimazole troches. Persistent infections may result from the continued use of colonized pacifiers or bottles. More extensive disease, involving the esophagus, for example, or infection that persists despite appropriate treatment should, however, raise the suspicion of an underlying immune defect, including both primary and acquired. For instance, mild esophagitis in the HIV-infected child can be treated with oral nystatin; however, many HIV-infected children with esophagitis have required treatment with either an azole or amphotericin B. Concurrent oral candidiasis is common and many HIV-infected children with esophagitis have a variety of associated signs and symptoms including odynophagia, retrosternal pain, drooling, fever, nausea and vomiting, dehydration, hoarseness, and occasionally upper gastrointestinal bleeding. Risk factors for *Candida* esophagitis in this population include prior oral pharyngeal candidiasis, low CD4 count and use of antibiotics (30). In neutropenic cancer patients, mucosal colonization with *Candida* may provide a portal through which dissemination and invasive infection can develop. Rarely, patients can develop necrotizing *Candida* esophagitis resulting in perforation. Management requires prompt surgical intervention and systemic antifungal therapy (31).

Chronic mucocutaneous candidiasis (CMC) is a rare inherited disease that results in severe candidal infection of the skin, nails, and mucosa. Invasive candidiasis in this syndrome, however, is distinctly uncommon. In approximately 50% of reported cases, an associated endocrine disorder is present (32). Recently, a gene
for autoimmune polyglandular syndrome Type 1 has been cloned and mapped to chromosome 21q22.3 (33). This autosomal recessive disorder is characterized by autoimmune polyendocrinopathy, chronic candidiasis, and ectodermal dystrophy. The syndrome frequently presents during infancy with persistent diaper rash. Case reports and reviews from the literature suggest that other distinct types of CMC might exist and that some families have a form of CMC without polyendocrinopathy.

2. Locally Invasive Candida Infection

In healthy children, locally invasive candidal infection occurs in three settings: laryngitis, otitis externa, and vulvovaginitis. Laryngeal candidal infection develops in children treated with inhaled corticosteroids for asthma. Presentation includes a hoarse voice and the diagnosis is confirmed by direct observation of the vocal cords. Chronic otitis externa occurs in children receiving extended courses of antibacterial therapy and/or local otic drops including corticosteroids. Systemic treatment is often indicated and on rare occasion, namely extension into the mastoid, surgery is indicated. Vulvovaginitis occurs in postpubertal females. Risk factors include diabetes, corticosteroid therapy, birth control pills, or extended use of tetracycline for acne. Vaginal troches are the first line of therapy, but relapse or recurrence is not uncommon.

3. Congenital Cutaneous Candidiasis

In contrast to neonatal candidiasis, which generally develops in an infant colonized with Candida, acquired either during passage through an infected birth canal or postnatally, congenital cutaneous candidiasis is acquired in utero by ascending infection, but this is relatively uncommon with fewer than 100 cases reported (34). The pathogenesis of this disorder is incompletely understood, though it is plausible that predisposing conditions include Candida chorioamnionitis. While fetal membranes are generally found to be intact, a possible role for subclinical rupture of membranes has been suggested. Once the amniotic fluid becomes infected, the Candida infection may spread to the skin, lungs, or gastrointestinal tract. Clinically, the disorder usually presents within the first 6 days of life with generalized erythematous papules and pustules caused by Candida spp. In full-term infants weighing more than 2500 g, it is generally benign and self-limited; management includes topical or oral antifungal therapy. However, it is critical to be vigilant, because dissemination can develop (35,36). In contrast to the benign course observed for this disease in full-term infants, preterm neonates weighing less than 1000 g with chronic cutaneous candidiasis are at higher risk of systemic infection and death. Diagnosis in the preterm infant warrants a thorough work-up, including blood, urine, and CSF cultures. The latter is particularly important because of the severe consequences of meningitis in the neonate (37). Systemically infected infants may have clinical signs of respiratory distress together with elevated white blood cell counts and hyperglycemia. With early recognition and aggressive treatment with systemic antifungal therapy, the mortality from this condition can be reduced. At this time, conventional amphotericin B therapy is the drug of choice.

4. Neonatal Candidiasis

This is a common form of disseminated candidiasis in premature infants, particularly those hospitalized in the neonatal intensive care unit (NICU) for an extended period of time (2). Overall Candida species account for approximately 10% of infections
observed in the NICU (7,38–40). Risk factors for systemic candidiasis in the NICU include gestational age (< 32 weeks), low Apgars scores, shock, disseminated intravascular coagulopathy, prior use of intralipid therapy, parenteral nutrition, H2 blockers, extended intubation, and central venous catheters (41,42). As with most infections in this setting, clinical signs and symptoms are frequently nonspecific; in some circumstances, persistent candidemia is observed (43). Severe infection can present with respiratory distress, episodes of apnea and bradycardia, hyperglycemia, or temperature instability. Other Candida spp. appear to be on the rise (40). There is some controversy as to whether infection with C.albicans carries a higher or lower risk for adverse outcomes, compared to C.parapsilosis. The recent trends indicate that non-Albicans species represent a greater percentage of blood-borne infection in the NICU (44).

The consequences of systemic infection are significant, which have led many to advocate sampling of urine and cerebrospinal fluid in addition to blood cultures (2). For instance, the risk for neurodevelopmental abnormalities is high, as is mortality. Renal ultrasound, echocardiogram, and ophthalmologic exam can reveal the presence of disease foci such as renal fungal balls or hydronephrosis, endocarditis, or endophthalmitis (45,46). Conventional amphotericin B has traditionally been the drug of choice for the treatment of neonatal candidiasis, and is generally well tolerated in this age group (47). Some investigators advocate the addition of oral 5-fluorocytosine (5-FC) to amphotericin B therapy when there is persistent candidemia, or the presence of either meningitis or endocarditis, but one should be cautious in monitoring for hematotoxicity. This has prompted investigation of fluconazole prophylaxis in preterm infants, which indicates that the prophylactic administration of fluconazole to very low birth weight infants can decrease the rate of fungal colonization (48). While larger studies are required to definitely show that prophylaxis decreases the risk of invasive Candida, results from one of these studies suggests that prophylaxis also decreases the risk for serious infection. While these findings are provocative, based on current data, it is premature to recommend universal adoption of fluconazole prophylaxis in all NICUs, although units with particularly high rates of fungal infection might consider this approach. The impact of prophylaxis on isolation of Candida species with intrinsic resistance to fluconazole will also need to be explored in detail.

5. Catheter- and Device-Associated Candidemia

Most children with life-threatening illness who require frequent blood sampling or infusional therapy have an indwelling venous catheter placed to facilitate venous access. However, there is risk for development of catheter-associated infection, which is usually due to bacterial pathogens, but on occasions, can be fungal, most commonly Candida spp. Vigorous activity often results in movement of the catheter, which can increase risk for infection. Usually lines are placed in the thoracic and neck region, but on some occasions are placed in the femoral region, which presents an especially daunting challenge for maintaining cleanliness. Given the high frequency of Candida diaper dermatitis in young patients, femoral lines may be at a higher risk of colonization or infection with Candida spp.

The diagnosis of a fungal infection associated with an indwelling device such as a vascular catheter or an intraventricular reservoir should lead to prompt removal of the infected hardware (49). Indwelling hardware infected with Candida or other fungal pathogens are notoriously difficult to clear with antifungal therapy and are
frequently identified as a source for persistent infection. Infected catheters should be removed promptly to prevent dissemination (50–52). Following removal of the catheter, follow-up blood cultures should be performed. Patients with persistent candidemia should be evaluated for suppurative phlebitis, thrombosis, endocarditis, or other foci of residual infection (36).

Candiduria in children is a distinctly rare event and usually associated with an underlying structural abnormality or recent instrumentation (53). In the newborn, it is unusual and should be confirmed by suprapubic tap in the evaluation for possible disseminated infection. Follow-up radiographic studies, including possible CT or ultrasound as well as dynamic studies are indicated if there is evidence for persistent candiduria.

6. Neutropenia and Candidemia

Many of the original studies that established the efficacy of empiric antibiotic therapy in febrile neutropenic patients included children. The risk for serious bacterial infection is significant enough to warrant empirical antibiotics when the absolute neutrophil count (ANC) falls below 500/μL, but the risk is even higher when the ANC is below 100/μL (54). Standard algorithms for choosing antibacterial agents vary by institutional bias and experience, but should take into account local antibiotic sensitivity patterns of infection. The use of antibiotics is warranted in this setting, but a major consequence of broad spectrum antibiotics results in alteration of normal colonization patterns, favoring fungal pathogens. Like adults, children with persistent fever and neutropenia are at a high risk for serious fungal infection, especially after 5–7 days. Diagnosis of fungal infections in the neutropenic child is as challenging as in the adult; a delay can lead to disseminated disease, which is associated with increased morbidity and mortality. Studies in both children (age > 2) and adults have suggested that liposomal amphotericin B is as effective as conventional amphotericin B in patients with fever and neutropenia (55–57). However, in most institutions, the drug has primarily been reserved for children who are either refractory to or intolerant of conventional antifungal therapy. New azoles, such as voriconazole, can be administered to children for either prophylaxis or treatment of documented infection (58).

7. Hepatosplenic Candidiasis

This is a particularly difficult infectious complication observed in children and adults undergoing therapy for lymphoreticular malignancies. It is also known as chronic disseminated candidiasis, a distinct form of invasive candidiasis, primarily seen in granulocytopenic patients (59,60). The infection often becomes clinically apparent in children who remain persistently febrile despite recovery from myelosuppression, and in fact, can progress despite resolution of neutropenia. In adults with acute leukemia, the incidence has been reported to be as high as 7% (61); pediatric risks are probably comparable. Suggested risk factors include the dose intensity of chemotherapy, use of steroids, duration of neutropenia, relapsed leukemia, detection of Candida species on surveillance cultures, and central venous catheterization (59–61). Extended therapy with conventional amphotericin B may be required for as long as 6–12 months; treatment failures are not uncommon. Amphotericin B lipid complex (ABLC), which is highly concentrated in the reticuloendothelial system of the liver and spleen, has garnered interest in the treatment of this infection (62). In a published report, six children (ages 4–17 years) with HSC were treated with
ABLC, which was well tolerated for a short course (4–6 weeks). Sustained radiologic response of liver and spleen lesions was observed even after discontinuation of ABLC, because of loading of the reticuloendothelial system.

B. *Aspergillus* Species

1. Invasive *Aspergillosis*

   The diagnosis and management of invasive *Aspergillus* infection is challenging in pediatric patients, for many of the same reasons observed in adults, clinical signs and symptoms can evolve slowly and come to medical attention at a critical juncture. Presentation can be insidious or acute. In children, invasive *Aspergillus* infection is generally associated with defects in host defenses such as CGD, HIV infection, Job’s Syndrome, neutropenia, aplastic anemia, corticosteroids, T-cell abnormalities, and indwelling foreign bodies such as catheters or vascular grafts (28,63). The most common site is the respiratory tract, especially the lungs. Children with CGD also present with sinus infection and osteomyelitis, which underscore the two routes of infection—direct inoculation and dissemination of infection, probably via the bloodstream. Outbreaks of aspergillosis have been described in pediatric oncology wards, transplant centers and NICUs, often associated with nearby construction work (64). Aerosolization of spores results in colonization of the sinopulmonary tract and skin, which can be the source of infection.

   *Aspergillus* pulmonary infection in the pediatric patient can be detected by radiographic studies, in particular, computerized tomography studies of the chest (65). The diagnosis of aspergillosis in children is established on the basis of sampling of tissue or body fluids. With the advent of modern bronchoscopic techniques suitable for small children, bronchoalveolar lavage is routinely performed in children, but not always neonates. *Aspergillus* species are commonly isolated from the upper respiratory tract, even among healthy individuals. In this regard, isolation of *Aspergillus* from bronchoalveolar lavage specimens will provide suggestive evidence of infection. Definitive diagnosis of *Aspergillus* requires histopathologic evidence or a positive sterile site culture as well as clinical or radiologic evidence of infection.

   New antifungal agents with activity against aspergillosis, such as voriconazole, have been quickly introduced into the pediatric setting. The efficacy and toxicity profiles do not appear to differ between adult and child. Already, several pediatric studies have established its safety in neutropenic children and ongoing studies are investigating its utility in primary immunodeficiencies, such as CGD, as well as in the oncology and transplant setting.

2. Hypersensitivity *Aspergillosis*

   Older children are also prone to develop allergic hypersensitivity aspergillosis, especially if there is a strong history for reactive airway disease. Occasionally, children with chronic lung disease, such as those with cystic fibrosis, can develop manifestations consistent with hypersensitivity aspergillosis. This condition develops most likely in the setting of asthma, recurrent cough with positive culture for *Aspergillus* species, chest radiogram with fleeting infiltrates, IgE, and *Aspergillus* precipitants. Therapy is long term and includes at least one course of corticosteroids and antifungal therapy, though frequent relapse often requires repeated courses of therapy.
C. Other Fungal Infections

Critically ill children and neonates are at risk for nosocomial and endemic fungal infections. In the NICU, the risk for acquisition of a severe fungal infection is high and can be related to catheters, especially for candidal infection as detailed above. Infection with *Malassezia* spp. can occur in children receiving lipid supplementation or from colonized health care workers (66–68). Like adults, infection with *Alternaria-Fusarium*, or *Trichosporon* spp. occurs in immunocompromised children, particularly in transplant, oncology, or NICU environments.

Most children exposed to *Histoplasma capsulation* in an endemic region, namely the central United States, do not develop clinical symptoms. In a healthy child, infection with *Histoplasma capsulation* is often a mild illness with few clinical manifestations. Symptoms of acute localized pulmonary histoplasmosis may resemble those of influenza and resolve within days. A unique manifestation of the disease occurs among infants and young children, generally between the ages of 5 and 25 months (11). Following exposure to a large inoculum of *Histoplasma capsulatum*, these otherwise healthy infants may develop an overwhelming primary infection with disseminated histoplasmosis. This syndrome has a case fatality rate as high as 40–50% if not diagnosed expeditiously. Major clinical features include fever, failure to thrive, hepatosplenomegaly, and pancytopenia. The diagnosis is established by isolation or visualization of the organism in samples obtained from one or more of the following sites: bone marrow, spleen, liver, lymph node, cerebral spinal fluid, or bronchoalveolar lavage fluids. In children with a defect in cellular immunity, such as those observed with the acquired immunodeficiency syndrome (AIDS), or with solid organ transplants, acute disseminated histoplasmosis can occur, and is associated with significant morbidity and mortality.

In regions endemic for *Blastomyces dermatididis* (Ohio, Mississippi, and Missouri River Basins) both pulmonary and extrapulmonary manifestations can develop in healthy children. Illness is usually mild, but on occasion, respiratory distress has been described. Chronic infection can present with night sweats and failure to thrive (i.e., failure to develop and gain weight). Radiographic changes in the lung are often more significant than the clinical manifestations. Long-term therapy with azoles can be given, but many still recommend amphotericin B (69).

Infection with *Coccidioides immitis* occurs most frequently in the southwest United States. Most children do not have clinical manifestations following primary exposure, but less than 5% develop severe pulmonary disease. Disseminated infection is rare in older children, but more common in neonates and young children. Immunocompromised children are at risk for disseminated infection, which includes both pulmonary and extrapulmonary disease (e.g., osteomyelitis, meningitis, and cutaneous disease). Extended therapy is required to eradicate infection.

*Zygomycetes* infections are distinctly uncommon in children, but when encountered are usually observed in the setting of immunosuppression or an underlying metabolic disorder (e.g., diabetes or acidemia). Additional predisposing factors for rhinocerebral mucormycosis include environmental exposure and extended use of corticosteroids. Amphotericin B is typically used for treatment as the azoles and echinocandins are frequently inactive, but the cornerstone of therapy is surgical debridement.

Children rarely develop meningitis due to *Cryptococcus neoformans*, a ubiquitous yeast-like fungus. Infection with *C. neoformans* occurs through inhalation of the acapsular yeast cells. Although depression of cell-mediated immunity has been
identified as a major factor predisposing individuals for invasive cryptococcal disease, humoral factors, such as specific antibodies may also be important in immunity. In the pre-HAART era, the prevalence of cryptococcal infection in HIV-1-infected children was about 1%. Infection in the neonate is very uncommon (70).

D. Therapeutic Considerations

Similar to the adult population, the development of new antifungal agents, such as the liposomal amphotericins, echinocandins, and voriconazole, represent important advances for the management of invasive fungal infections in children. Parallel studies in children have shown comparable toxicity profiles for lipid formulations of amphotericin, voriconazole, and the echinocandins. For example, based on data from adult studies, many pediatric centers now use voriconazole as first-line therapy for invasive pulmonary aspergillosis.

A particular note of caution, however, is warranted with regard to the use of newer antifungals agents in the treatment of children, especially neonates, with invasive fungal infection. While these agents may well become the standard of care for children, the experience with many of these agents in children is limited and often is extrapolated from the experience in adult populations (71,72). This concern is particularly true among neonates and preterm infants, in whom toxicity profiles and pharmacokinetic parameters differ substantially from adults and even older children. The pharmacokinetics of antifungal agents can differ between children and adults, and in fact, between children and infants (73). Many advocate a judicious and careful dosing schedule for amphotericin B and its lipid formulations in the neonate (74–76). Generally, increased excretion of compounds is observed in older children; for instance, fluconazole should be given at higher doses or twice a day to older children (77).

It is likely that most serious fungal infections will lead to consultation with infectious disease consultants with expertise in pediatric issues, such as diagnosis and pharmacology. The importance of future research to establish the safety, efficacy, and pharmacokinetics of these agents among pediatric patients cannot be overemphasized. The value of enrolling children with life-threatening diseases on clinical trials is illustrated by the major advances that have been made in the management of pediatric cancers through participation in co-operative clinical trials.

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Fungal Infections in Immunocompromised Hosts

Clinical Manifestations of Invasive Fungal Infections

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I. UNEXPLAINED FEVER

Invasive fungal infections are principally encountered in patients who are seriously immunosuppressed over a long period of time (1). These opportunistic infections occur particularly in those with an impaired cellular immunity and/or a severe granulocytopenia (2,3). This association has crucial repercussions for both establishing the diagnosis and instituting an appropriate therapy. Granulocytes are supposed to protect an individual against opportunistic pathogens and this reaction accounts under normal circumstances for most signs and symptoms that may accompany a serious local and, subsequently, invasive fungal infection. As a consequence of the low number of granulocytes, the inflammatory reaction is muted and the absence of infiltrating white cells around the germinating fungi prohibits an early radiological diagnosis, which has in turn consequences for the management of these diseases (4). More than one-third of patients in whom an invasive fungal infection was found at autopsy never received any antifungal therapy, which indicates that the symptoms of an active and, obviously, lethal fungal infection are neither alarming nor very typical (1). Even in non-neutropenic patients, the diagnosis of an invasive fungal infection may be problematic because the clinical presentation is nonspecific and variable, related to the organs afflicted. Moreover, sometimes patients do not appear to be seriously endangered because the accompanying symptoms are ameliorated by concomitantly administered anti-inflammatory drugs. Because of the suppression of the fever by concurrent corticosteroids, even patients suffering from chronic disseminated mycoses may feel relatively well until the infection progresses and organ failure becomes evident. The fact that a large proportion of these opportunistic infections affect critically ill patients at the extremes of age is a further explanation for the paucity of symptoms. In addition, it has to be emphasized that the clinical picture
can be disturbed by coexisting other infections too; they appear to play a role in a significant proportion of patients (5). The perturbed inflammatory reaction as a result of a low number of granulocytes, often in combination with immune modulating drugs, is responsible for a very wide spectrum of diseases, especially because chemotherapy-induced granulocytopenia is not a constant factor. It is a rather dynamic process with a time-dependent increase or decrease in the number of immune reactive cells. Upon return of the granulocytes, the clinical signs and symptoms will become readily detectable but in many cases, the infection would have reached an advanced stage by then (6). As is apparent from Table 1, there are very few clinical signs that can be regarded as characteristic for a particular invasive fungal infection. Fever, being present in 99% of the episodes, appears to be the only consistent signal of a possible, acute infection. This applies not merely to common organisms such as Candida and Aspergillus species but also to endemic mycoses, as well as to the emerging, more rare fungi like zygomycetes (mucormycosis), Fusarium, Scedosporium/Pseudallescheria, Saccharomyces, and Rhodotorula species. All of these organisms have occasionally been associated with life-threatening systemic symptoms and shock, but quite commonly unexplained fever is the first manifestation (7–14).

Candidemia can manifest itself with a sudden onset of fever and a sepsis syndrome accompanied by chills, hypotension, and myalgia and skin lesions. In patients eventually diagnosed with invasive candidiasis, unexplained fever was the first indication of infection in 88% of cases, whereas clinically documented organ involvement was encountered in barely 10% (15). Life-threatening organ infections may follow episodes of unexplained fever with numerous blood cultures that remained negative in spite of the fact that the blood is probably the vehicle for transport of the organisms from the gastrointestinal tract to the deep organs (16,17). Patients who develop candidemia during prophylaxis tend to be more acutely ill at the time of presentation and have a higher rate of disseminated disease, as well as pneumonia (18). Whilst acute disseminated candidiasis can arise within a few hours, the chronic form of this infection usually takes several days or even weeks to evolve to the full picture (Fig. 1) (19,20). The initial signs and symptoms of chronic disseminated candidiasis are

Table 1  Clinical Symptoms Suggestive of an Invasive Fungal Infection

<table>
<thead>
<tr>
<th>All fungal infections</th>
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</thead>
<tbody>
<tr>
<td>Persisting or new fever in patients known to be colonized by a fungus</td>
</tr>
<tr>
<td>Yeast infections</td>
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<tr>
<td>Retrosternal pain, upper abdominal discomfort</td>
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<tr>
<td>Increasing alkaline phosphatase with persisting, undulating fever</td>
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<tr>
<td>Multiple small hepatosplenic lesions on ultrasound when granulocytes recover</td>
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<tr>
<td>Chorioretinal lesions when granulocytes recover</td>
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<tr>
<td>Fever in combination with unexplained rash and muscular tenderness</td>
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<tr>
<td>Growing, sometimes partly necrotic, macronodular cutaneous lesions</td>
</tr>
<tr>
<td>Unexplained pain in spine and other bones; arthralgia</td>
</tr>
<tr>
<td>Mold infections</td>
</tr>
<tr>
<td>Dry cough with persisting fever</td>
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<tr>
<td>Chest pain related to respiration movements</td>
</tr>
<tr>
<td>Single or multiple pulmonary infiltrates, particularly wedge-shaped and halo sign</td>
</tr>
<tr>
<td>Air crescent sign when granulocytes recover</td>
</tr>
<tr>
<td>Elevated antigen levels, notably increasing titers</td>
</tr>
<tr>
<td>Facial pain with abnormality on nasal sinus x-ray</td>
</tr>
</tbody>
</table>
nonspecific, the most common presentation being persistent, sometimes intermittent, fever despite empirical broad-spectrum antibiotics with a gradual clinical deterioration and incremental dysfunction of the organs affected.

Over 30 years ago, it was shown that the diagnosis of invasive aspergillosis was not made during life in approximately two-thirds of patients who had evidence of disease at autopsy (21). More than three decades later, this percentage has not changed significantly. Invasive pulmonary aspergillosis in bone marrow transplant recipients often has an insidious inception such as fever unresponsive to empirical antibiotic therapy (22–24). However, the incidence of invasive aspergillosis amongst neutropenic patients with persisting fever is extremely low if other symptoms such as dyspnea, cough, and pleuritic chest pain are completely lacking. In a population without specific risk factors, which include prolonged deep granulocytopenia and exposure to moderate or high doses of corticosteroids, the frequency of invasive aspergillosis is well below 1%. In specific risk groups, such as allogeneic bone marrow transplant recipients with graft-vs.-host disease and cytomegalovirus reactivation, the incidence of invasive fungal infections may amount to more than 25%, but as a rule the persisting fever will be accompanied by another symptom indicative of a localized infection (23,24). The same is true for virtually all other mold infections.

Notwithstanding the lack of specificity of continuing fever as a trademark of invasive fungal infections, the clinicians’ behavior in the prescription of systemically active antifungals is often guided by this rather aspecific early clinical sign given the dismal prognosis of a firmly established invasive fungal infection (25). In some categories of patients, it might be justifiable to start a broad-spectrum antifungal agent after 5–7 days of adequate antibacterial treatment if fever persists, provided that attempts to make a more precise diagnosis are not delayed in the false confidence that a possible mycotic infection has been covered.

II. BRONCHOPNEUMONIA

A. Aspergillus and Aspergillus-like Pulmonary Fungal Infections

Pulmonary infections, either as the primary focus or as a complication of septicemia, offer granulocytopenic patients a gloomy prospect as they have been blamed for 70%
of all fatal infections (26). Both Gram-negative and Gram-positive bacteria have been identified as causative agents of bronchopneumonia in the normal population, as well as in the immunocompromised host. However, although micro-organisms like *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* can show a strikingly similar clinical picture, a pneumonia caused by *Aspergillus fumigatus* or another mold ought to be the leading diagnostic consideration when a chest radiograph displays a gradually progressive pulmonary infiltrate in conjunction with antibacterial-refractory fever and chest pain (21–24). Mold infections are typically airborne and acquired by inhalation of spores into the airways of the sinuses and bronchial tree prior to or during immunosuppressive therapy. Particularly, smokers show a high prevalence of colonization of the airways by *Aspergillus* species.

Therefore, pneumonia, which evolves over days to weeks is by far the most common manifestation of invasive aspergillosis and other molds (21–24). Classically, the patient presents with fever, tachycardia, and new pulmonary infiltrates, preferably in the upper lobes. Pulmonary infiltrates deserve a careful work-up that encompasses all clinical, laboratory, as well as radiological findings. An example is given in Figure 2. Symptoms other than fever may be absent in the early stages of infection, while some patients with invasive aspergillosis will present with dyspnea or nonproductive cough. Suspicion should rise to a red alert zone in a patient with pleuritic pain and rubbing, or radiographic evidence of a pleural effusion or localized pulmonary infiltrates. *Aspergillus* species and other fungi have a propensity for invading blood vessels and surrounding tissue such as ribs, muscles, pericardium, and pleura, thereby causing local thrombosis, hemorrhage, and tissue damage. This behavior explains the principal symptoms because fungal invasion causes extensive necrosis and occlusion of small blood vessels that ultimately can lead to infarction of lung tissue. It is often difficult or even impossible to discriminate between a beginning invasive pulmonary fungal infection and pulmonary embolism and infarction on clinical grounds.

Typically, chest radiographs performed early in the evolution of infection fail to show infiltrates; it may take more than 3 days for the infection to generate enough

![Algorithm for the diagnostic approach of pulmonary infiltrates.](image-url)
damage or for the few remaining granulocytes to concentrate around the infectious focus to allow recognition on a radiograph (6). By then, nodular patchy densities are the most prominent radiological manifestations of invasive pulmonary aspergillosis. In contrast, chest computed tomographs are already abnormal in a very early stage of development. Pulmonary nodules or areas of wedge-shaped consolidation on a chest radiograph are regarded as highly suspicious (27). The most characteristic findings on a high-resolution computed tomograph of the chest in neutropenic patients is a distinct halo of ground–glass attenuation around focal nodules (Fig. 3), which corresponds pathologically to hemorrhage around a focus of pulmonary infarction and a so-called air crescent sign (6,27). Notably upon engraftment and return of neutrophils, cavitation of the pneumonic process may occur as the normal lung previously infiltrated by fungal hyphae undergoes ischemic necrosis and separates from surrounding tissue. Under these circumstances, a so-called mycotic lung sequestrum may evolve with a risk of fatal massive hemoptysis that is exceptional in other patients.

While computerized tomography of the chest has shown to be an important aid to an early diagnosis, the pattern of the pulmonary infiltrate is merely suggestive of and not specific for a fungal infection. Unfortunately, cultures of specimens from bronchoalveolar lavage or bronchoscopic biopsy, if obtained at all, are often inconclusive or negative. On the other hand, nonculture techniques may assist in the interpretation of the radiographic findings. Serial serological monitoring of fungal antigens such as galactomannan or glucan, as well as screening for the possible presence of fungal nucleic acid sequences in blood or bronchoalveolar material by means of PCR assays may help to identify the origin of a lung infiltrate (28–31). Conversely, a positive test may indicate the necessity to perform a high-resolution computerized tomography. Efforts to establish a microbiological diagnosis should be vigorously pursued. It remains to be emphasized that it is impossible to distinguish pulmonary infections caused by Aspergillus species from infections with other molds such as Rhizopus, Absidia, and Mucor without cultures or histology. This also applies to Bipolaris, Exserohilum, and Alternaria species and, to a lesser extent, to increasingly encountered Fusarium, Scopulariopsis, and Scedosporium species (12,32,33). Pulmonary aspergillosis in immunocompromised patients has many faces (2,21,34). In patients with only mild abnormalities of their immune system, a more slowly progressive form of invasive pulmonary aspergillosis may develop. Among

Figure 3 ‘Halo’-sign type of a pulmonary infiltrate. Courtesy of Dr Siem de Marie, University Medical Center Erasmus, Rotterdam, The Netherlands.
lung transplant recipients, the spectrum of disease encompasses bronchitis, both ulcerative and pseudomembranous, invasive pneumonia, empyema, disseminated infection, bronchocentric granulomatosis, aspergilloma, surgical wound infection, and allergic bronchopulmonary aspergillosis (35). Ulcerative tracheobronchitis is a form of invasive aspergillosis characteristically found in lung transplant recipients. It is usually seen within 1 month after transplant on routine bronchoscopy. While patients are asymptomatic and chest radiographs generally unchanged from baseline, the bronchoscopic picture reveals a pattern of severe tracheobronchitis progressing to multiple ulcers at the site of anastomosis. Possible sequelae vary from superficial scarring to bronchial necrosis with anastomotic dehiscence and invasive pulmonary disease. Endobronchial aspergillosis occurs rarely in bone marrow transplant recipients.

B. Other Pathogens

Primary pulmonary candidiasis without evidence of other systemic candidal infection is extremely exceptional, although incidentally isolated bronchopneumonia and lung abscesses have been reported (36). Candida glabrata apparently causing pneumonia has been reported in patients with longstanding IV catheters who were treated with broad-spectrum antibiotics after cytotoxic chemotherapy and long-term corticosteroid therapy (37).

Occasionally, Cryptococcus neoformans stays confined to the lungs, presenting with dull chest pain, dyspnea, and cough; a chest x-ray will show miliary or nodular shadows in these cases (38).

Fever, cough, and chest pain and bilateral infiltrates visible on chest radiographs are rather germane signs and symptoms, which, however, can be caused by an array of fungal organisms. In the respective endemic areas, Histoplasma capsulatum, Coccidioides immitis, and Paracoccidioides brasiliensis (Fig. 4) have been held responsible for debilitating and even life-threatening pulmonary infections (39,40). A segmental pneumonia can be seen in approximately half of patients, whereas pleural effusions, nodules, or intrapulmonary cavities remain limited to a small number of cases. Symptomatic patients infected with Blastomyces dermatitidis usually have an influenza-like syndrome with fever, chills, arthralgias, cough, pleuritic chest pain, and hemoptysis (41). In acute blastomycosis, chest radiographs may

![Figure 4](image)

Figure 4  Pulmonary pattern of a Paracoccidioides infection.
show massive lobar or segmental consolidation that resembles a pulmonary malignancy, whereas the chronic form may mimic tuberculosis. Pleural thickening and small pleural effusions do occur but large pleural effusions are uncommon. Miliary disease and diffuse pneumonitis, often associated with respiratory failure, have been reported and are associated with a high mortality. *Pneumocystis carinii/jerovici*, now classified as a fungus, is the predominant cause of opportunistic interstitial pneumonia in transplant recipients. Typically, a patient becomes increasingly dyspneic with fever and malaise followed by nonproductive cough in association with the chest radiography exhibiting bilateral infiltrates or asymmetric abnormalities.

Management of pulmonary infiltrates is complex. Many infiltrates do have a noninfectious etiology and can be due to adverse effects of cytotoxic therapy, irradiation or pulmonary hemorrhage. Furthermore, it has to be emphasized that conditions favorable for development of fungal infections also facilitate other pathogenic micro-organisms. Alangaden et al. (5) described a survey on 88 bone marrow or peripheral stem cell recipients who suffered from graft-versus-host disease. From this group, 12 had to be readmitted to the hospital with a suspected pulmonary infection. Cough and fever had been the initial symptoms in the majority of cases; 10 out of 12 were shown to have pulmonary aspergillosis, although Gram-negative pathogens, including *P. aeruginosa* and *Enterobacter cloacae*, were concurrently isolated from the sputum or blood of 6 out of 10 patients. Moreover, for 5 of the 6 patients, not only the initial presentation but also the chest radiographic findings had been compatible with bacterial pneumonia. Without a computerized scan of the chest, half of the patients might have been treated as pure bacterial pneumonia and the coexisting pulmonary aspergillosis might have been missed. This observation indicates the complexity of the problem, as well as the urge to maximize the diagnostic efforts. The critical decision faced by the clinician at the bedside of patients with pulmonary infiltrates is whether or not to undertake invasive procedures. Sputum cultures are rarely diagnostic and the yield of bronchoscopy with bronchoalveolar lavage is disappointingly low. Percutaneous needle aspiration appears a better method to obtain adequate specimens for histological examination and/or culture, but this procedure is frequently considered precluded because of a concurrent thrombocytopenia. The exact role of these diagnostic approaches for the optimal management of patients remains controversial because the yield depends on the collaboration and skills of various specialists.

III. SKIN LESIONS

Mycotic infections of the skin have a rather common occurrence. However, sometimes skin lesions reflect serious, often life-threatening underlying fungal infections. Fungi that are known to involve the skin are listed in Table 2. In about 10% of patients, acute invasive candidiasis is accompanied by severe myalgia and typical pinkish-purple, painless subcutaneous nodules that may arise anywhere on the body (36). *Candida* species may also produce lesions that resemble ecthyma gangrenosum or purpura fulminans, but such a distinctive appearance is more common in disseminated fusariosis (Fig. 5). The clinical signs and symptoms of infections by *Saccharomyces cerevisiae*, also known as baker’s yeast, *Rhodotorula*, and *Malassezia furfur*, the causative agent of pityriasis versicolor, are in essence not different from those encountered in acute disseminated candidiasis. The correct diagnosis of these more rare fungi is seldom suspected before the organism is recovered from blood, urine,
Table 2  Differential Diagnosis of Skin Lesions in Febrile Immunosuppressed Patients

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>Differential Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maculo-papulomatous lesions</td>
<td>Drug allergy (allopurinol, cotrimoxazole, penicillins, cephalosporins, etc.)</td>
</tr>
<tr>
<td></td>
<td>Reaction to blood transfusion</td>
</tr>
<tr>
<td></td>
<td>Fungi (Candida species, Malassezia furfur, Cryptococcus neoformans, Trichosporon,</td>
</tr>
<tr>
<td></td>
<td>Histoplasma, Coccidioides immitis, Penicillium marneffi)</td>
</tr>
<tr>
<td></td>
<td>Viral (measles, rubeola)</td>
</tr>
<tr>
<td></td>
<td>Graft-versus-host disease</td>
</tr>
<tr>
<td>Pustulae, folliculitis</td>
<td>Fungi (Candida species, Malassezia furfur, Fusarium species, Blastomyces, Penicillium</td>
</tr>
<tr>
<td></td>
<td>marneffi)</td>
</tr>
<tr>
<td></td>
<td>Bacterial (staphylococci, streptococci)</td>
</tr>
<tr>
<td></td>
<td>Viral (Herpes viruses)</td>
</tr>
<tr>
<td>Ulcerative lesions and necrosis</td>
<td>Bacterial (staphylococci, streptococci, enterococci, Pseudomonas aeruginosa, etc.)</td>
</tr>
<tr>
<td></td>
<td>Anaerobic organisms (Clostridium perfringens, Actinomyces)</td>
</tr>
<tr>
<td></td>
<td>Fungi (Trichosporon, Blastomyces, Aspergillus, Alternaria, and Scedosporium species)</td>
</tr>
<tr>
<td>Erythema</td>
<td>Drug allergy (allopurinol, cotrimoxazole, penicillins, cephalosporins, etc.)</td>
</tr>
<tr>
<td></td>
<td>Fungi (Candida species, particularly in skin folds, Coccidioides immitis)</td>
</tr>
<tr>
<td></td>
<td>Viral (measles, rubeola)</td>
</tr>
<tr>
<td></td>
<td>Graft-versus-host disease</td>
</tr>
<tr>
<td></td>
<td>Overheating</td>
</tr>
</tbody>
</table>

Figure 5  Fusarium lesions of the skin.
or a cutaneous lesion. *Penicillium marneffei*, endemic in South-East Asia can cause disseminated infections with papular skin lesions in immunocompromised patients (42).

Cutaneous disease accounts for 75–80% of cases of sporotrichosis. *Trichosporon beigelii* and *Sporothrix schenckii* naturally gain access to the body through minor injuries of fingers and hands. Subsequently small, erythematous papules or subcutaneous nodules at a site of injury may emerge. These lesions often wax and wane for months or years but eventually the organism will reach the local lymph nodes. If IV catheters are the porte d’entree for *T. beigelii*, an overwhelming disseminated infection may ensue in patients with neutrophil defects (43).

In blastomycosis, skin disease usually occurs in conjunction with pulmonary disease (41). The well-demarcated and indurated lesions tend to appear on sunlight-exposed parts of the body, notably the face and distal extremities. In the early phase, a small papule or pustule is seen that gradually enlarges over a period of weeks or months, becoming elevated, verrucous, and crusted. Removal of the crust reveals a granulomatous base with numerous small abscesses that exude purulent material. Sometimes, central healing and scarring go together with active expansion at the outer border of the lesion.

Within the first days of onset of an infection with *C. immitis*, a fine, generalized maculopapular rash, sometimes urticarial in appearance develops in 10–40% of patients (39). The development of cutaneous hypersensitivity may be manifest as erythema nodosum or erythema multiforme, which occurs in less than 25% of infected individuals.

Nonspecific skin lesions, usually firm nodules, are suggestive but uncommon in neutropenic patients with disseminated histoplasmosis.

*A. fumigatus* and *A. flavus* have been described as the cause of necrotizing infections at the insertion site of long-standing central venous catheters (2,21,24). Involvement of the skin with Mucorales like *Rhizopus arrhizus* may be primary or secondary, following dissemination from another site (7,10). Other fungi with a *Aspergillus*-like behavior such as *Fusarium*, *Curvularia*, *Pseudallescheria*, and *Alternaria* species have also been held responsible for similar cutaneous infections in the compromised host (Fig. 5) (8,13,44). Moreover, *Pseudallescheria boydii* is a well-recognized cause of mycetoma—a localized noncontagious infection that can progress very slowly and involves cutaneous tissue, fascia, and bone (12). Mycetoma may be caused by either various other fungi or actinomycetes and generally follows a local trauma.

### IV. SINUSITIS AND CENTRAL NERVOUS SYSTEM DISEASE

Hematogenous spread of *Aspergillus* species from the lung to the brain can occur. Differential diagnostic considerations are summarized in Table 3. As a rule, affected patients develop headache, seizures, or other focal neurological signs, depending on the localization of the infection. Aspergillosis of the central nervous system may also originate from an aggressive *Aspergillus* sinusitis with or without a pulmonary infection (2,21). Presenting symptoms include fever, orbital swelling, facial pain, and nasal congestion. In bone marrow transplant recipients and other severely immunocompromised patients, *Aspergillus* sinusitis is frequently destructive, extending beyond the sinuses to the orbit or brain. The clinical picture of zygomycosis, fusariosis, and alternariosis resembles aspergillosis. However, in zygomycosis, the
rhinocerebral form is more pronounced and aggressive than in aspergillosis, featuring painful unilateral facial swelling, ptosis, proptosis, and dilatation or fixation of the pupil, together with a dark, serosanguinous nasal discharge (Fig. 5) (7,10). Drainage of black material from the eye is sometimes seen. Local symptoms of sinusitis and palatal or orbital cellulitis may be encountered or in more advanced disease ulceration of the nasal septum with even necrosis or perforation. The nasal turbinate bones are often black and necrotic. Neurologic sequelae evolve after a few days and usually are rapidly progressive and include blindness, cranial nerve involvement, and finally, contralateral hemiplegia because of thrombosis of the carotid artery or cerebral abscesses. Progressive lethargy develops and coma follows. Severe myalgias, sinusitis, ocular symptoms, and multiorgan system involvement are distinctive symptoms of disseminated fusariosis. *Pseudallescheria boydii* may also infect the eye and central nervous system, particularly in severely immunosuppressed patients and after accidents (45,46).

In cases of acute disseminated candidiasis, involvement of the central nervous system, presenting with headache, lethargy, and disorientation as a manifestation of meningitis, encephalitis, abscess, or hemorrhage is not an uncommon feature in children (47,48). It is frustrating that in fewer than 50% of such cases, examination of cerebrospinal fluid will reveal yeast cells.

Central nervous system involvement is reported in less than 5% of cases of blastomycosis. Abscesses presenting as massive lesions are most common; meningitis is usually a late complication and frequently associated with multiorgan disease. Short-term mortality rates associated with fungal infections of the central nervous system are extremely high, with exception of cryptococcal meningitis. Usually *C. neoformans* spreads from the lungs to central nervous system causing a highly variable clinical pattern of signs and symptoms. The most common signs and symptoms include headache, fever, nuchal rigidity, cranial nerve palsies, impaired memory and judgment, lethargy, obtundation, and coma (38). Patients may present with acute symptoms of only a few days’ duration, particularly when they are immunosuppressed, whereas others may have subtle symptoms for weeks or months before the diagnosis is made.

### Table 3  Differential Diagnosis Central Nervous System Disease in Febrile Immunosuppressed Patients

<table>
<thead>
<tr>
<th>Meningitis/encephalitis-like</th>
<th>Bacteria (meningococcus, pneumococcus, <em>Listeria monocytogenes</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi (Candida species and other yeasts, <em>Cryptococcus neoformans</em>, endemic fungi)</td>
<td></td>
</tr>
<tr>
<td>Viral (cytomegalovirus, herpes)</td>
<td></td>
</tr>
<tr>
<td>Graft-versus-host disease</td>
<td></td>
</tr>
<tr>
<td>Radiotherapy- and chemotherapy-induced tissue damage</td>
<td></td>
</tr>
<tr>
<td>Cyclosporin toxicity</td>
<td></td>
</tr>
<tr>
<td><strong>Intracerebral</strong></td>
<td></td>
</tr>
<tr>
<td>Bacterial (staphylococci, streptococci, <em>Pseudomonas aeruginosa</em>, etc.)</td>
<td></td>
</tr>
<tr>
<td>Fungi (mainly molds like <em>Aspergillus</em> species, zygomycetes, blastomycosis)</td>
<td></td>
</tr>
<tr>
<td>Parasites (<em>Toxoplasma gondii</em>)</td>
<td></td>
</tr>
<tr>
<td>Lymphoma, including post-transplant lymphoma</td>
<td></td>
</tr>
<tr>
<td>Cyclosporin toxicity</td>
<td></td>
</tr>
<tr>
<td>Primary brain tumor</td>
<td></td>
</tr>
</tbody>
</table>
Many fungi may trigger serious, acute disseminated infections with potential invasion of many vital organs. *Candida* species, *Aspergillus* species, *Fusarium* species, and *Pseudallescheria boydii* have been incriminated in a wide range of clinical syndromes, varying from esophagitis to disseminated sepsis-like infections. Severe myalgias and polyarthralgias, with or without disseminated ecthyma gangrenosum-like skin lesions, and multiorgan system involvement are starting symptoms of an acute disseminated infection by any of these organisms.

Alternatively, a disseminated fungal infection may mimic a single organ infection, but in most cases, a limited number of organs seem to be infected; in these cases, the clinical and laboratory symptomatology is determined by the affected organ. Ophthalmologic examination is a valuable tool for monitoring patients at risk of disseminated candidiasis and can establish infection in patients with negative blood cultures and no detectable colonization at other sites. Fundoscopic abnormalities, either early or mature, are present at baseline in approximately 10–20% of patients (49,50). The characteristic fluffy exudates may precede a loss of visual acuity and, if inadequately treated, blindness. *Candida* endophthalmitis seldom occurs in neutropenic patients since the lesions are the result of an inflammatory response that requires granulocytes. Other organs that serve as favorite destinations of a disseminated *Candida* infection or other fungi are the bones and the heart. Arthritis and osteomyelitis usually have an insidious onset, the long bones, vertebrae, hip or knee being most commonly affected (16). Endocarditis is associated with indwelling intravascular catheters and the typical, large vegetations may subsequently be shed as fungal emboli all over the body. Surgical replacement of the valve is essential but patients are often not able to undergo open heart surgery.

Since the introduction of mucosa damaging chemotherapy, chronic disseminated candidiasis is being recognized rather frequently (20,51). A schematic course of the evolution of such an infection is depicted in Figure 1. Typically, the patient has an irregular fever, complaints of abdominal discomfort with anorexia, vomiting, right upper quadrant tenderness, and elevation of alkaline phosphatase levels with or without hepatosplenomegaly (52). After recovery from neutropenia, an abdominal ultrasound or CT scan will demonstrate rather unique multiple abscesses in the liver and/or spleen, known as “bull’s eyes.” Intra-abdominal abscesses or peritonitis caused by *Candida* species are serious complications of recurrent surgery for acute pancreatitis or of continuous ambulatory peritoneal dialysis.

Patients with candidal esophagitis typically complain of a burning retrosternal pain that becomes worse on swallowing. The diagnosis of oropharyngeal candidiasis is often made clinically but it has to be taken into account that it is impossible to distinguish between *Candida* and other infections, particularly Herpes simplex. Blastomycetes can be found in painless osteolytic lesions. This organism can also infect the prostate and epididymis, whereas the kidney is usually spared. Patients note a painful swelling of the testis or epididymis, a perineal ache, or symptoms of urinary obstruction.

By virtue of the organism’s propensity for vascular invasion, inadequately treated pulmonary aspergillosis often disseminates to other sites, including the pericardium (Fig. 6) and myocardium, brain, eyes, kidneys, and gastrointestinal tract. *Pseudallescheria boydii* may also infect the eye, sinuses, ear, lungs, heart, bone, joints, and skin, particularly in the immunosuppressed patient. In addition, *Fusarium*, *Curvularia*, *Bipolaris*, *Exserohilum*, and *Alternaria* species have been held responsible for
disseminated infections very similar to those caused by *Aspergillus* species. In fact, often only on the basis of culture and histology, the distinction between the various fungi can be made.

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Clinical Syndromes by *Candida* Species

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I. INTRODUCTION

Candidiasis refers to infections caused by any of the >150 species of the genus *Candida*, mainly *Candida albicans*. The history of candidiasis dates back to the fourth century B.C. Since the 1940s, the frequency and severity of these infections have been increasing sharply as a result of the widespread use of broad spectrum antibiotics, steroids, and other immunosuppressive drugs (1). *Candida* species are ubiquitous human commensals. They become pathogens in situations where the host’s resistance to infection is lowered locally or systemically. In such circumstances, *Candida* spp. can cause superficial, locally invasive, or disseminated infection. *Candida* spp. are the fourth most common cause of bloodstream infections in the United States (2). These bloodstream infections are associated with an estimated annual national cost that ranges from US$200 million to $1 billion (3,4).

II. THE PATHOGEN

*Candida* spp. are thin-walled, small yeasts (4–6 μm) that reproduce by budding. The genus *Candida* belongs to the order Saccharomycetales, family Saccharomycetaceae. Seven species in the genus *Candida* are well-known opportunistic causes of infection (Table 1), while many others have been described as pathogens in individual case reports or short case series (5).

In 1995, a new species, *C. dubliniensis*, was defined. Morphologically and physiologically, the phenotype of *C. dubliniensis* resembles *C. albicans*. Germ tubes formed by *C. dubliniensis* are indistinguishable from those of *C. albicans*. *Candida dubliniensis* colonies sometimes appear as a darker green hue than those of *C. albicans* on a commercial differential isolation medium (6). It differs from *C. albicans* principally by the nonreactivity of its DNA with a *C. albicans*-specific molecular probe (7), but the presence of intracellular β-glucosidase activity in *C. dubliniensis* is otherwise the only phenotypic difference found with high consistency (6).

*Candida albicans* can grow over a very wide pH range, from below 2.0 to almost 8.0 and under aerobic, microaerophilic, and even anaerobic conditions of incubation.
The *Candida* spp. colonize primarily the gastrointestinal tract, and can also be found in the vagina, urethra, skin, and under the finger nails. *Candida albicans* has been recovered from different environmental sources that include fresh and seawater, soil, and any items that have contact with humans directly, such as clothing, bedding, and toothbrushes. Places where *C. albicans* is found are almost invariably the result of human and animal contamination (1,8,9).

*Candida* spp. can be part of the normal oral flora in 25–50% of healthy subjects (10). In hospitalized patients, the oral carriage rates are higher (50–70%) (11). Oral carriage rates are also higher in certain settings, such as HIV-infected patients (12), denture users with denture stomatitis (13), diabetic patients (14), patients on chemotherapy for malignant conditions (15,16), and children (17). The species that predominate in skin samples are *C. guilliermondii* and *C. parapsilosis*, rather than *C. albicans*.

Colonization at a specific site by more than one species of *Candida* can be as high as 44% (11,12,18–21). Simultaneous *Candida* colonization of more than one site may involve the same or different *Candida* strains. Concurrent isolation of similar species is the most common finding when the sites are anatomically related. More than 90% of *Candida* strains isolated simultaneously from vagina, urethra, and anus represent the same species. By contrast, only 61–75% of simultaneously isolated anal and oral *Candida* strains were the same (18,22).

The predominant source of infection in all types of candidiasis is the patient himself or herself. Transmission of *Candida* spp. from the gastrointestinal tract to the bloodstream requires prior overgrowth of the number of yeasts in their commensal habitat (23), and is favored by loss of the integrity of the gastrointestinal mucosa (24,25).

### Table 1  List of *Candida* spp. that are Opportunistic Human Pathogens

<table>
<thead>
<tr>
<th>Species commonly implicated in human infections</th>
<th>Species uncommonly implicated in human infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td><em>C. catelunata</em></td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td><em>C. chiropterorum</em></td>
</tr>
<tr>
<td><em>C. guilliermondii</em></td>
<td><em>C. ciferrii</em></td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td><em>C. dubliniensis</em></td>
</tr>
<tr>
<td><em>C. lusitaniae</em></td>
<td><em>C. famata</em></td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td><em>C. haemulonii</em></td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td><em>C. humicola</em></td>
</tr>
<tr>
<td><em>C. guilliermondii</em></td>
<td><em>C. inconspicua</em></td>
</tr>
<tr>
<td></td>
<td><em>C. kefyr</em></td>
</tr>
<tr>
<td></td>
<td><em>C. lambica</em></td>
</tr>
<tr>
<td></td>
<td><em>C. lipolytica</em></td>
</tr>
<tr>
<td></td>
<td><em>C. norvegensis</em></td>
</tr>
<tr>
<td></td>
<td><em>C. pelliculosa</em></td>
</tr>
<tr>
<td></td>
<td><em>C. pintolopesii</em></td>
</tr>
<tr>
<td></td>
<td><em>C. pulcherrima</em></td>
</tr>
<tr>
<td></td>
<td><em>C. rugosa</em></td>
</tr>
<tr>
<td></td>
<td><em>C. utilis</em></td>
</tr>
<tr>
<td></td>
<td><em>C. zeylanoides</em></td>
</tr>
</tbody>
</table>
Exogenous transmission of Candida also occurs. Outbreaks of Candida spp. infection resulting from contaminated materials have been described (26–29). Transmission of Candida species from staff to patient and from patient to patient has been demonstrated (30,31), mainly in specialized, relatively closed settings, such as burn (32), geriatric (21), hematology (33,34), intensive care (medical, surgical, adult, and neonatal) (35–37), and transplantation units (38,39).

The acquisition of Candida spp. by neonates can be the result of two mechanisms. Most newborn babies acquire a Candida flora from the maternal vagina at the time of birth or during gestation. However, nonperinatal nosocomial transmission can also occur (40). The hands of the hospital personnel may be a potential reservoir for nosocomial Candida spp. acquisition by neonates (41).

The survival of clinical isolates of five species of Candida was determined on the palms of human volunteers (42). Transmission from one hand to a second and to a third hand was observed in 69% and 38% of the experiments, respectively. Candida albicans was able to survive for 24 hr on inanimate surfaces, and transmission to and from inanimate surfaces was successful in 90% of the experiments.

Although most women who suffer from Candida spp. vulvovaginitis are infected with an endogenous commensal strain, there is a possibility of sexual transmission between partners (43,44), especially in the setting of receptive oral sex (45,46). Most cases of recurrence of vaginal Candida spp. infection have been ascribed to relapse with the same strain (47), rather than to infection with a new strain.

Among heroin abusers, hematogenous candidiasis (48) is usually acquired through the IV injection of a solution of heroin dissolved in contaminated lemon juice. The lemon juice originally becomes contaminated most probably with yeasts from the heroin users themselves an example of indirect transmission of an endogenous strain (49–51).

Candida albicans is the most commonly implicated organism in human candidiasis (52). The most frequent nonalbicans species regarded as pathogens are C. dublinensis, C. glabrata, C. guilliermondii, C. krusei, C. lusitaniae, C. parapsilosis, C. pseudotropicalis, and C. tropicalis.

The widespread use of the antifungal agent fluconazole for therapy and prophylaxis in HIV-infected patients has been associated with the appearance of fluconazole-resistant strains of C. albicans (53,54) and increasing frequency of non-albicans Candida strains in the oral mucosa (55,56). However, since the time the highly active antiretroviral therapy (HAART) became available, the rate of carriage of fluconazole-resistant C. albicans has significantly declined as a function of the host’s immune status (57).

Candida spp. are now the fourth most common organism isolated from blood of hospitalized patients in the United States (58). A survey of 1591 cases of hematogenous candidiasis found that the prevalence of non-albicans Candida species was 46% and was largely unchanged from 1952 to 1992 (59). However, more recent data suggest that in certain settings a reduction in the rates of C. albicans in favor of the non-albicans spp., may be occurring (60–63). These changes may be a consequence of increased immunosuppression, the use of prophylactic antifungal treatments, or the lack of adequate infection control measures. The use of prophylactic antifungal treatments with azoles has been associated with the likelihood of infections by C. glabrata and C. krusei (64,65), not C. parapsilosis (62). A strong association between C. parapsilosis and IV catheters has been suggested (66).
IV. PATHOGENESIS

The pathogenicity of Candida spp. relies mainly on the state of the host. Candida spp. are considered opportunistic pathogens because they are usually benign colonizers of mucosal surfaces, and disease occurs when there is a breakdown in the host defense.

Factors associated with the organism also contribute to its ability to cause disease. The most relevant virulence factors for Candida spp. include adherence to a wide range of tissue types and inanimate surfaces, dimorphism, enzyme production, phenotypic switching, and modulation of cytokine production by human monocytes.

Candida albicans adheres more strongly to epithelial cells than C. tropicalis, followed by C. parapsilosis. These findings are in agreement with the virulence ranking of these species (67). The different ability of each Candida species or strains to produce biofilm in vitro may be considered a virulence factor responsible for catheter-related candidemia in patients receiving total parenteral nutrition. Non-albicans Candida species are more likely to produce biofilm than Candida albicans strains (68).

Candida albicans is able to grow in a variety of cell shapes and forms. These range from spheroidal, budding blastoconidia through short and long pseudohyphal forms, to true hyphae and refractile chlamydospores. This phenomenon is commonly referred to as “dimorphism,” although the term “pleomorphism” would be more appropriate. Pleomorphism appears to be important (but not essential) in causing disease (69), while hyphal forms may be more virulent. Both forms (yeasts and hyphae) can penetrate host tissues and express virulence attributes (70).

One of the most extensively studied groups of Candida enzymes are the secreted aspartyl proteinases produced by C. albicans, C. dublinensis (71), C. guilliermondii (72), C. parapsilosis (73), and C. tropicalis (74). These enzymes produce nonspecific proteolysis of host proteins involved in defenses against infection. C. albicans, C. dublinensis C. glabrata, C. krusei, C. lusitaniae, C. parapsilosis, and C. tropicalis (75–77) also produce phospholipases. Such enzymes are important in invasion through hydrolysis of phospholipids of the host tissues (78,79). Phospholipase B has proved to be essential for C. albicans virulence (77).

Colonies of C. albicans grown on agar media sometimes show variations in form, particularly after long periods of incubation. This is the expression of a phenomenon called phenotypic switching, which may be related to the relative virulence of the species (80). The rate of phenotype switching is higher among strains of C. albicans from patients with invasive infections than among those colonizing superficial sites (81). Phenotypic switching contributes to the virulence of C. albicans by facilitating its ability to survive, invade tissues, and escape from host defenses (80). On the other end, neutrophils can augment the switching process toward a more susceptible strain (82).

Virulence of C. albicans may also be related to its ability to induce secretion of Interleukin 10 (IL-10) by monocytes, with selective inhibition of IL-12 and interferon gamma resulting in impaired immune response to Candida (83).

V. SYNDROMES

A. Disseminated candidiasis

Incidence: Hematogenous infections with a Candida spp. can be chronic or acute in nature, and the pattern of disease varies in different types of patients.
The overall incidence of candidemia has increased persistently worldwide during the last few decades. This increase is the consequence of increasing populations of individuals who are immunosuppressed as a result of their underlying disease (malignancy, AIDS, and newborns with very low weight birth) or as a consequence of their immunosuppressive treatment (chemotherapy, radiation, transplantation, prophylaxis, and treatment of graft rejection or graft versus host disease). Data from the United States showed an 11-fold increase in incidence of hematogenous Candida infections between 1980 and 1989, from 0.013 to 0.15 cases per 1000 admissions (84) and an increase from 2.0 to 3.8 fungal infections per 1000 hospital discharges, with Candida species accounting for 78.3% of the 30,477 fungal infections reported (85). In a population based surveillance, the average annual incidence of candidemia in two cities in the United States from 1992 to 1993 was 8 per 100,000 population (86). Currently in the United States, Candida species represent approximately 8% of all organisms isolated in blood cultures and Candida spp. are the fourth leading cause of bloodstream infection (58). The magnitude of the increase in the incidence of candidemia may vary in different medical settings and geographic areas. For examples, in Finland, Candida spp. are the eighth leading cause of bloodstream infections (87). As another example, the rate of candidemia increased >11-fold (2.5–28.5 cases/1000 admissions) from 1981 to 1995 in a neonatal intensive care unit in the United States (63), while the rate of candidemia only doubled to 0.71/10,000 patients/days between 1987 and 1995 in five Dutch University hospitals (88).

The increased incidence of candidemia probably achieved its maximum in the 1990s and is starting to decline after the availability and widespread use of fluconazole. In a study where the characteristics of candidemia before and after the newer antifungal triazoles were compared in a tertiary care community hospital, the incidence of candidemia dropped from 13% (1986–1989) to as low as 0.06% after the introduction of fluconazole (1994–1997) (89). In another study that included cancer patients, the incidence of candidemia decreased from 7.1% (1972–1973) to 3.4% (1998) (90).

The incidence of candidemia in patients receiving total parenteral nutrition has been reported to be as high as 22% (91), similar to the one observed among burn patients colonized with Candida strains (12–21%) (92–94).

**Morbidity and mortality:** The mortality attributable to hematogenous candidiasis was estimated to be 38% among a group of patients hospitalized during the 1983–1986 period (95). A second analysis done in the same institution during the 1997–2001 period showed a comparably high (49%) attributable mortality (96). In another institution, the mortality of patients with candidiasis in 1998 was reduced (33%) compared to the one observed during the 1974–1982 period (77%) (90). The crude mortality of disseminated candidiasis ranges from 26% to 75% (63,97–104). Candidemia is also associated with a 30 day prolongation of hospital stay (95).

The most important prognostic factors for outcome of hematogenous candidiasis include: older age, poor performance status, presence and persistence of neutropenia, corticosteroid therapy, extensive organ involvement with candidiasis, and lack of antifungal treatment (101,102,105,106). Central venous catheter retention appears to play a limited role if any (107–110). Different species of Candida have been associated with different attributable mortality, lowest with C. parapsilosis, and highest with C. tropicalis and C. glabrata (40–70%). Candida krusei has similar mortality to C. albicans (15–35%) (111).

The reported response rate of chronic disseminated candidiasis has varied from 54% to 90% series and does not seem to be improved by splenectomy (60%) (112–115).
Risk factors: Colonization with Candida spp. (mainly in the gastrointestinal tract) seems to be an essential step for the development of invasive candidiasis (23). This is supported by studies that show colonization of the gut by the same strain that subsequently causes candidemia (116–119). A higher density of colonization was associated with a higher risk of infection among patients with acute lymphocytic leukemia (120), other hematological cancers (121), infants with very low birth weight (122), and patients admitted to surgical intensive care units (123). Hematogenous candidiasis developed in >30% of neutropenic cancer patients colonized with Candida spp. at multiple sites compared with no infection among those who were not colonized (124–126). Several other factors also contribute to the development of hematogenous candidiasis through one or more mechanisms (see Table 2). The presence of central venous catheters has been found to be a risk factor for candidemia in some but not all series. The mechanism by which the catheter can be a risk factor for candidiasis is thought to be through contamination of the skin leading to catheter infection and subsequent dissemination. However, in contrast to the gut colonization by Candida spp. as a source for candidemia, published data do not fully support skin colonization by Candida spp. as a source of catheter-related candidemia (127). It is possible that the presence of CVC represented more a marker of severity of illness (in the studies that identified the CVC as risk factors) rather than a risk factor for the development of candidiasis. On the other hand, CVCs are associated with an increased incidence of thrombophlebitis. These thrombi may get seeding in the setting of hematogenous candidiasis and thus become a source of persistent infection (128–130).

Clinical Presentation

Acute disseminated: The clinical presentation of hematogenous candidiasis varies in different patient populations. In neonates, the clinical picture of hematogenous candidiasis is similar to that of bacterial sepsis, and spread of the infection to different organs is a common event. The most frequent sites of Candida involvement are the skin (66%) (131), followed by the central nervous system (CNS) (up to 64%)

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**Table 2** Risk Factors for Hematogenous Candidiasis

<table>
<thead>
<tr>
<th>Increased colonization</th>
<th>Increased translocation</th>
<th>Increased invasion of deep tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endogenous</strong></td>
<td><strong>Intestinal graft-vs host disease</strong></td>
<td><strong>Immunosuppression</strong></td>
</tr>
<tr>
<td>• Broad spectrum antibiotics</td>
<td>• Malnutrition</td>
<td>• Cancer</td>
</tr>
<tr>
<td><strong>Exogenous</strong></td>
<td>• Mucositis</td>
<td>• Corticosteroid treatment</td>
</tr>
<tr>
<td>• Heroin users*</td>
<td>• Surgery</td>
<td>• Hemodialysis</td>
</tr>
<tr>
<td>• Hospital stay</td>
<td>• Severe burns</td>
<td>• Neutropenia</td>
</tr>
<tr>
<td>• Contaminated TPN</td>
<td>• TPN</td>
<td>• Premature neonates (&lt;32 weeks, 5' Apgar &lt;5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• TPN</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Transplantation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Severe burns</td>
</tr>
</tbody>
</table>

Abbreviations: TPN, total parenteral nutrition.

*In the case of heroin abusers with hematogenous candidiasis, the infection may be transmitted through IV injection of a solution of heroin dissolved in contaminated lemon juice (48).
Respiratory dysfunction and apnea are the most common presenting signs (70% of cases) (104,134). The most common pattern of disseminated \emph{Candida} infection in adults is the acute type seen typically in non-neutropenic patients in intensive care units, or in patients with hematologic malignancies during chemotherapy-induced neutropenia. Fever unresponsive to antibacterial drugs is the usual presenting symptom. However, other manifestations may include those of sepsis (135). Disseminated candidiasis can involve any organ in the body. Endophthalmitis is a common result of \emph{Candida} dissemination. \emph{Candida} spp. gain access to the eye via the capillaries of the choroid and the retina, where they proliferate and induce focal inflammation and abscess formation. The frequency of ocular involvement by \emph{Candida} spp. varies from 3% (136) to 78% (137–141) depending on the patient population (less in neutropenic patients probably because of their inability to develop an inflammatory response), the diagnostic criteria used, the study design (prospective versus retrospective), and the physician who performed the ophthalmologic examination (ophthalmologist vs. nonophthalmologist). A recent prospective study conducted among 31 patients with candidemia showed a 26% rate of ocular candidiasis. Of note, only five patients were found to have ocular involvement at the time of diagnosis of candidemia, while the remaining three patients had documented chorioretinitis within 2 weeks of diagnosis (141). This study suggests that patients with candidemia should have an ophthalmologic evaluation at baseline and 2 weeks after diagnosis. Another recent prospective study conducted among patients with candidemia found that 20 of 180 patients (15%) had retinal lesions. Most of these lesions were nonspecific; even though they could have been because of candidiasis, other etiologies could not be ruled out (142). None of the patients with retinal lesions had ocular symptoms at the time of the ophthalmologic exam.

Skin lesions may be present in 10–15% of patients with hematogenous candidiasis in neutropenic patients along with myalgias. These lesions may present like pink nodules (143,144), ecthyma gangrenosum (145), or other nonspecific lesions that resemble a drug rash (135,146).

IV heroin users who suffer from disseminated candidiasis develop a unique pattern of organ involvement. They acquire the infection by IV injection of contaminated drug solutions (48,147). The initial symptoms may last from a few hours to even a month, and the patients complain of fever, shivering, sweating, asthenia, or headache (148). Within 1–4 days of candidemia, 75–80% of patients will develop nodular cutaneous lesions affecting mainly the scalp (147,149). Fifty percent of patients may develop ocular involvement (chorioretinitis, hyalitis, episcleritis, anterior uveitis, and endophthalmitis) within a few days to up to 3 weeks after onset of the infection (147,150). At a later time (from 15 days to 5 months after the infection), osteoarticular lesions (mainly costochondritis and vertebral lesions) (147) may develop in up to 42% of patients.

\emph{Chronic disseminated candidiasis}: Less common than the acute disseminated disease, chronic disseminated candidiasis (CDC) (previously known as hepatosplenic candidiasis) is almost always associated with recovery from neutropenia and may arise subsequent to a treated episode of acute hematogenous candidiasis. The condition occurs mainly among patients with acute leukemia undergoing cytotoxic chemotherapy, and those undergoing allogeneic bone marrow transplant, and is characterized by persistent fever nonresponsive to broad-spectrum antibiotics, negative blood cultures, abdominal pain (mainly right upper quadrant pain), increased liver function tests, in particular serum alkaline phosphatase, and multiple
abscesses in the liver, spleen, lungs, and kidneys. Hepatomegaly and/or splenomegaly detected by abdominal examination is found in half of patients with CDC while abdominal tenderness is found in about two-thirds of these patients (113). Response to treatment is low. The median number of days for disappearance of fever among patients who will have a favorable outcome ranges from 4 to 26 days (median 19) (114). Usually, only a minority of patients with CDC (20–30%) develop positive blood cultures for Candida spp. (113–115).

Candida abscesses are usually detectable on ultrasonography, computed tomographic scan (CT), or magnetic resonance imaging (MRI) (151). Four patterns of CDC have been described by ultrasonography. Early in the disease, the Candida microabscesses may show a “wheel within a wheel” image (first pattern) or a “typical bull’s eye” (second pattern) and/or uniformly hypoechoic lesions (third pattern). Late in the course of the disease, fibrosis or calcification of the lesions may show as echogenic foci with variable degrees of acoustic shadowing (fourth pattern). On CT, only the third and fourth patterns are commonly seen. MRI imaging is more sensitive than CT for the detection of the presence and number of CDC lesions (152) and is accurate for assessing different stages of the disease (153). Three patterns of CDC have been described by MRI imaging. The acute pattern (within 2 weeks of therapy) consists of lesions <1 cm in diameter appearing as well-defined-high-intensity foci on T2-weighted images. The subacute pattern (from 2 weeks to 3 months of therapy) shows similar size lesions that are mildly hyperintense on T1-weighted images, along with a perilesional ring. The chronic pattern reveals lesions (1–3 cm in diameter) with irregular margins, with decreased enhancement on images obtained after gadolinium injection.

Catheter-associated candidemia: The term catheter-related candidemia implies that a catheter can have a role in the pathogenesis of candidemia, either as a primary source of the organism (primary catheter-related candidemia as a result of CVC colonization from skin) or as a factor that can perpetuate candidemia originating from another site (secondary catheter-related candidemia as a result of CVC seeding from blood).

Primary catheter-associated candidemia: This is a rather uncommon entity, because as mentioned earlier under the section of risk factors, the gut, and not the skin, appears to be the primary source of hematogenous candidiasis (127). Further complicating this issue are the widely varying definitions of catheter-related candidemia used by different authors. Examples of definitions used include: candidemia in a patient with: (a) central venous catheter in place (154); (b) central venous catheter in place without any other source of infection (155,156); (c) central venous catheter in place with positive CVC tip culture for the same Candida spp. causing fungemia (154–157) or positive catheter-related thrombus for the same Candida spp. (155); (d) central venous catheter in place with CVC exit site positive for the same Candida spp. causing candidemia (155).

The lack of a standard definition (including lack of established methodology for catheter culture) makes understanding the pathogenesis, clinical features, and outcome of this entity very difficult.

Bodey et al. (158) defined catheter-related candidemia as candidemia that occurs in a patient with an intravascular catheter, and no other obvious origin for the infection after careful clinical and laboratory evaluation. If the catheter is removed, a quantitative culture of the tip should recover ≥15 CFU of the same Candida spp. by the roll plate or ≥100 CFU by the sonication technique. If the catheter is not removed, a quantitative blood culture collected through a CVC should
contain at least a 10-fold greater concentration of *Candida* spp. than a simultaneously collected quantitative peripheral blood culture.

To be more precise, there are two issues that should be included in the definition of catheter-related candidemia: (a) lack of recovery of the same *Candida* spp. from other colonizing sites (because of the high likelihood that sources other than CVC such as gut, contaminated TPN solution, or other are the primary source of candidemia in a large proportion of patients) and (b) presence of molecular relatedness between colonizing (skin or CVC tip) and infecting strains (blood), as the mere recovery of the same species of *Candida* does not necessarily mean that the organisms are genotypically related (159).

**Secondary catheter-associated candidemia:** In this entity, the primary source of the candidemia is not the catheter, but the catheter can become a secondary source. This is the case of patients with candidemia of other origin. *Candida* spp. adheres to a CVC-related thrombus, the vessel walls and the CVC, and these infected sites become a source for subsequent candidemia. This is the case of septic venous thrombophlebitis.

*Candida* thrombophlebitis of the central veins is rare (130,160,161) and occurs mainly in severely ill patients. Risk factors include CVC in place, treatment with multiple antibiotics and TPN, admission to ICU, and abdominal surgery. The most common sites of thrombophlebitis include the subclavian, the innominate, and the superior cava vein. In most cases, *C. albicans* is the causative pathogen. Clinical presentation includes fever, edema of the area involved, and persistent candidemia (2–3 weeks), even after removal of the CVC and appropriate antifungal chemotherapy (130).

*Candida* thrombophlebitis of the peripheral veins is also a rare entity. Risk factors are the same as those for thrombophlebitis of the central veins. Patients usually present with fever or sepsis. Locally, symptoms may range from a noninflamed thrombosed vein, to a warm, tender, erythematous vein with or without purulent drainage (128,129,162–164).

### B. Local Infections

Local infections by *Candida* spp. can be divided into two groups: mucocutaneous infections and locally invasive ones. The most common mucocutaneous forms of candidiasis involve the female genitalia, the skin and nails, and the oral cavity (thrush) (sometimes with concomitant esophageal invasion). Locally invasive candidiasis involving deep tissues are almost always the result of hematogenous spread of a *Candida* organism from an endogenous or, less often, an exogenous site. Table 3 summarizes the recognized forms of local *Candida* infection and lists the settings in which they are most commonly encountered.

#### 1. Mucocutaneous Infections

**Oral candidiasis:** Oral *Candida* infections occur predominantly among immunosuppressed patients or from populations at risk exposed to other factors that favor the overgrowth, and invasiveness of this fungus include immunosuppressed individuals such as newborns with birth asphyxia, diabetic patients, patients infected with HIV, patients receiving corticosteroid or cytotoxic chemotherapy particularly for hematological malignancies, patients undergoing maxillofacial radiotherapy, and recipients of organ and/or stem cell transplantation. Oral *Candida* infections also
<table>
<thead>
<tr>
<th>Type of disease</th>
<th>Major predisposing/risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oropharyngeal infection</td>
<td>Age extremes</td>
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<td></td>
<td>Denture wearers</td>
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<td></td>
<td>Diabetes mellitus</td>
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<td></td>
<td>Antibiotic use</td>
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<td></td>
<td>Radiotherapy for head and neck cancer</td>
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<td></td>
<td>Inhaled and systemic corticosteroids</td>
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<td></td>
<td>Cytotoxic chemotherapy</td>
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<tr>
<td></td>
<td>HIV infection</td>
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<td></td>
<td>Hematologic malignancies</td>
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<td></td>
<td>Stem cell or solid organ transplantation</td>
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<td>Esophagitis</td>
<td>Systemic corticosteroids</td>
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<td>AIDS</td>
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<td>Cancer</td>
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<td></td>
<td>Stem cell or solid organ transplantation</td>
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<tr>
<td>Lower gastrointestinal infection</td>
<td>Cancer</td>
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<td></td>
<td>Surgery</td>
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<tr>
<td>Vulvovaginal infection</td>
<td>Oral contraceptives</td>
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<td></td>
<td>Pregnancy</td>
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<td></td>
<td>Diabetes mellitus</td>
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<td></td>
<td>Systemic corticosteroids</td>
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<td></td>
<td>Antibiotic use</td>
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<tr>
<td>Infections of the skin and nails</td>
<td>Local moisture and occlusion</td>
</tr>
<tr>
<td></td>
<td>Immersion of hands in water</td>
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<tr>
<td></td>
<td>Peripheral vascular disease</td>
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<tr>
<td>Cutaneous congenital candidiasis</td>
<td>Intrauterine foreign body</td>
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<tr>
<td></td>
<td>Prematurity</td>
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<tr>
<td>Chronic mucocutaneous candidiasis</td>
<td>T-lymphocyte defects</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>Indwelling urinary catheter</td>
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<td></td>
<td>Urinary obstruction</td>
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<tr>
<td></td>
<td>Urinary tract procedures</td>
</tr>
<tr>
<td></td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Aspiration</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>Major surgery</td>
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<tr>
<td></td>
<td>Previous bacterial endocarditis or valvular disease</td>
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<td></td>
<td>IV drug abuse</td>
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<td></td>
<td>Long-term central venous catheter</td>
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<tr>
<td>Pericarditis</td>
<td>Thoracic surgery</td>
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<td></td>
<td>Immunosuppression</td>
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<tr>
<td>Central nervous system (CNS) infection</td>
<td>CNS surgery</td>
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<td></td>
<td>Ventricular-peritoneal shunt</td>
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<tr>
<td>Ocular infection</td>
<td>Ocular surgery</td>
</tr>
<tr>
<td></td>
<td>Trauma</td>
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<tr>
<td>Bone and joint infection</td>
<td>Surgery</td>
</tr>
<tr>
<td></td>
<td>Trauma</td>
</tr>
<tr>
<td></td>
<td>Intra articular injections</td>
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<tr>
<td></td>
<td>Diabetic foot</td>
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<tr>
<td>Abdominal infection</td>
<td>Recurrent perforation</td>
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<tr>
<td></td>
<td>Repeat abdominal surgery</td>
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<tr>
<td></td>
<td>Anastomotic leaks</td>
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<tr>
<td></td>
<td>Pancreatitis</td>
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<tr>
<td></td>
<td>Continuous ambulatory peritoneal dialysis</td>
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<td>Solid organ transplantation</td>
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develop in nonimmunosuppressed individuals exposed to prolonged antibiotic treatment (165,166), inhaled steroids (167), and those who wear dentures (166).

The prevalence of oral *Candida* infection (thrush) in association with AIDS approaches 100%, when CD4 counts decrease below 200/μL. The improvement of the immune system of HIV-infected patients associated with the administration of HAART has resulted in a significant decrease in the incidence of oropharyngeal candidiasis (57,168). Thrush can be present in 28–38% of cancer patients undergoing therapy with corticosteroids or cytotoxic agents (169,170).

**Esophageal candidiasis:** This accounts for up to 15% of the AIDS-defining illnesses (171). Of note, up to 30% of patients with *Candida* esophagitis may not have oral thrush (172,173), while 64–88% of patients with thrush have concomitant esophageal candidiasis (172,174).

The usual presentation of oral and esophageal infections is in the form of white “cottage cheese” patches. Other presentations include the pseudomembranous type (“thrush”) that reveal a raw bleeding surface when scraped; the erythematous type, which are flat, red, sometimes sore areas; the candidal leukoplakia, consisting of nonremovable white thickening of epithelium because of *Candida* spp., and angular cheilitis, presenting as sore fissures at corners of mouth. In addition to all of these, median rhomboid glossitis, an abnormality of tongue associated with ovoid, denuded area in median posterior portion of the tongue, can be associated with candidiasis. In elderly patients, particularly those who wear dentures, a more chronic form of disease is seen that is characterized principally by areas of non-specific erythema, often beneath denture surfaces (9).

**Other gastrointestinal sites:** Candidiasis can involve any site of the gastrointestinal tract with the esophagus and the small bowel as most common sites. These lesions may be clinically significant and may progress to hematogenous infection. The pathology of infection of the lower GI tract by *Candida* spp. ranges from mucosal ulceration with or without pseudomembrane to exophytic lesions. In deep invasive lesions, pseudohyphae may extend beyond the muscular layer and reach the serosa (175). Direct vascular invasion through the bowel wall has been reported only in patients receiving immunosuppressive chemotherapy (175,176). These patients may have extensive involvement of the GI tract from mouth to anus, while non-neutropenic surgical patients exhibit a more localized involvement (177). The histologic criteria for diagnosing this form of the disease include the presence of budding yeast forms, mycelial forms, or both on KOH smears or culture; a disrupted epithelium; and a submucosal inflammatory reaction.

**Candida infections of the genitalia:** *Candida vulvovaginitis* (CVV) is the second most frequent genital complaint in women, with around 75% experiencing at least one episode of CVV in their life time, and half by 25 years of age (178). Several risk factors have been associated with CVV including oral contraceptives, corticosteroids and antibiotics (179), diabetes (180), and pregnancy (181). Sexual transmission of *Candida* strains may occur after receptive oral sex. CVV does not appear to correlate with vaginal intercourse (43,46).

In most cases, the presentation is acute, the symptoms are not severe, and the condition responds readily to treatment. However, around 5% of women develop a chronic or recurrent form of *Candida* vulvovaginitis (RCVV) resistant to antifungal treatment (182). The majority of women with RCVV do not suffer any obvious underlying immune deficit or illness. A local change in vaginal immune defenses appears to increase the susceptibility to this infection (183). Most cases of RCVV...
are caused by the same strain of *Candida* that developed subtle genetic variations (47). Drug resistance does not seem to be an important factor in RCVV (183).

Genital infections in males are less common than in females and can be caused by the yeast itself or by an allergic reaction to the presence of *Candida* antigen after unprotected intercourse. *Candida* balanoposthitis is a recognized entity, usually presenting only as mild irritation with focal signs of erythema but sometimes becoming severe, even leading to phimosis in rare instances (184). Although *Candida* yeasts can be sexually transmitted, it is practically impossible to differentiate between transmission from a sexual partner and that from the patient’s own flora via the anus. Among partners of patients with RVCC, strain typing of the infecting *Candida* strains have shown identity or near identity between strains recovered from both partners (47).

*Candida* infections of the skin and nails: *Candida* spp. inhabits the skin and mucus membranes in around 75% of the population without causing harm (185). In occluded sites of the body where the surface remains moist (typically the groins and the armpits, or the spaces between toes and the breastfolds) *Candida* infections can occur. These infections present as a pruritic rash with a poorly defined edge and abundant erythematous vesiculo-pustular lesions. Fissures may occur in interdigital spaces (186).

Invasive infections of the fingernails (onychomycosis) are mainly caused by *C. albicans* and *C. parapsilosis* (less commonly *C. glabrata* and *C. guilliermondii*) (187–189). *Candida* spp. are the most common etiology of onychomycosis of the fingernails while dermatophytes are the most common cause of onychomycosis of the toenails (190,191).

Chronic swelling and inflammation of the nail fold (paronychia) is a condition characterized by the presence under the fold of a mixed microbial flora of normally commensal organisms, including yeast flora and the most common species is *C. albicans* (186,192).

Neonates may develop a rare entity referred to as cutaneous congenital candidiasis. Among neonates weighing >1000 g, the condition usually presents with a generalized macular erythematous rash that may become pustular, papular, or vesicular, with subsequent desquamation. Among premature neonates weighing <1000 g, this entity presents with a widespread desquamating or erosive dermatitis that can evolve to hematogenous candidiasis and increased risk of death. Presence of intrauterine foreign body is considered a major risk factor for the development of this infection (193).

Skin lesions by *Candida* spp. can also represent a manifestation of hematogenous candidiasis. They are lesions of major diagnostic value in the immunocompromised host.

**Chronic mucocutaneous candidiasis:** In rare cases, individuals are chronically susceptible to superficial *Candida* infections, as a result of a defect in T-lymphocyte responsiveness to the fungus. However, the defect varies from case to case with no single predominant deficiency in cellular immunity.

Through childhood and into adulthood, such patients suffer from unremitting mucocutaneous *Candida* lesions, including severe nail and skin involvement and vaginitis. The lesions sometimes develop into a gross, disfiguring granulomatous appearance (194,195).

2. **Locally Invasive Infections**

The most common situation when deep tissues are infected with *Candida* spp. is in the setting of hematogenous candidiasis. In this setting we may have the impression
that only one organ is involved when any of these possibilities occur: (1) all tissues except one eradicate the yeast; or (2) one tissue succumbs to infection more rapidly and extensively than the others. The physician should therefore be alert to the possibility of disseminated infection even in cases where only a single organ shows signs of disease. Some examples of single-organ Candida infections without concomitant disseminated disease are certainly known, but these are greatly outnumbered by instances of disseminated disease.

Urinary tract infections: Candida micro-organisms frequently exist as saprophytes on the external genitalia or urethra; however, yeasts in measurable quantities are present in <1% of clean voided urine specimens (196). The isolation of Candida specimens from a urine sample may represent contamination, colonization, or lower or upper urinary tract infection. Contamination is more common in female patients with vulvovaginal candidiasis, and it can be excluded by repeating the urine sample with proper collection techniques.

Colonization (asymptomatic candiduria) and infection occurs only in patients with local or systemic predisposing risk factors (Table 4) (197,198).

Asymptomatic candiduria: The incidence of asymptomatic candiduria has increased dramatically during the last decade among hospitalized patients, especially those with indwelling bladder or drainage devices. The prevalence of candiduria varies considerably in the same hospital and ranges from 2% to 11% and is more prevalent among patients with leukemia, bone marrow transplantation, and in those patients admitted to ICU (199).

Although C. albicans is the most common species recovered in candiduria (50%), non-albicans species are common particularly with C. glabrata. (200,201).

Distinguishing colonization from infection may be difficult. In catheterized patients, the presence of pyuria and a high colony count (10^7 cfu/mL) may not help in the diagnosis of infection, while a high colony count in urine suggests infection among non-catheterized patients.

Asymptomatic candiduria in nonseverely immunocompromised patients usually follows a benign course (198,201). In a series of 316 patients, none developed complications (urinary tract infection or candidemia) (201), while in another series of 816 patients, only 1% (six patients) developed candidemia (198). No antifungal therapy is required for asymptomatic candiduria. This condition is usually transient and associated with very low morbidity. Antifungal treatment resulted in a comparable

Table 4   Predisposing Factors for Colonization or Infection of the Urinary Tract by Candida Species (338)

<table>
<thead>
<tr>
<th>Predisposing factors</th>
<th>Candiduria</th>
<th>Lower UTI</th>
<th>Upper UTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extreme age</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Female sex</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>Broad-spectrum antibiotics</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>Indwelling bladder catheter in place</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>Immunosuppression</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Renal transplantation</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>Urinary tract obstruction</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Urinary tract instrumentation</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
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<td>Surgery</td>
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rate of eradication of candiduria 2 weeks after discontinuation of the antifungal agent (201). Discontinuation of the predisposing factors when possible is the recommended strategy. In catheterized subjects, removal of the catheter and discontinuation of broad-spectrum antibiotics eliminates candiduria in 40% of patients, while catheter exchange alone resolves candiduria in 20% of patients (201).

The favorable outcome of asymptomatic candiduria does not, however, apply to severely immunosuppressed patients (such as those with prolonged neutropenia) and recipients of renal transplantation, in whom the development of urinary tract infection and candidemia is more likely to occur. Asymptomatic candiduria in renal transplant recipients requires antifungal treatment. Candiduria in the neutropenic and the critically ill patient has been of controversial value as a marker of hematogenous candidiasis (202–204) and certainly implies a site of Candida colonization that as such contributes to the likelihood of invasive infection. Although candiduria alone cannot predict hematogenous candidiasis with accuracy, persistent candiduria in a severely immunocompromised patient with fever should lead to the investigation of disseminated candidiasis and evaluation for empiric antifungal treatment.

Eradication of candiduria is also recommended for patients undergoing urologic instrumental procedures caused by the high risk of disseminated candidiasis (205,206).

**Lower urinary tract infections:** Symptoms are comparable to those observed in patients with bacterial cystitis. If a cystoscopy is performed, it reveals soft, pearly white elevated patches with hyperemic and friable mucosa underneath. Emphysematous cystitis and prostatic abscess are occasional complications of *Candida* cystitis (207). Antifungal treatment is always indicated and the outcome is usually favorable. Oral fluconazole (200 mg/day × 7–14 days) is the recommended treatment because of its efficacy, high urine concentration, and low toxicity. For fluconazole resistant *Candida* species, intravenous (IV) amphotericin B (0.3–1 mg/kg/day for 1–7 days) is also effective (208). Of note, even a single dose of IV amphotericin B, 0.3 mg/kg, has been shown to be highly efficacious in lower urinary tract candidiasis (209). Other effective but less used alternatives include bladder instillation with Amphotericin B 50–200 μg/mL and oral flucytosine 25 mg/kg qid (208). Amphotericin B instillation is not recommended because it requires an indwelling catheter and hospitalization. Monotherapy with oral flucytosine cannot be used in patients with renal failure but may be very useful for treating *C. glabrata* infections.

**Upper urinary tract infections:** Ascending pyelonephritis and urosepsis by *Candida* species are indistinguishable from bacterial pyelonephritis and urosepsis. These infections occur mainly among patients with urinary obstruction and stasis. Complications include pyonephrosis, focal abscesses, development of fungal balls (bezoars) resulting in obstruction and renal colic, and papillary necrosis. Fungal bezoars are rare and develop mainly in the pelvis and upper ureters. Candidemia is also an uncommon complication of ascending infection and occurs mainly among patients with obstruction, or following urologic procedure or manipulation. Ultrasonography and CT scanning are useful in the diagnosis of fungal balls, hydronephrosis, and intrarenal and perinephric abscesses (207). These infections require systemic antifungal treatment, typically with fluconazole. The duration of the treatment will depend on the severity of the infection. Failures of medical treatment are usually because of obstruction, and resolution of the obstruction may require percutaneous nephrostomy, drainage, placement of ureteral stents, or surgical removal of bezoars.

**Renal candidiasis:** This infection is usually secondary to the hematogenous spread of *Candida* species. It is accompanied by fever and other constitutional
manifestations of *Candida* sepsis. These patients may develop candiduria without any other symptom of renal involvement other than variable reduction in renal function (207). This entity is described in detail in the section on hematogenous candidiasis.

A clinical approach to the patient with candiduria is described in Figure 1.

**Pneumonia:** *Candida* pneumonia is classified as primary in the absence of other manifestations of hematogenous candidiasis. The infection is very rare and results from aspiration of food contents. Secondary *Candida* pneumonia, the most frequent presentation, is associated with hematogenous candidiasis (210).

**Cardiovascular infections:** Cardiovascular infections caused by *Candida* spp. can present as endocarditis, myocarditis, or pericarditis.

**Endocarditis:** *Candida* spp. are responsible for 65% of fungal endocarditis (9,211). *Candida* spp. cause 2–10% of prosthetic valve endocarditis (212), and among patients with prosthetic valve, those who develop candidemia have a 25% risk of developing *Candida* endocarditis (213). Fourteen percent of infective endocarditis among IV drug abusers are caused by *Candida* spp. (9,211,214,215).

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**Figure 1** Clinical approach to the patient with candiduria. *Also check for genital candidiasis. *Also check for genital candidiasis. **Lower, upper or systemic symptoms that cannot be associated to other etiology.
Reported risk factors for *Candida* endocarditis include: (1) major surgery (cardiac and others), (2) pre-existent bacterial endocarditis or valvular disease; (3) in situ pacemaker, or long-term CVC. Other populations that also reported cases of *Candida* endocarditis include neonates and occasionally immunosuppressed patients. Among neonates, *Candida* endocarditis is a less common event than hematogenous infection and seems to affect mainly the right side of the heart (216).

The clinical presentation of *Candida* endocarditis resembles bacterial endocarditis with fever at presentation (75%), new or changing heart murmur (50%), and/or heart failure (25%). Unlike bacterial endocarditis, however, the risk of embolization of major arteries is very high (2/3 patients). Embolic lesions are usually detected in brain, kidneys, spleen, liver, skin, eyes, and the coronary arteries. Classic signs of infective endocarditis like finger clubbing, Osler’s nodes, splinter hemorrhage Roth’s spot, and splenomegaly are uncommon (214,217–219). The most commonly involved valves are the aortic and mitral valves, even among IV drug abusers and among patients with a central venous catheter in place (218).

*Myocarditis:* This infection almost always occurs in the setting of hematogenous dissemination, mainly among immunocompromized patients (220,221), and is associated with conduction disturbances, hypotension, and shock (222).

*Pericarditis:* Pericarditis is a rare condition that is associated with serious complications, including hematogenous spread and tamponade (9,211,223). The infection is associated with immunosuppression (including AIDS), previous antibiotic therapy, pericardectomy, thoracic surgery, and hematogenous *Candida* infection (224).

*Central nervous system infections:* CNS infections by *Candida* spp. are rare and can present as meningitis or abscesses. *Candida* infections of the CNS are usually secondary to hematogenous disease or associated with CNS surgery or ventricular-peritoneal shunt (9). In the setting of hematogenous disease, meningitis is more frequent in neonates (64%) (131) than among adults (225). Prematurity is one of the principal risk factors for neonatal meningitis (226). *Candida* meningitis usually presents as an acute infection among neonates, while its course may be indolent or chronic in adults. Neurosurgery-related candidiasis can present with the features of bacterial meningitis in adults (227,228).

Although the pathogenesis of *Candida* meningitis is unclear, recent data suggest that *C. albicans* is able to adhere, invade, and trancytose across human brain microvascular endothelial cells without affecting the monolayer integrity (229).

*Ocular infections:* Ocular *Candida* infections include keratitis, chorioretinitis, and endophthalmitis. Most cases of chorioretinitis and endophthalmitis are secondary to hematogenous spread and may be the earliest manifestation of hematogenous candidiasis. A few of these infections are secondary to trauma (mainly after ocular surgery), while keratitis is usually associated with local trauma.

*Bone and joint infections:* Most cases of bone and joint *Candida* infections are secondary to hematogenous candidiasis. Primary *Candida* osteitis and arthritis are rare and result from accidental implantation of the fungus by traumatic means (e.g., surgery, intra-articular injection of corticosteroids) or by contiguity in patients with infected diabetic foot ulcers. The infection typically involves a single joint or bone. Diagnosis is best achieved by culturing the organism from the joint fluid.

*Abdominal infections:* *Candida* spp. are frequently cultured from intra-abdominal material but should be considered significant only in certain subsets of patients, such as those receiving TPN or broad-spectrum antibiotics, patients with recurrent perforations or abdominal infections, necrotizing pancreatitis, and anastomotic leak (230–233).
Primary biliary candidiasis (infection of gallbladder and/or the biliary tree by *Candida* spp.) is very rare. In the only series of biliary candidiasis, risk factors included candidemia (3 of 27 patients), and the known risk factors for hematogenous candidiasis, including colonization and poor physiologic scores, suggest that in most of these patients the biliary involvement was secondary to hematogenous spread (234). The diagnosis is usually made with pure or persistent growth of *Candida* spp. from the biliary tract and response to antifungal treatment. Histopathological documentation of tissue invasion is desirable but not always feasible.

There is an increasing appreciation for the role of *Candida* in infections following acute necrotizing pancreatitis (235).

Liver, gallbladder, and subphrenic abscesses have been described in cancer patients with percutaneously placed drainage catheters (236).

*Candida* peritonitis is seen in patients on continuous ambulatory peritoneal dialysis (CAPD). A review of 105 cases of peritonitis in patients undergoing CAPD indicated that fungi accounted for 8% of all infections (237). Another series of 20 cases of CAPD-related fungal peritonitis revealed that 75% of these infections were caused by *Candida* spp, mostly *C. albicans* (238). *Candida* peritonitis has also been reported in a patient with liver cirrhosis (239) and in patients with intra-abdominal malignancies (240). In *Candida* peritonitis, the infection tends to remain localized and presents with low grade fever, abdominal pain and tenderness. The peritoneal dialysate is usually cloudy and contains more than 100 neutrophils/mm³. If untreated, *Candida* peritonitis may lead to hematogenous candidiasis (232).

Wound infections: The diagnosis of candida wound infections is difficult. Recovering *Candida* spp. from wounds does not necessarily imply that this organism is causing tissue infection and should not compel physicians to use systemic antifungal therapy. However, such therapy should be considered in those patients whose wound infections do not respond to appropriate antibacterial therapy, particularly if the same *Candida* spp. is repeatedly isolated from the wound site. In a recent survey *C. albicans* was found in 0.43% of 2458 wound swabs in patients at a teaching hospital (241). Patients undergoing coronary artery bypass grafting may develop deep sternal wound infections by *Candida* spp. with or without concomitant sternal osteomyelitis. This infection may present up to 150 days after surgery and is characterized by a chronic, indolent course, requiring surgery and prolonged antifungal treatment (median 6 months) and may recur (242). *Candida* spp. have been also reported to cause ostomy wound infections (243), necrotizing fasciitis following renal transplantation (244), and postlaminectomy wound infection (245).

**VI. DIAGNOSIS**

**A. Direct Microscopy**

Direct microscopy is a simple and economic approach to the detection of *Candida* spp.; however, negative results from microscopy should not be regarded as definitive. Yeast cells (and pseudohyphal or hyphal forms when present) are easily visualized by phase-contrast microscopy of any wet specimen. Like all fungi, *Candida* spp. are Gram-positive and can usually be visualized with a Gram stain. They do not show up well with hematoxylin–eosin or Giemsa stains, and are better evaluated by periodic acid-Schiff’s reaction and Gomori’s methenamine silver stains. The presence of *Candida* spp. in the urine is often detectable by direct microscopy. In cases of
suspected hematogenous Candida infection, microscopic examination of blood smears will occasionally reveal the fungal cells.

B. Isolation Methods

Blood cultures with hematogenous candidiasis are negative in one-quarter to one-third of patients. Biphasic blood culture media and vented culture bottles are optimal for the detection of Candida yeasts. Pretreating blood samples by cell lysis and centrifugation enhances the yield of blood cultures (246). Combination of lysis-centrifugation and the automated BACTEC® system for identification offers an average time to detection of around 3–4 days. Candida albicans, C. parapsilosis, and C. tropicalis usually appear within 3 days in blood cultures, whereas C. krusei and C. glabrata often take longer to grow.

The Candida spp. are all able to grow on standard mycologic isolation media at 35°C. Sabouraud agar pH 5.6 with chloramphenicol and gentamicin added to minimize bacterial contamination is widely used for the culture of Candida organisms from clinical samples. Note that cycloheximide (“Actidione”) should not be incorporated in isolation media for Candida yeasts because it inhibits the growth of some species. Most pathogenic Candida spp. also grow on many bacteriologic isolation media, including blood agar, brain–heart infusion agar and tryptose agar.

One commercial product, CHROMagar Candida®, allows the rapid presumptive identification of C. albicans, C. tropicalis, and C. krusei at the time of isolation, and may distinguish colonies of C. dubliniensis from those of C. albicans (6). However, the shelf life of the medium in a refrigerator is short (maximally 2 months), and the volume of medium poured in the Petri plate must be sufficient (at least 20 mL) to ensure formation of the correct colony color.

C. Identification Methods

The methods currently adopted in most routine clinical laboratories remain those based on morphologic and physiologic testing.

The most common strategy for identification of yeasts is to use rapid, simple, and specific tests to identify isolates of C. albicans. For non-C. albicans species, a battery of physiologic tests combined with scrutiny of microscopic morphology will ensure correct identification of all but a few of the yeasts recovered from clinical material.

The preliminary identification of C. albicans can most easily be done by recognition of its characteristic colony color on a suitable differential isolation medium. An alternative specific test for C. albicans is its ability to produce germ tubes (short hyphal outgrowths) in serum after 3 h at 37°C (more than 90% of C. albicans isolates produce positive germ tube) and detection of its N-acetyl-β-D-galactosaminidase and L-proline aminopeptidase activities: only C. albicans among Candida spp. expresses both enzymes (247,248). Commercial systems based on this property are also available. The newly described species C. dubliniensis closely resembles C. albicans in all of these screening tests. Testing of apparent C. albicans isolates for the absence of β-glucosidase activity (6) can confirm the identity of an isolate as C. albicans.

Isolates that cannot be recognized as C. albicans or C. dubliniensis at this prescreening stage should be examined for the morphology of their blastoconidia and for their ability to produce pseudohyphae and chlamydospores on suitable semistarvation media such as corn meal or cream of rice–Tween agars, and their carbohydrate assimilation profiles should be determined. Several commercial kits are
available, of which the API 20C kit is very widely used internationally. A recently
developed kit (ID 32C) in the API series offers more rapid yeast identification and
the possibility of automated reading.

To establish unequivocally that two strains are identical would theoretically
require determination of the entire DNA base sequence from both isolates.

D. Serological Methods of Detection

1. Antibody Detection

A plethora of test methods have been tried, including agglutination of coated latex
particles, immunodiffusion and immunoelectrophoretic test systems, radioimmunoas-
say and enzyme immunoassay, often with sophisticated refinements (249). Unfortu-
nately, detection of *Candida* antibodies, falls short of discriminative specificity and
sensitivity for prospective diagnostic purposes regardless of the detection method used.

2. Antigen Detection

Methods for antigen detection failed to achieve satisfactory diagnostic results in a
routine prospective diagnostic setting. Three groups of antigens have been studied:
(1) Mannan, (2) (1–3)-β-D-glucan, and (3) *Candida* enolase.

3. Polymerase Chain Reaction

Amplification of DNA of *Candida* spp. appears to be a quick and specific diagnostic
tool. While multiple approaches have been pursued, several limitations still need to
be overcome before this methodology can be routinely used.

4. Detection of Metabolites

Another approach to diagnosis of *Candida* infections is the detection of specific
*Candida* metabolites by chemical methods. D-arabinitol is the most promising
metabolite in terms of its near unique specificity as a *Candida* product. Because levels
of the metabolite may become elevated in patients with impaired renal function,
results of D-arabinitol testing are usually expressed as a serum D-arabinitol: creati-
nine (Ara/Cre) ratio. However, serial Ara/Cre determinations for detection of
*Candida* infection and for monitoring therapeutic responses among patients at risk
of such infections did not perform better than blood culture (250).

D-arabinitol/L-arabinitol ratio was evaluated among urine samples of 61
patients undergoing treatment for hematologic malignancies (251) and 117 neonates
(252), but was found to be of limited diagnostic value.

Mannose is another metabolite of *Candida* spp. that could be useful for the
early detection of candidal infection. This test however requires a complicated
gas–liquid chromatography system and suffers low sensitivity (39%) (253).

VII. THERAPY

A. Disseminated Candidiasis

1. Acute Disseminated

Hematogenous candidiasis is associated with significant mortality and morbidity (see
earlier section Morbidity and Mortality). As a result, all patients with positive blood
cultures for *Candida* spp. should receive antifungal treatment (254).
Fluconazole, a well-tolerated triazole, has good activity against *Candida* spp. Five studies compared fluconazole to IV amphotericin B. These studies were randomized (156,255,256) prospective observational (99) and matched cohort (257). Fluconazole dosages were generally around 400 mg/day (range 200–800) orally or intravenously, while IV amphotericin B was given at doses of 0.3–1.2 mg/kg. All these studies showed that fluconazole was as effective as and better tolerated than amphotericin B. It is currently recommended that fluconazole, be administered at 600–800 mg/day IV for 3 days, particularly if the infecting organism is known to be or is likely to be *C. albicans*. If the patient responds rapidly to this regimen, the dosage may be decreased to 400 mg/day and the drug given orally (258).

Itraconazole therapy for hematogenous candidiasis has not been adequately evaluated mainly because of the low bioavailability of the itraconazole capsules. A new solution of itraconazole has been recently developed. This formulation has improved the solubility of itraconazole, leading to enhanced absorption and bioavailability compared with the original capsule formulation and making this formulation potentially suitable for the treatment of hematogenous candidiasis. An IV formulation of itraconazole is now available and was found to be effective against hematogenous candidiasis in animal models (259). Clinical data are not available yet.

Voriconazole is a new azole that has been recently marketed. Its spectrum includes *Candida* spp. (including some that are fluconazole-resistant strains), and the drug has shown good clinical activity in the treatment of esophageal candidiasis (260). It is likely that voriconazole will be effective in candidal bloodstream infections.

Lipid-associated formulations of amphotericin B are less nephrotoxic than the parent compound. Three lipid products of amphotericin B are available: amphotericin B colloidal dispersion (ABCD) (Amphotil), amphotericin B lipid complex (ABLC) (Abelcet), and liposomal amphotericin B (L-AMB) (AmBisome). Several studies have evaluated the utility of the lipid amphotericin B preparations for hematogenous candidiasis. A large prospective randomized trial has shown that ABLC at a dose of 5 mg/kg/day was as effective as, and probably less nephrotoxic than 0.7–1 mg/kg/day of, conventional amphotericin B in hematogenous candidiasis (261). Data regarding the treatment of hematogenous candidiasis with ABCD are limited to trials where the drug was used because of intolerance to, or failure of conventional amphotericin B. In such studies, the response rate was 70% among evaluable patients (no. 107), and 50% among the intent-to-treat-population (no. 239) (262). The efficacy of L-AMB in hematogenous candidiasis has been studied in the neonatal population with response rates ranging between 72 and 100% (263,265) Among 52 adults who were intolerant to or failed to respond to conventional Amphotericin B, 83% responded to L-AMB (266). Among the three lipid formulations of amphotericin B, L-AMB (AmBisome) is the least nephrotoxic and appears to result in significantly less infusion-related reactions (267,268). There is no consensus about optimal dosing of the lipid formulations of amphotericin B. However, doses of L-AMB as low as 1–3 mg/kg/day and those of ABLC and ABCD of 5 mg/kg/day seem to be adequate for the treatment of *Candida* infections. The substantial cost of the lipid formulation of amphotericin B limits their use to patients at risk for renal failure and those intolerant to conventional amphotericin B who are infected by an azole-resistant strain and are unable to receive caspofungin (see later).

A new class of antifungal agents called echinocandin has been developed, and caspofungin is the first marketed agent of this class. Caspofungin is approved for treating candidemia and invasive candidiasis and was as effective as and better
tolerated than amphotericin B in a randomized, double-blind trial that enrolled 239 (mainly non-neutropenic) patients (269). Caspofungin is better tolerated than the lipid formulations amphotericin B for the treatment of Fluconazole-resistant Candida infections. Micafungin and anidulafungin, other members of the Echinocandins class, are currently being evaluated under study for the treatment of candidemia (270).

**Therapeutic recommendations**: For patients who are hemodynamically unstable and those with high-grade persistent fungemia, two-drug antifungal regimens may be considered for faster clearance of the infection (258). Recently, a randomized, blinded, multicenter trial in 219 non-neutropenic subjects with candidemia showed that the combination of fluconazole plus amphotericin B was not antagonistic when compared to fluconazole, and resulted in more rapid clearance of the organism from the bloodstream (197).

Duration of therapy depends on the extent and seriousness of the infection. Therapy can be limited to 7–10 days for patients with low-grade fungemia, without evidence of organ involvement or hemodynamic instability. On the other hand, patients with high-grade fungemia, organ involvement, or hemodynamic instability should receive antifungal therapy for 10–14 days after resolution of all signs and symptoms of infection (258).

2. **Chronic Disseminated Candidiasis (CDC)**

Amphotericin treatment of 23 patients with CDC (median 112 days, mean total dose 4.5 g) resulted in a response rate of 82% (114). Alternative agents included fluconazole with a response rate of 88% (n = 20) (113) to 100% (n = 5) (271), and the lipid formulations of amphotericin B. ABLC has been used in 11 patients as primary treatment in doses ranging from 2.5 mg/kg day for 6 weeks (272) to 5 to 11 mg/kg/day for a median of 4 months (273). L-AMB has been successfully used in two patients who failed to respond conventional amphotericin B (274,275).

Treatment duration is usually prolonged until 1–2 months after complete resolution of clinical findings of infection (258,276).

*Patients with candidemia and CVCs in place*: Removing all CVCs in patients with candidemia is considered standard practice (208,277), based on the belief that the CVC is the primary source of candidemia and that its removal reduces the morbidity and mortality associated with candidemia. However, arguments against removal of all CVCs in patients with candidemia include: (1) the gastrointestinal origin of most candidemias (see section on hematogenous candidiasis, risk factors); (2) the cost, difficulties, and complications associated with CVC replacement in certain settings (patients with difficult venous access, multiple CVCs in place, high risk for bleeding or pneumothorax, others); and (3) the lack of randomized trials designed to specifically answer the question of CVC removal or retention among patients with candidemia.

In a recent evidence-based review, Nucci and Anaissie (110) showed that among 203 candidemia studies, only 14 evaluated outcome in relation to CVC removal or retention, and among those, only four performed multivariate analysis and included confounding variables such as severity of illness. Analysis of these four studies showed that the beneficial effect of CVC removal on mortality was not present in one study, was marginal in two studies, and was only significant in a subset of 21 neutropenic patients in the fourth study.
Removal of CVC is recommended when its access is no longer needed, or when its replacement is easy and safe (nontunneled, nonimplanted CVC). In addition, the CVCs should be removed in the presence of high-grade fungemia, hemodynamic instability, organ infection (endophthalmitis and other) or pocket site infection, or when the patient remains with a pyrexia of unknown etiology despite 72 hr of adequate antifungal treatment (appropriate dose of an agent usually active against the infecting Candida strain). Removal of all CVCs in infections by Candida parapsilosis is also recommended unless other sources such as contaminated TPN are responsible for the candidemia. In patients with candidemia and implantable or semi-implantable CVCs, medical antifungal treatment without CVC removal should be considered first, especially in the absence of any of the clinical settings mentioned above. A detailed approach to managing CVC in candidemia is presented in Figure 2 (258).

In the setting of secondary CVC-related candidemia (septic thrombophlebitis or endocarditis), removal of the CVC in all patients should be done. Excision of the infected vein, when possible, may shorten the duration of the bloodstream infection. Duration of antifungal therapy in the setting of thrombophlebitis should be determined by the clinical response. Patients should continue to receive therapy until 2 weeks after resolution of all signs and symptoms of infection (129,162). Repeat surgery may be needed if the vein excision was not complete and blood cultures remain positive (163). Patients with thrombophlebitis of the central veins are not surgical candidates. In those cases, aggressive medical treatment alone has been reported to be successful in 8 of 10 patients (130).

**Figure 2** Proposed management of central venous catheters (CVCs) in nonneutropenic patients with candidemia. 

- **High risk of bleeding or pneumothorax; serious complication with bleeding or pneumothorax (such as patients with limited lung function).**
- **Value of quantitative blood cultures not established.**
- **Especially in patients with Candida parapsilosis (typically associated with CVC-related candidemia).**
- **Most cases of cellulitis at the CVC site are not infectious and occur within a few days of CVC insertion. Patients with severe neutropenia and mucositis are unlikely to benefit from CVC removal.**

_Candidemia caused by contaminated IV fluids and total parenteral nutrition may occur. Removal of CVC recommended in addition to elimination of source of contamination. Source: Vascular catheters and candidemia, CID, 2002; 34(1 March):597. (Figure is from Clin Infect Dis 34(5): 591–599)._
The average total dose of amphotericin B given to patients who survived was 2 g. Despite CVC removal and appropriate antifungal therapy, blood cultures may remain positive for up to 3 weeks (278).

3. Cytokine Therapy for Hematogenous Candidiasis

The immune status of the host plays a predominant role in the pathogenesis of opportunistic fungal infections, including hematogenous candidiasis (279). Polymorphonuclear leukocytes and macrophages are the predominant host defenses against candidal infections. Four cytokines, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage stimulating factor (GM-CSF), macrophage-stimulating factor (M-CSF), and interferon gamma, seem to be promising as adjuvant therapy for proven fungal infections including candidiasis (270,271).

Interferon gamma enhances the candidacidal activity of phagocytes probably through increased production of reactive oxygen radicals and modulates the phagocytosis of Candida albicans by endothelial cells. Both G-CSF and GM-CSF activate phagocytic cells and restrict the growth of C. albicans. Despite the promising in vitro and experimental data, the clinical experience with cytokines for the treatment of fungal infections remains limited. Anecdotal reports showed favorable outcome of cytokine therapy (interferon-gamma plus G- or GM-CSF) in three patients with hematologic malignancies and refractory chronic disseminated candidiasis (unpublished, Anaissie EJ et al. in press).

B. Local Infections

1. Mucocutaneous Infections

Oropharyngeal candidiasis: In general, systemic treatments with fluconazole, itraconazole, amphotericin B, and caspofungin are more effective than the topical treatments with nystatin or clotrimazole (282).

Elimination of the colonization of the denture by extensive and regular cleaning is also recommended in denture-related oropharyngeal candidiasis. (283).

Esophageal candidiasis: Randomized studies showed that caspofungin and voriconazole were as effective as fluconazole (200 mg/day) (260,284) or amphotericin B (0.5 mg/kg/day) (285) for the treatment of esophageal candidiasis. Duration of therapy is at least 10 to 14 days.

Lower gastrointestinal candidiasis: Because stool cultures do not differentiate between colonization and infection, candidiasis in the lower gastrointestinal tract is usually a postmortem diagnosis; hence, there are no reliable criteria governing when and how to treat this condition. Patients with diarrhea caused by heavy colonization with Candida spp. may respond dramatically to two to four days of nystatin therapy (286).

Candida infections of the genitalia: In uncomplicated cases, most cases of vulvovaginal candidiasis can be successfully treated with topical azoles, a single day treatment of oral fluconazole (150 mg) or itraconazole capsules (200 mg bid), or 5 days of ketoconazole (400 mg/day) (287). A recent randomized double-blind study showed that fluconazole 150 mg single dose was as effective as 3 days of itraconazole 200 mg/day (288). In cases of recurrent vulvovaginal candidiasis (defined as more than four episodes per year), acute severe attacks, or failure to conventional therapy, treatment (fluconazole, itraconazole, or ketoconazole) should be prolonged for 14 days, followed by a maintenance regimen (fluconazole 150 or 100 mg weekly for 6 months). Candida balanitis responds quickly with twice-a-day application of
miconazole, clotrimazole, or other topical antifungal agents. Relief is almost immediate, but treatment should be continued for 10 days. Preparations containing topical steroids give temporary relief by suppressing inflammation, but the eruption rebounds and worsens, sometimes even before the steroid cream has been discontinued.

*Candida* infections of the skin and nails: Oral fluconazole (50 mg/day, or 150 mg/week) (289), itraconazole (100 or 200 mg/day), ketoconazole (200 mg/day), or terbinafine (125–250 mg bid) can be effective (290,291). Terbinafine seems to be more active against infections caused by *C. parapsilosis* and *C. albicans* (292). Treatment also includes adequate hygiene and measures directed at promoting dryness and avoidance of occlusion.

Congenital cutaneous candidiasis may resolve with topical and oral nystatin treatment. However, in patients with burnlike dermatitis, or positive cultures for *Candida* spp. from any site, respiratory distress, or laboratory finding consistent with sepsis, systemic antifungal treatment is recommended because of the high likelihood of developing hematogenous infection (196).

Topical treatments for onychomycosis by *Candida* spp. are usually of little value and oral therapy should be used. Topical antifungal agents may have a role in preventing relapse of the infection after successful oral therapy. Oral treatment options include: itraconazole (200 bid × 7 day per month × 3 pulses), terbinafine (250 mg/day × 12 weeks), and fluconazole (150 mg/week × 9–18 months) (293).

Antifungal treatments can ameliorate the morbidity of patients suffering of chronic mucocutaneous candidiasis. Effective agents include itraconazole (200 mg/day) and fluconazole (50–200 mg/day) given for months continuously or intermittently (294–296). Relapses will occur because of persistence of the immune defect.

2. Locally Invasive Infections

**Urinary tract infections:** Treatment of urinary tract infections usually require the same treatment given for hematogenous candidiasis. Release of obstruction when present is essential for successful outcome (see earlier section Urinary tract infections).

**Pneumonia:** The recommended treatment for *Candida* pneumonia is the one that applies to hematogenous candidiasis.

**Cardiovascular infections Candida:** endocarditis is associated with high mortality and high frequency of relapse. In two reports of fungal endocarditis mostly caused by *C. albicans*, the 5-year survival ranged from 67% to 75%, with a relapse of 19–33%. Relapses may develop up to 9 years after the first episode (214,297).

Treatment of *Candida* endocarditis includes surgery and antifungal chemotherapy given prior (1–2 weeks), during, and after (6–8 weeks) surgery followed by prolonged (1–2 years) suppressive antifungal therapy for periods as long as 2 years after surgery. The recommended drugs include amphotericin B or its lipid formulation, and fluconazole for suppressive therapy. Doses and standard duration of therapy have not been established (211,218). Caspofungin is a less toxic option than amphotericin B but has not been investigational in this setting.

The rationale for valve replacement in *Candida* endocarditis relies on the fact that antifungal agents are fungistatic and may penetrate poorly into the vegetations. Furthermore, candidal vegetations tend to be large, leading to a higher rate of embolic complications compared to bacterial endocarditis. Successful outcome with medical treatment alone has been reported even in the setting of prosthetic valve endocarditis (298).
**Candida** myocarditis is usually treated like an acute condition; therefore, there are no standard recommendations for treatment. The same treatment that applies to hematogenous candidiasis is recommended, because 62% of cases of hematogenous candidiasis have myocardial involvement without valvulitis (222,258).

*Candida* pericarditis is best treated with a combined surgical and medical approach, including pericardial drainage or/and pericardiectomy and prolonged antifungal therapy (224,299). Amphotericin B has been the most commonly used antifungal agent and achieves high concentration within pericardium (50% of the serum concentration) (300). Other antifungal agents such as fluconazole, caspofungin, and the lipid formulations of amphotericin B are also likely to be effective.

**Central nervous system infections:** Standard therapy for *Candida* meningitis is amphotericin B plus flucytosine (301). The combination of high-dose fluconazole (800 mg/day) and flucytosine (50 mg/kg/day) is a particularly attractive approach because of the high cerebral spinal fluid concentrations achieved with both agents (302,303). Therapy should be given until all signs and symptoms of infection have resolved.

**Ocular infections:** The management of *Candida* endophthalmitis consists of prompt initiation of antifungal therapy (to prevent blindness) with an agent that has high intraocular penetration and surgical consultation. Fluconazole is currently the drug of choice because of its proven efficacy and its higher concentration in ocular tissue. It is recommended to give 800 mg/day of fluconazole until a major response is observed at which time it may be possible to reduce the dose to 400 mg/day. If the infecting organism is potentially resistant to fluconazole, then high-dose amphotericin B (0.7–1 mg/kg/day) should be given, preferably in conjunction with flucytosine because of the poor intraocular penetration of amphotericin B. Therapy should be continued for at least 10–14 days after resolution of all signs and symptoms of infection. In the presence of vitreal or severe ocular infection, amphotericin B may have to be administered intraocularly (258).

**Bone and joint infections:** Early diagnosis of *Candida* arthritis and systemic antifungal therapy are important to prevent destruction of the cartilage or loosening of the prosthesis. For the treatment of *Candida* osteomyelitis, surgical drainage of pus is essential for a good response; however, debridement of bony lesions may not be needed. Fluconazole 400–800 mg/day or amphotericin B for 6 months is recommended (304–308).

**Abdominal infections:** Because fluconazole (400–800 mg/day for 2–6 weeks) is safe and achieves high concentration in the peritoneal fluid, it is likely to be useful in the management of candidal peritonitis and other intra-abdominal infections. The use of peritoneal amphotericin B is discouraged because of the local toxicity (local irritation and fibrosis). Liver, gallbladder, or subphrenic abscesses have been reported in patients with percutaneously placed drainage catheters for malignancy (236). Catheter exchange or removal may be indicated in this setting.

Patients with candidal peritonitis after long-term ambulatory peritoneal dialysis should receive therapy with systemic antifungal agents and the peritoneal catheter may need to be removed (309,310).

**VIII. PREVENTION**

The strategies for the prevention of severe candidal infections should be effective and safe, and not associated with a high likelihood of development of resistance to antifungal agents. The best strategy should focus on identification of the target
population (those at highest risk for developing severe candidiasis), implementing simple but effective infection control measures and, when needed, providing antifungal chemoprophylaxis.

A. Identification of Patients at Risk

As mentioned earlier, patients at highest risk for severe candidal infections include those who are colonized with *Candida* spp. at two or more sites, who have or likely to have significant disruption of the integrity of the gut mucosa (leading to increased translocation of *Candida* spp.), and who are immunosuppressed (either locally such as after organ transplantation or systemically following immunosuppressive therapy). The patient populations at risk include neonates; critically ill adults (surgical, burn, hemodialysis patients, among others); cancer patients; bone marrow/stem cell, and solid organ transplantation recipients; and advanced stages of AIDS.

B. Infection Control Measures

*Handwashing:* Up to 58% of health care workers can carry *Candida* spp. on their hands, and transmission from staff to patients and from patient to patient has been documented in several studies (see section on epidemiology). Strict handwashing remains the simplest and most effective measures to prevent the acquisition of organisms by patients. The use of artificial fingernails should be discouraged in areas housing high-risk patients as artificial fingernails may harbor yeasts (245,311).

*Equipment, devices, and IV solutions:* Cleaning, sterilization, and disinfection of all medical equipment and care of intravascular devices should follow the Centers for Diseases Control and Prevention guidelines for the prevention of nosocomial infections. Preparation of TPN infusates should follow strict aseptic techniques to avoid exogenous contamination.

C. Antifungal Chemoprophylaxis

Chemoprophylaxis with systemic antifungal agents should be limited to high-risk patients.

*Hematologic and bone marrow (BMT)/stem cell transplant (HSCT) patients:* Patients with acute leukemia and/or bone marrow/stem cell transplantation and any patients with anticipated prolonged neutropenia are more likely to benefit from antifungal chemoprophylaxis. Fluconazole has been the most commonly used agent for prophylaxis because of its excellent safety profile. Two randomized, prospective, double-blinded, placebo controlled trials that enrolled HSCT recipients showed that prophylactic Fluconazole given in doses of 400 mg/day significantly reduced the incidence of superficial and invasive candidiasis (312,313), improved overall survival (312), and was associated with fewer fungal-related deaths (313). Continuation of Fluconazol to day 75 after transplant was shown to improve survival in one study of high-risk allogeneic patients (314). Similar studies were also conducted among patients with acute leukemia. Fluconazol was effective in reducing colonization and superficial infection, but studies did not show a decrease in invasive fungal infections (315–317). In a recent randomized placebo-controlled study, fluconazole 400 mg/day was as effective as 200 mg/day for prophylaxis of yeast infections in recipients of bone marrow transplantation (318). This study also shows no major benefit of prolonging fluconazole prophylaxis beyond day 100 after transplantation. Another randomized trial showed that fluconazole 200 mg/day was as effective as
low dose amphotericin B (0.2 mg/kg/day) for prophylaxis of fungal infections in HSCT recipients (319).

Itraconazole capsules (200 mg/day) (320) and solution (5 mg/kg/day) (321,322) are also effective in reducing documented fungal infections (mostly hematogenous candidiasis) in these patients. In a nonblinded randomized study that compared Itraconazole solution (5 mg/kg/day) to Fluconazole suspension (100 mg/day), a trend favoring Itraconazole was observed albeit at the cost of additional gastrointestinal toxicity (323).

Conventional amphotericin B is not an adequate drug for prophylaxis because of its high toxicity. Among the lipid formulations of amphotericin B, L-AMB (AmBisome) is the least toxic (267,268) and, therefore, the most appropriate for prophylaxis when prophylaxis with an azole is not possible. This agent has been used in doses of 1 mg/kg/day (324) to 2 mg/kg/day thrice weekly (325), although the incidence of fungal infections was too low in the placebo groups to detect any statistically significant benefit from this agent. The high cost of the lipid formulations of Amphotericin B, the lack of data that support significant reduction in morbidity and mortality, and the availability of new and safer antifungal agents such as the Echinocandins, and the new azole voriconazole, limit the use of lipid formulations of amphotericin B for prophylaxis of invasive candidiasis.

Patients who develop chronic disseminated candidiasis may need to undergo further cytotoxic chemotherapy or bone marrow transplantation while radiological findings may not have been completely resolved. In this setting, two reports suggest that keeping patients on antifungal therapy (secondary prophylaxis) during subsequent periods of neutropenia, including after myeloablative chemotherapy and stem cell transplantation, may result in a successful outcome in ≥80% of the patients (326,327).

*Surgical and critically ill patients:* A prospective randomized, double-blind, placebo-controlled study showed that IV Fluconazole (400 mg/day) significantly reduced colonization, and intra-abdominal infections by *Candida* spp. among patients undergoing surgery for recurrent gastrointestinal perforation or anastomotic leaks (328). Among 260 critically ill surgical patients with a length of ICU stay of at least three days, Fluconazole 400 mg/day reduced the risk of fungal infection by 55%, although there was no difference in death between the Fluconazole and the placebo group (329).

*Liver transplant patients:* Prophylaxis with liposomal Amphotericin B (AmBisome) was evaluated in a prospective randomized, double-blind, placebo-controlled study. This study showed that a dose of 1 mg/kg/day for 5 days after transplantation was effective in preventing fungal infections in this patient population. This benefit was still present even one year after transplantation (11% incidence of fungal infections in the AmBisome group versus 29% in the placebo group, p < 0.05) (330).

Fluconazole is also effective for prophylaxis of fungal infections in this patient population. One study compared Fluconazole 100 mg/day with nystatin from days 3 to 28 after transplantation. The incidence of fungal infections was significantly lower in the fluconazole group (13%) than in the nystatin group (34%) (331). In a prospective randomized, double-blind, placebo-controlled study, fluconazole (400 mg/day until 10 weeks after transplantation), significantly reduced colonization, and superficial and invasive fungal infections (332). Of note, no patient had to discontinue Fluconazole because of hepatotoxicity.

*Very low birth weight infants:* A randomized trial designed to look at rectal colonization of infants by *Candida* spp. compared placebo to fluconazole (6 mg/kg
every 72 hr during the first 7 days, and every 24 hr from days 8 to 28 of life) and showed a significant reduction of rectal colonization by *Candida* spp. through day 28 of life among infants of all weights randomized to fluconazole. This effect persisted through day 56 among infants weighing <1,250 g (333). This study suggested but did not demonstrate that fluconazole may be useful in reducing candidal infections among infants weighing <1,250 g. Later, another prospective, randomized, double-blind clinical trial conducted in 100 preterm infants with birth weights <1000 g showed that Fluconazole given during the first 6 weeks of life was effective in preventing fungal colonization and invasive fungal infection. Invasive fungal infections developed in 20% and in none of the infants that belonged to the placebo and Fluconazole group, respectively (334).

**D. Preemptive Antifungal Therapy**

Systemic antifungal chemoprophylaxis can be associated with toxicity, cost, and emergence of resistance. An alternative approach is give pre-emptive antifungal therapy which consists of limiting antifungal therapy only to those patients at high risk for serious candidal infections and who also exhibit evidence of significant colonization with *Candida* spp. at two or more sites. This strategy has been proposed in the setting of cancer chemotherapy (335) and surgery (336,337).

**REFERENCES**

Clinical Syndromes by *Candida* Species

Clinical Syndromes by Candida Species


Aspergillosis refers to infection with any of the >150 recognized species of the genus *Aspergillus*. These are in mould form in the environment, on artificial media, and when invading tissues. Aspergilli are the second most common of the fungi infecting immunocompromised hosts, but are the most common cause of mortality caused by invasive mycoses in the USA; these infections, moreover, are increasing in frequency relative to *Candida* infections, possibly because of success with prophylactic regimens for the latter mycoses. In 1996, annual hospital costs associated with aspergillosis were estimated to be >US $633 million in the USA (1).

**I. ETIOLOGY AND EPIDEMIOLOGY**

Aspergilli are ubiquitous in the environment and are associated with decaying matter with growth in temperatures of 40–50°C, e.g., self-heating organic compost. They have been easily isolated from soil, air, and even swimming pools and saunas. They are also isolated from houses, particularly from basements, crawl spaces, bedding, humidifiers, ventilation ducts, potted plants, wicker or straw material, and house dust. In surveys, they have even been found in condiments, pasta, and marijuana samples. This pervasiveness should not make it surprising that they are sometimes found in normal expectorated sputa.

In tissues, Aspergilli may be seen as septate hyphae, dichotomously branched (resembling the divergence of fingers from one another), and may produce their characteristic conidia in tissues or artificial media. If the septation can be seen, they can be differentiated from the zygomycetes, but Aspergilli may be confused with *Pseudallescheria boydii* unless the characteristic terminal spores of the latter are seen.
Several putative *Aspergillus* virulence factors have been identified, including melanin and secreted proteases, toxins, and hemolysins (2). However, gene disruption studies have rarely identified prominent virulence factors, suggesting that the true virulence of *A. fumigatus* is likely multifactorial and dependent on host immune status.

Aspergillosis generally results from airborne conidia and is not contagious. The threat to hospitalized patients has been revealed in outbreaks of infection, particularly pulmonary infection in immunocompromised hosts, associated with building renovation and new construction. The suspected vector has been unfiltered air, as from inlets contaminated with bird excreta and fireproofing materials. Hospital water, which may become aerosolized during such activities as patient showering, is a newly described possible source (3).

The most common species infecting humans are *A. fumigatus* (64–67% in two series) (4,5), *A. flavus*, *A. niger*, and *A. terreus*. However, some patient isolates are speciated by the clinical laboratory only with difficulty, and they may be reported only as “*Aspergillus* species.” Molecular biology tools will be a help in speciation in the future. For example, Rath et al. (6) have been able to identify five different *Aspergillus* species by SSCP (single-strand conformational polymorphism, a sensitive method that in conjunction with PCR, is able to distinguish amplicons that differ in sizes) using 27 culture collection strains and 55 patient isolates. *Aspergillus fumigatus* has a small spore size, enabling it to penetrate deeply into the lung alveoli (7). Most pulmonary diseases are caused by *A. fumigatus*, although isolated sinus disease is frequently caused by *A. niger* or *A. flavus* (8). *Aspergillus terreus*, which can be resistant to polyene antifungals (e.g., amphotericin B), has been reported to cause approximately 3% of cases of invasive aspergillosis (IA) (4).

*Aspergillus* aerosolizes conidia readily; while immunocompetent people breathe and clear conidia everyday (2,4), immunocompromised patients are at risk for the development of IA. Since the route of infection appears to be pulmonary, the first line of defense is formed by alveolar macrophages. In vitro studies with murine cells have suggested that resident pulmonary macrophages are responsible for digesting inhaled *Aspergillus* conidia (9,10). If conidia escape and germinate into hyphae, then the hyphae become susceptible to neutrophil killing through the release of toxic oxygen radicals (11). Thus, disease risk is associated with neutropenia, challenge with overwhelming microbial doses, and/or corticosteroid suppression of macrophage conidiacidal activity (12). More recent studies have shown that CD4 T-cell responses are important for both protection against and effective therapy of invasive infection (13–15). The mechanism by which T-cells function to protect against invasive aspergillosis is not clear, but they may enhance phagocyte killing of conidia (14).

## II. SYNDROMES

### A. Invasive Aspergillosis

Invasive aspergillosis is generally a problem of immunocompromised hosts, while more aggressive immunosuppression and anticancer therapy are the most important factors contributing to the rise of *Aspergillus* infections. Usually, several of the following factors are present: leukopenia, glucocorticoid therapy, cytotoxic chemotherapy, and broad-spectrum antibacterials. Neutropenia is the time-honored risk factor for invasive mold infections; the risk of IA is calculated to increase from 1% per day
after the first 3 weeks of neutropenia to 4-5% per day after 5 weeks (16). The incidence of IA can be as high as 70% if neutropenia exceeds 34 days (17). Repeated cycles of neutropenia may be an added risk factor. Corticosteroids suppress the ability of monocytes/macrophages to kill conidia through inhibition of nonoxidative processes and impairment of lysosomal activity, and also inhibit polymorphonuclear neutrophils in their chemotaxis, oxidative burst, and antifungal activity against hyphae (18). The results of one in vitro study suggest that corticosteroids may actually accelerate the growth of *A. fumigatus* (19).

Some series have reported an incidence as high as 41% at autopsy in patients with acute leukemia, and notably in 89% of these cases it played a significant role in the death of the patient. Pulmonary involvement was present in 97% of patients, and the infection was widely disseminated to various organs in 25% of patients. In patients with leukemia, there is particularly an association with relapses of the malignancy. In heart transplant patients, the incidence of infection goes up to 28%. The incidence in bone marrow transplant patients ranges from 5% to 20%, with a higher frequency in certain groups, such as patients undergoing allogeneic transplantation or suffering from graft-versus-host disease (GVHD), and mortality is 68 to >95% in various series. Numerous studies have repeatedly identified the risk factors for IA as older age, receipt of a hematopoietic stem cell transplant (HSCT) from an HLA-mismatched or unrelated donor, and underlying disease, and infections are more common in the summer (8,20). Pediatric patients undergoing transplantation are generally less vulnerable to infections than their adult counterparts (21), although recent reviews have reported substantial incidences of IA, ranging from 6% to 19% after HSCT (22,23).

The primary risk periods for invasive mould infections are during the early, preengraftment time period, and then again later during therapy of GVHD. Over the last decade, the late time period has become increasingly significant, with most mould infections occurring during GVHD therapy (20,24,25) while in the outpatient setting. In reviews of patients undergoing allogeneic HSCT, IA was diagnosed at a median of 88–115 days post-transplant (26–29), with mortality exceeding 80% (30). Recent studies that focused on the risks for infection late after allogeneic HSCT identified GVHD, corticosteroid exposure, secondary neutropenia and lymphopenia, and CMV disease as important variables (8). It is unknown whether CMV represents a risk in itself or signifies an underlying immune defect that is not well controlled in multivariable analyses. IA is also commonly seen in lung and liver transplant recipients (in the former also associated with CMV), and other steroid-treated patients. Mortality in solid organ transplant patients ranges from 70% to 93% (31). IA is also a problem in patients with the neutrophil defect of chronic granulomatous disease and in patients with the cell-mediated immunity depression seen in AIDS (32).

Radiographic presentation varies from single nodular lesions to bilateral diffuse pulmonary infiltrates. The classic picture is that of fever and pulmonary infiltrates or nodules, especially progressing to a cavity (usually when granulocytopenia is reversed), or wedge-shaped densities resembling infarcts. The pulmonary pathology in all these entities is that of hemorrhagic infarction and pneumonia. Pulmonary emboli are common because of the organism’s tendency to invade blood vessel walls. These processes often combine to produce a “target lesion” pathologically, consisting of a necrotic center surrounded by a ring of hemorrhage.

As with most invasive mould infections, the clinical signs and symptoms are very nonspecific. The most common clinical symptom triggering evaluation is
unremitting fever (26), but high fever may be absent in those patients receiving corticosteroid therapy (33). Other early symptoms of pulmonary disease include dry cough and possibly chest pain. The chest pain may be misinterpreted as esophagitis or viral pleuritis. Dyspnea is more common in patients with diffuse disease, and the presentation in some patients is similar to a pulmonary embolism. Hemoptysis can occur and may be fatal with the first presenting episode (34), while in neutropenic patients a pneumothorax is also an occasional presenting feature (7).

B. Invasive Tracheobronchitis

Invasive airway disease with ulcerative, pseudomembranous, or plaque-like tracheobronchitis occurs, particularly in immunocompromised hosts, and may presage parenchymal invasion. Cases of infection of the larynx, trachea, or epiglottis have been reported. Localized infection that has commonly been limited to the anastomotic site has been described in heart–lung and lung transplant recipients (35).

C. Disseminated Aspergillosis

Mycelia invading blood vessels may produce a microangiopathic hemolytic anemia. Dissemination can result in Budd–Chiari syndrome, myocardial infarction, gastrointestinal disease, or skin lesions. Esophageal ulcers may produce gastrointestinal bleeding. Abscesses are common in the kidney, liver, and myocardium.

A frequent target of disseminated disease is the central nervous system (CNS), where hematogenous spread results in occlusion of intracranial vessels and infarction (36). This may manifest as the characteristic single or multiple cerebral abscesses, or meningitis, an epidural abscess, or a subarachnoid hemorrhage (37). Cerebral aspergillosis has been noted in 25–40% of patients with invasive pulmonary disease (8,26,28,38). The classical presenting features of abscesses such as headache, nausea, and vomiting are rare (<10% of cases). More frequently, presenting signs and symptoms include altered mental status, confusion, hemiparesis, and cranial nerve palsies (39). Computed tomography (CT) of the head often reveals one or multiple hypodense, well-demarcated lesions. Hemorrhage and mass effect are unusual, but for patients with adequate peripheral white blood cell counts, ring enhancement and surrounding edema are frequent (7). The cerebrospinal fluid (CSF) glucose level is normal, and cultures of the CSF are negative. Biopsy of these lesions, if feasible, is warranted to differentiate Aspergillus infections from those caused by other fungi, such as Pseudallescheria, dematiaceous fungi, Mucorales or Fusarium, which may alter one’s choice of antifungal therapy. A surgical approach leads to laboratory characterization of the causative agent together with removal of nonviable tissue, which may not be well penetrated by systemic antifungals (40,41). Stereotactic procedures for abscess drainage have also been used (42). However, systemic antifungal therapy is used in almost every case. Meningitis is unusual, cases have been reported in neutropenic patients or those on prolonged corticosteroid therapy. It may present as an extension of paranasal sinus disease. Intrathecal therapy (via a reservoir) has been used as an adjunct to systemic therapy. Epidural abscesses are usually secondary to a contiguous site of infection, such as in a vertebral body. Surgical drainage along with systemic therapy is indicated. The published literature suggests that mortality in CNS infection exceeds 90% (43).
D. Locally Invasive Aspergillosis

Examples of locally invasive disease abound and are usually severe. These include invasion of burn wounds, focal rhinitis (particularly in immunosuppressed and/or granulocytopenic hosts), sinusitis (in these hosts or following dental procedures), and osteomyelitis or endophthalmitis (after fungemia, trauma, or surgery).

E. Invasive Sinusitis

Invasive Aspergillus sinusitis is likely underdiagnosed because of lack of detailed examination, but patients can present with sinus congestion, facial pain or swelling, orbital swelling, headache, or epistaxis (7,33). A high index of suspicion is necessary in immunocompromised patients. These infections are characterized by mucosal invasion with infarction and spread of infection in centrifugal fashion to contiguous structures. Early diagnosis is imperative, and the onset of new local symptoms, such as epistaxis, naso-orbital pain, a positive nasal swab culture in a febrile, susceptible host, or an abnormal sinus radiographic finding should lead to immediate otolaryngologic evaluation, including careful inspection of the nasal turbinates. Rhinoscopic examination may reveal insensitive areas with decreased blood flow, localized pallor of the nasal septum or turbinate mucosa, frank crusting or ulceration, or blackened necrotic foci. Although surveillance nasal cultures are of questionable value, baseline sinus radiographs or limited CT should be considered in these high-risk patients. T2-weighted magnetic resonance imaging (MRI) images may show decreased signal intensity compared to those of bacterial sinusitis, which show increased signal intensity (7). The maxillary sinus is most commonly involved, followed by the ethmoid, sphenoid, and frontal sinuses (44). Biopsy and subsequent fungal culture of suspicious lesions are important not only to demonstrate mucosal invasion but also to differentiate Aspergillus infections from those caused by other fungi, such as Mucorales or Alternaria species. Mortality is high, ranging from 20% in patients with leukemia in remission who are undergoing maintenance therapy, up to 100% in patients with relapsed leukemia or those undergoing HSCT (45,46).

F. Cutaneous Aspergillosis

This can be either primary, resulting from skin injury or traumatic inoculation, or secondary, from contiguous extension or hematogenous dissemination. The majority of cutaneous infections are caused by A. fumigatus, A. flavus, or A. terreus, but A. chevalieri has recently been reported to cause morphologically distinct skin lesions (47). In general, primary infection is most common in burn victims, neonates, and solid organ transplant recipients, whereas skin lesions in HSCT recipients usually result from contiguous extension of infected structures under the skin or from hematogenous dissemination (48). Recent reviews have reported that skin lesions may occur in 4–11% of patients with documented pulmonary aspergillosis, and may be the first presenting sign of disease (49–51).

Cutaneous aspergillosis often begins as an area of raised erythema which progresses from red to purple, then pustulates, developing a central ulceration with an elevated border covered by a black eschar, and may ulcerate (7,52,53). These lesions may be single or multiple, may not be tender, and occur most commonly on the extremities. Although the late stages are characteristic of Aspergillus infections, a skin biopsy with fungal culture is indicated to rule out other infections that may
manifest in a similar fashion. Infections arising at the site of an IV catheter puncture typically begin with erythema and induration and progress to necrosis that extends radially (53). The *A. chevalieri* lesions are erythematous, hyperkeratotic, and vesiculopapular. Cutaneous disease has been associated with the use of adhesive tape; erythematous indurated plaque-like lesions progress to necrotic ulcers.

G. Other Sites

Vertebral osteomyelitis or diskitis is the most common bone infection, with joint infections being distinctly uncommon (43,54). Surgical debridement is generally required in addition to systemic antifungal therapy.

Urinary tract infections are generally a consequence of hematogenous spread. Infections that involve the renal parenchyma are more common in immunocompromised hosts. The appearance of a fungus ball usually represents renal papillary necrosis; prostatic abscesses with or without a fungus ball also occur (55). For management of abscesses and fungus balls, surgical removal is usually indicated as an adjunct to systemic antifungal therapy. Local irrigation has also been used for urinary bladder and renal pelvis infections.

III. DIAGNOSIS

Diagnosis is difficult because aspergilli are frequently contaminants in sputum and even in other cultures during handling. Despite many efforts developing new and exciting detection tools, such as PCR assays, the diagnosis of IA still remains very difficult (Table 1). Several reasons are responsible for these limitations. First, IA often shows nonspecific and variable clinical signs, the manifestations are subtle and occur late in the course of disease. Second, IA occurs in many different patient cohorts, those at risk for a short period of time or for years. Because of residual defects and tissue infarcts, the disease has a potential to reactivate, mainly during prolonged or continuous immunosuppression and it may occur as a subacute or chronic infection. Third, no unique universally applicable test with sufficient sensitivity and specificity exists yet, and in consequence, IA is often diagnosed late leading to a delayed initiation of antifungal therapy, with a fatal outcome.

A. Microscopy, Culture, and Histopathology

Direct microscopy can be useful for the detection of *Aspergillus* spp. in bronchoalveolar lavage (BAL) or endotracheal aspirates. This method is a relatively fast, moderately sensitive tool with good specificity for fungal infection. Microscopy might be considered with material aspirated from pulmonary lesions. In wound aspergillosis, sometimes fruiting bodies can be seen. However, in the absence of these, microscopy does not allow an unequivocal diagnosis of IA, because other moulds, such as *Fusarium*, *Pseudallescheria*, and *Scopulariopsis* species may have identical appearances in microscopy and histology. The use of stains, such as calcofluor white, may be helpful in achieving a greater sensitivity. Combining microscopy and culture increases the diagnostic yield for IA in pulmonary specimens by 15–20% (56–58).

The yield of premortem and even autopsy cultures in autopsy-proven IA is extremely low (59). Culture of respiratory secretions from patients with IA is also often negative (57), and the predictive value of cultures for *Aspergillus* spp. in
<table>
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<th>Authors</th>
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<th>Type of clinical specimen</th>
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<td>Cancer</td>
<td>Whole blood</td>
<td>100% (with proven IA)</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(proven IA)</td>
<td></td>
</tr>
<tr>
<td>Ferns et al. (101)</td>
<td>Aspergillus mitochondrial</td>
<td>Leukemia/BMT</td>
<td>Whole blood</td>
<td>86%</td>
<td>n.d.</td>
</tr>
<tr>
<td>Buchheidt et al. (102)</td>
<td>Aspergillus-specific nested PCR</td>
<td>Hematological</td>
<td>BAL</td>
<td>93.9%</td>
<td>94.4%</td>
</tr>
<tr>
<td>Raad et al. (103)</td>
<td>Aspergillus mitochondrial</td>
<td>Cancer</td>
<td>BAL</td>
<td>80%</td>
<td>93%</td>
</tr>
<tr>
<td>Hayette et al. (104)</td>
<td>Aspergillus protease</td>
<td>Intensive care</td>
<td>BAL</td>
<td>100%</td>
<td>96%</td>
</tr>
<tr>
<td>Einsele et al. (105)</td>
<td>Panfungal</td>
<td>BMT</td>
<td>Whole blood</td>
<td>100% (2 specimens)</td>
<td>98%</td>
</tr>
</tbody>
</table>

**Abbreviations:** TX, transplantation; n.d., not determined; BMT, bone marrow transplantation; IA, invasive aspergillosis; Galactoman, Galactomannan antigen assay; SCT, stem cell transplantation; BAL bronchoalveolar lavage.
IA varies widely from 40% to 100% (60). The sputum culture, while having good positive predictive value in the appropriate setting (especially, neutropenic patients, particularly if febrile), is positive in only 8–34% of cases, and obtaining tissue is advisable to make the diagnosis. Prospective culturing of the nose of granulocytic patients has been of some value, because a positive nasal culture (and particularly the presence of nasal Aspergillus lesions) has led to the early diagnosis of concurrent pulmonary or sinus disease. However, negative nasal cultures are common in pulmonary aspergillosis.

Culture or cytology of BAL fluid may also be useful in diagnosis of invasive disease. However, BAL samples may only be positive in 30–50% of IA patients (61). A positive culture of Aspergillus from an otherwise sterile site provides proof of the disease. However, culture may have a reduced ability to detect Aspergillus at an advanced stage of the disease, owing to necrosis occupying a large portion of a lesion. Aspergillus species are very rarely isolated from blood (62). Aspergillus-positive blood cultures very frequently represent environmental contamination, especially when taken from a nonsterile site (63). The invasion of blood vessels, resulting in thrombosis and distal infarction, may possibly account for the low sensitivity of blood or tissue cultures, as blood flow through the affected areas may be reduced thereby. Additionally, immune responses of the host, such as cytotoxic effects, may reduce viability of the fungal cells.

Although Aspergillus species are able to grow on blood agar and other agar media, the sensitivity is higher when specific fungal media are used (64). Modifications of blood culture systems, such as aeration by shaking or adding hydrogen peroxide to prevent low oxygen pressure, have not been associated with increased isolation rates of Aspergillus.

### B. Antibodies

Although almost all persons carry anti-Aspergillus antibodies because of environmental exposure, the titers are low (except in certain patient cohorts such as patients with cystic fibrosis) and infection might correlate with an increasing number of antibodies (65–68). Data from the more commonly reported techniques suggest a high degree of sensitivity in allergic disease or aspergillomas, but generally a low sensitivity in invasive disease. Because the frequency of false-positive reactions, even in the presence of other mycoses, is low, a positive test in invasive disease may be useful. In immunocompromised patients, the presence of anti-Aspergillus antibodies is often not a result of an acute invasive infection, and it is likely that antibodies have been present before the onset of immunosuppression. Growth of A. fumigatus in tissues of these patients is not correlated with an increase of the antibody titer. Thus, the use of antibody detection in immunosuppressed patients might be limited.

Sridhar et al. (69) reported a retrospective analysis of 10 cases of confirmed IA. Serology performed by gel diffusion precipitin test was positive in only one case of sinonasal aspergillosis. The presence of serum IgG or IgA antibodies against seven Aspergillus recombinant antigens was assessed in patients with IA (70). Superoxide dismutase and a 94 kDa antigen were the most immunogenic for IgA, while the IgG pattern varied from patient to patient. The authors conclude from their study that the detection of antibodies against these antigens should not be used as a diagnostic method.

More recently, Chan et al. (71) presented data from an antibody assay with a purified recombinant antigenic cell wall galactomannoprotein of A. fumigatus, Afmmp. Clinical evaluation revealed that the test was 100% sensitive for patients
with aspergilloma and 33% sensitive for patients with IA. No false-positive results were found for serum samples from 80 healthy persons and 39 patients infected with other fungi or bacteria indicating a high specificity. This new antibody assay lends hope for the future of antibody testing in IA.

Haynes et al. (72) reported the presence of an immunodominant antigen, a 18-kDa protein in urine samples from patients with IA, whereas urine samples from patients without evidence of IA were unreactive. They proposed that these antigens should play a valuable role in the diagnosis of IA. Over a decade later, Weig et al. (73) showed in their study using serum samples that recombinant mitogillin (the 18-kDa protein studied by Haynes et al.) improves the serodiagnosis of *A. fumigatus* infection. They detected positive IgG-titers in 31 out of 42 sera from patients with pulmonary IA, but only in 1% of the serum samples of healthy persons.

### C. Antigens

Serological tests are an important tool for rapid diagnosis of IA. Except for the study of Haynes et al. (72) mentioned above, the use of these assays has focused on the *Aspergillus* galactomannan (GM) antigen. GM is a polysaccharide with a linear mannan core containing 1–2- and 1–6-linked residues. The antigenic side chains that branch from 1–2-linked mannose residues are composed of 1–3-galactofuranosyl residues (74). GM was the first antigen detected in animal models as well as in patients with IA (75–77). Two commercially available kits are available for the detection of GM in clinical samples.

The latex agglutination kit is commercially available in Europe (Pastorex® *Aspergillus*, Sanofi Diagnostics Pasteur, Marnes La Coquette, France) and has a detection threshold of 15 ng/mL (2,78). The early latex agglutination tests showed only a low sensitivity (79) but a high specificity. The detection threshold of the Pastorex assay was reported to be 15 ng of GM per mL with a sensitivity of 25–70% and a specificity of 90–100%. However, Kappe et al. (80) reported that the antibody used in this assay cross-reacted with *Penicillium* and *Acremonium* species and *Alternaria alternata*, which are all potential laboratory contaminants.

Later, an enzyme-linked immunosorbent assay (ELISA) technique was introduced using a rat anti-GM monoclonal antibody, EB-A2, which recognizes the 1–5-β-D-galactofuranoside side chains of the GM molecule. The threshold of detection with ELISA improved to 5 ng/mL (78). A sandwich ELISA technique was introduced in 1995 (81) and by using the same antibody as both a capture and detector antibody in the sandwich ELISA (Platelia® *Aspergillus*, Bio-Rad, France), the threshold for detection can be lowered to 1 ng/mL. The Platelia assay was approved for use in the United States in May 2003. The sandwich ELISA, Platelia *Aspergillus*, is widely available and has a sensitivity of 50–90% and a specificity of 81–93% when serum is used (26,78). However, when samples were serially analyzed, both the sensitivity (93%) and the specificity (95%) were higher (78). GM was detected in 63% of the patients before onset of clinical disease (82,83). The sandwich ELISA was able to detect GM at least 2 weeks earlier than the latex agglutination test (84–86). For patients receiving empiric antifungals, the sensitivity of GM detection appears to be lower, but specificity is preserved. Administration of some antibacterials can lead to false positive results.

Antigenemia can be observed from 1 week up to 2 months, depending on the type of patient (85,87,88). Furthermore, a decrease of the antigen titer in serum is indicative of treatment efficacy (83,85,89,90). Animal models suggest a decline in
titer with echinocandin therapy appears less common. There have been several clinical studies utilizing the GM assays in various populations (Table 1), generally with useful sensitivities and specificities. One continued debate is the exact cut-off value used in serum vs. urine, as well as adult vs. pediatric patients.

Another major component of the *Aspergillus* cell wall is the polysaccharide 1–3-glucan. An ELISA-based assay (G-test) has been established for the detection of 1–3-glucan (106). The components of this assay are purified from a lysate of the horseshoe crab, *Limulus polyphemus* (107), including factor G, which triggers the 1–3-glucan-sensitive hemo-lymph-clotting pathway. The detection threshold of the assay is 10–20 pg/mL serum. The G-test has been used for the detection of 1–3-glucan during various systemic fungal infections (108–110) in humans and animals (108). Unlike the GM assays, this assay is not able to distinguish amongst fungi.

**D. Biochemical Methods**

Francis et al. (111) reported a study detecting the hexitol D-mannitol in BAL by gas–liquid chromatography–mass spectroscopy, in rabbits with pulmonary IA. Measuring D-mannitol levels was significantly more diagnostically sensitive than culture and levels were significantly elevated in lavage of infected rabbits compared to controls. Serum concentrations were not useful because of high-background levels. Wong et al. (112) described a rat model of IA and postulated that *A. fumigatus* can produce and release sufficient D-mannitol in the tissues of infected animals to raise serum D-mannitol levels. The value of this diagnostic marker seems to remain limited and further investigation in patients with IA is mandatory.

**E. Radiological Signs**

Different radiological tools, such as chest radiography, ultrasonography, CT, and MR imaging, are available. The appearance of IA on chest radiographs is extremely heterogeneous. The most distinctive appearances are cavitations and pleural-based, wedge-shaped lesions. In addition, nodular shadows with and without cavitation and thin- or thick-walled cavities (especially in patients with AIDS) are typical signs of IA. However, pulmonary IA often results in false-negative chest radiographs. Therefore, high-resolution CT scans often play an important role in the detection of IA (113–119). Early lesions in the lung of neutropenic patients are small nodules with the so-called halo sign: small, often pleural-based lesions with surrounding low attenuation. These nodules may further cavitate leading to the air crescent sign. Both signs are highly distinctive for IA of the lung, probably representing radiographic correlates of edema or hemorrhage, and infarction, related to the organism’s vascu-tropism. The duration of the halo sign is short and demonstrates the value of early CT.

Miaux et al. (120) reviewed imaging data of five patients with cerebral aspergillosis. They concluded that lesions are often located in the basal ganglia and demonstrate an intermediate signal intensity within surrounding high-signal areas on long-repetition-time MR scans. These lesions were multiple and more numerous on MR images than on CT scans.

**F. Nucleic Acid-Based Diagnosis in Clinical Materials**

Since the first presentation of the polymerase chain reaction (PCR) by Saiki et al. (121) in 1988, several hundred papers have been published dealing with the
detection of fungal DNA. However, no PCR kit system is currently on the market.

The critical and important issues for the detection of fungal DNA by PCR from clinical material are the type of clinical material and its sampling, the DNA extraction protocol, the PCR design, the detection and specification of the amplicon, the need for appropriate controls, especially to exclude contamination, and the question whether quantitative PCR assays are beneficial.

G. Choice of Clinical Material

The kind of clinical specimen that should be analyzed depends on the disease entity and the condition of the patient. Blood and blood fractions can be easily collected and this material contains circulating leukocytes with phagocytized conidia and hyphae, free fungal cell elements, as well as free circulating fungal DNA. Examination of the detection limit of plasma and whole blood samples shows that the assay is more sensitive when performed on whole blood rather than on plasma. Three patients with documented IA were negative with plasma PCR but positive when whole blood specimens were analyzed. Bronchoalveolar lavages are more difficult to obtain, and PCR from BAL has not yet been able to distinguish infection from contamination (122). Sputum shows a very low specificity. CSF fluid cannot be used in routine diagnosis based on weekly screening. Hendolin et al. (123) developed a panfungal PCR for the detection of *Aspergillus* DNA in tissue specimens from the paranasal sinuses. Jaeger et al. (124) described an assay based on a nested PCR for fungal endophthalmitis.

H. DNA Extraction

The essentials of successful fungal DNA extraction are an enrichment of fungal DNA, the elimination of potential Taq-inhibitors and the avoidance of contamination with airborne spores.

The release of fungal DNA can either be managed using an enzymatic or a mechanical approach. Many protocols rely on the enzymes zymolase and lyticase, 1,3-glucanases that generate fungal spheroplasts (125). However, an efficient release of DNA from many molds, such as *A. niger*, *A. terreus*, Mucorales, and *Fusarium* species, requires additional treatment, including boiling of the samples with NaOH (126), high-speed cell disruption, grinding with mortar and pestle, or repeated freeze-thawing using liquid nitrogen (127).

I. Target Genes

The selection of a target gene, and the design of the primers and probes are the most important issues when creating a PCR assay. Single or multicopy genes could be selected. Assays that amplify single copy genes are often highly specific (species-specific) but might not be sensitive enough to detect a low fungal load, whereas multicopy genes show high sensitivity but low specificity because of many highly conserved gene regions. In contrast to *Candida* species where a variety of PCR protocols based on single copy genes (lanosterol-14-x-demethylase, actin, chitin synthase) exist, the detection of mold DNA is limited to multicopy gene analysis. Recently, Kanbe et al. (128) described a nested PCR assay using a mixture of specific primers binding to the DNA topoisomerase II gene. Primers targeting multicopy
genes bind to the 18S or 28S subunits of the ribosomal DNA (97,98,129) or to the highly variable intergenic transcribed spacer regions (ITS 1–4) that flank the ribosomal gene regions (130–132). Furthermore, mitochondrial genes have been used for the design of primers for diagnostic assays (99). Recently, Luo et al. (132) described a multiplex PCR with five sets of species-specific primers binding to the ITS1 and ITS2 regions. They found that this multiplex PCR method provided 100% sensitivity and specificity testing a total of 242 fungal isolates. Raad et al. (100) performed a study on whole blood specimens from 54 patients with cancer and pulmonary infiltrates. PCR was performed by amplifying Aspergillus mitochondrial DNA. The sensitivity, specificity, positive and negative predictive value were 100% for proven IA and 57%, 100%, 100%, and 92%, respectively, for the probable and possible IA cases, indicating the high sensitivity of their PCR assay. Furthermore, Ferns et al. (101) described a PCR assay based on the amplification by nested PCR of a 135 bp fragment in the mitochondrial region of A. fumigatus or A. flavus (121 bp). They were able to detect 1 CFU/2 mL of blood. Six of seven patients with clinical evidence of IA were PCR positive. Buchheidt et al. (102) developed a two-step PCR assay enabling the authors to detect 10 fg of Aspergillus DNA, corresponding to 1–5 CFU/mL of spiked samples in vitro.

J. Amplicon Detection

After amplification of fungal DNA by PCR, species or genera can be distinguished by different techniques. The traditional methods involve gel electrophoresis followed by hybridization protocols. These techniques include Southern blots, Slot blots, or PCR-ELISA using species- or genus-specific probes. This step is especially important when using panfungal primer sets that recognize conserved gene regions in many pathogenic fungi. Southern blot assays were successfully applied to the detection of different Aspergillus species (103,133). An assay based on the commercially available PCR-ELISA format from Roche is able to specifically detect Aspergillus DNA extracted from 10 CFU (126). Fletcher et al. (134) compared the sensitivity of a plate hybridization assay with Southern blotting. They conclude that both assays showed an identical sensitivity of 1.5 pg; however, by plate assay, results were obtained within 3 hr Willinger et al. (135), analyzed maxillary sinus samples from patients with histologically proven fungal infections by PCR and culture. For identification and speciation of the fungi, three different techniques were studied. By sequence analysis of the amplicon, identification of the fungal DNA was successful in 90%; by hybridization, fungal DNA could be speciated in 77%, whereas culture was positive in only 52% of the analyzed samples. The differentiation of fungi by sequencing analysis has also been successfully explored by Turenne et al. (130), performing an automated fluorescent capillary electrophoresis system.

K. Real-Time PCR

These recently developed PCR assays combine rapid in vitro amplification of DNA with real-time speciation and quantification of DNA load. However, the number of protocols for the detection of Aspergillus-DNA by real-time PCR tests is very limited. The Tubingen group established a quantitative PCR protocol for the detection of A. fumigatus (136). The sensitivity of the assay was comparable to previously described PCR protocols (5 CFU/mL). The Light Cycler allowed a quantification
of fungal load in a limited number of clinical specimens from patients with hematological malignancies and histologically proven invasive fungal infection. Five out of nine positive samples showed a fungal load between 5 and 10 CFU/mL, 2/9 samples between 10 and 100 CFU/mL, and 2/9 samples were positive with more than 100 CFU/mL blood. Three additional quantitative PCR assays based on the TaqMan technology were described recently. Bowman et al. (137) developed a PCR to monitor disease progression and measure the efficacy of caspofungin acetate in a murine model of disseminated aspergillosis. They conclude that because of its much larger dynamic range and its higher sensitivity, the quantitative PCR assay is superior to traditional CFU determination. Costa et al. (138), developed two TaqMan PCR tests, targeting the mt gene and the FKS gene of A. fumigatus, including a quantification of the fungal DNA load in spiked blood samples. Finally, Pham et al. (139) report the design and evaluation of a real-time PCR assay for the detection of mould DNA in serum. The test has a lower limit of sensitivity of 110 fg (three genomes). Quantitative analysis of the positive serum samples showed a mean fungal load of 1.6 $\times$ 10^5 genomes and a maximum fungal load of 4.2 $\times$ 10^7 genomes.

L. Diagnostic Conclusions

In severe disease, an aggressive, invasive approach, as well as making a tissue diagnosis early in the illness, appears to be a key to survival. In the appropriate clinical setting, such as an immunocompromised host with fever and a pulmonary infiltrate, repeated isolation of the same species in culture, and particularly a BAL or other endobronchial culture, correlates with invasive disease. Sometimes, even a single sputum culture (especially with heavy growth) may have to be the stimulus for therapy if invasive procedures cannot be done. Negative cultures do not rule out invasive disease. CT scanning of the chest done at the earliest suspicion of this diagnosis initially may reveal a halo sign or, later, a lesion with an air crescent, which is highly predictive of this diagnosis. These are the situations where a positive galactomannan, PCR, or glucan test could be particularly helpful, in prompting therapy even if a specific microbiologic diagnosis from tissue is not possible.

IV. THERAPY

A. Prevention

Prophylaxis of susceptible patients, such as immunocompromised hosts, using intranasal, inhaled, or systemic antifungals, is an approach to avoid disease and the need for therapy. One strategy for this would be to identify the highest risk patients, such as those identified by screening respiratory cultures as colonized, or those with HSCT and GVHD, and targeting prophylaxis to them. Reducing airborne spores, such as by filtering hospital air, keeping patients in rooms with positive pressure relative to the corridor, and with frequent air changes and high unidirectional air flow in the room; reducing activities that increase spore counts, such as room maintenance, when the patient is in the room, separating patients from areas of construction, preventing bird access to ductwork, preventing dust, avoiding carpeting in patient areas, wet mopping, substituting sponge baths for showers and bottled water for tap water, and restricting contaminated materials (e.g., potted plants, sterilization of spices), are believed to be worthwhile efforts for patients who will be transiently immunosuppressed or neutropenic.
B. Therapy—Overview

In invasive disease, prompt, aggressive chemotherapy has produced superior survival statistics at some institutions, although recovery from neutropenia is a necessary accompaniment of recovery in almost every success. Therapy may need to be initiated on only a high degree of suspicion. Surgical excision has an important role in the invasion of bone, burn wounds, epidural abscesses, and vitreal disease. It may have a function in invasive pulmonary disease for which chemotherapy has failed or where disease impinges on major vascular structures, and there is a heightened risk of sudden, fatal exsanguination. In pleural disease, locally instilling antifungals may be useful.

Therapy should be continued after lesions resolve, cultures are negative, and reversible underlying predispositions have abated. Reinstating therapy, in patients who have responded, should be considered if immunosuppression is reinstituted or neutropenia recurs.

There is little agreement on the best systemic chemotherapy for IA, and the optimal therapy remains elusive. Amphotericin B deoxycholate (AmB) has been the “gold standard” since its approval in 1958 and a guideline-recommended treatment choice (140); but now after years of limited therapeutic options there are several new exciting possibilities. There has been a recent surge in the development of newer antifungals for treating IA, including new formulations of older drugs (amphotericin B lipid products, cyclodextrin–itraconazole) and entirely new classes of drugs (echinocandins) with novel targets (141) (Table 2). Newer strategies have also been explored with combination antifungal therapies as well as cytokine therapy to augment the host response system.

C. Amphotericin B Formulations

Amphotericin B has been the treatment of choice for IA as well as the standard of comparison for all newer antifungal agents for over 40 years. However, the fact that AmB remained at such a post is not by virtue of its effectiveness, but rather because of the lack of alternatives until recently (142).

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Drug Name (Brand / Investigational name)</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyene</td>
<td>Amphotericin B Deoxycholate (Figmizone®)</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td>Amphotericin B Lipid Complex (Abelcet®)</td>
<td>IV</td>
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<tr>
<td></td>
<td>Amphotericin B Colloidal Dispersion (Amphocil®, Amphotec®)</td>
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</tr>
<tr>
<td></td>
<td>Liposomal Amphotericin B (AMBisome®)</td>
<td>IV</td>
</tr>
<tr>
<td>Triazole</td>
<td>Itraconazole (Sporanox®)</td>
<td>PO, IV</td>
</tr>
<tr>
<td></td>
<td>Voriconazole (VFend®)</td>
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<td></td>
<td>Posaconazole (SCH 56592)</td>
<td>PO</td>
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<td></td>
<td>Ravuconazole (BMS-207147; ER-30346)</td>
<td>PO, IV</td>
</tr>
<tr>
<td>Echinocandin</td>
<td>Caspofungin (MK-0991)</td>
<td>IV</td>
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<tr>
<td></td>
<td>Anidulafungin (VER-002; LY303366)</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td>Micafungin (FK463)</td>
<td>IV</td>
</tr>
</tbody>
</table>

*Licensed for clinical use in the united states.

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In addition to conventional AmB, three fundamentally different lipid-associated formulations have been developed that offer the advantage of an increased daily dose of the parent drug, better delivery to the primary reticuloendothelial organs (lungs, liver, spleen) (141–144), and reduced toxicity. ABLC (Abelcet®, Enzon, Bridgewater, New Jersey, U.S.A.) is a tightly packed ribbon-like structure of a bilayered membrane formed by combining dimyristoyl phosphatidylcholine, dimyristoyl phosphatidylglycerol, and amphotericin B in a ratio of 7:3:3. ABCD (Amphocil®, AstraZeneca, London; or Amphotec®, Intermune Pharmaceuticals, Brisbane, California, U.S.A.) is composed of disk-like structures of cholesteryl sulfate complexed with amphotericin B in an equimolar ratio. L-AmB (AmBisome®, Fujisawa Healthcare, Inc., Deerfield, Illinois, U.S.A.), the only “true liposomal” product, consists of small uniformly sized unilamellar vesicles of a lipid bilayer of hydrogenated soy phosphatidylcholine-distcaryl phosphatidylglycerol–cholesterol–amphotericin B in the ratio 2:0:8:1:0:4. There are no data or consensus opinions among authorities indicating improved efficacy of any new AmB lipid formulation over conventional AmB (143,145,146). This leaves the clearest indication for a lipid formulation over AmB to be reducing glomerular toxicity.

Animal studies clearly indicate that on a similar dosing schedule the lipid products are almost always not as potent as AmB, but that the ability to safely administer higher daily doses of the parent drug improves their efficacy (147). Experimental in vitro and in vivo studies support concentration-dependent killing with a prolonged postantifungal effect, suggesting large daily doses will be most effective and achieving optimal peak concentrations is important (148). A multicenter maximum tolerated dose study of liposomal AmB including 39 patients with IA using doses from 7.5 to 15 mg/kg/day found no demonstrable dose-limiting nephrotoxicity or infusion-related toxicity, but the study was not statistically powered for dose-dependent efficacy (149). Other authors point out the scant support for higher doses of AmB (150), emphasizing the varying experimental conditions in published studies and no evidence of a clinical dose effect to support higher doses of AmB.

D. Itraconazole

First publicly described in 1983 (151) and available for treatment of *Aspergillus* in 1990, itraconazole (Sporanox®, Ortho-Biotech, Raritan, New Jersey, U.S.A.) adopted a triazole nucleus with higher specificity for the fungal cytochrome enzyme system over the older imidazoles. Historically, there have been several constraints with itraconazole: no parenteral formulation, erratic oral absorption in high-risk patients, and significant drug interactions. Azole–drug interactions may lead to decreased plasma concentration of the azole, related to either decreased absorption or increased metabolism, or increased concentration of coadministered drugs (152).

To overcome problems with variable capsule absorption, itraconazole has now been solubilized in cyclodextrin with substantial improvement as an oral solution (153,154). A new IV formulation of itraconazole was also approved in the US for pulmonary and extrapulmonary aspergillosis in patients who are intolerant of or refractory to AmB (155). The IV formulation can rapidly achieve high and steady-state plasma concentrations (156) as opposed to the 7–10 day period needed for the capsule or oral formulation (157). The IV formulation was shown to be effective in experimental IA with dose-dependent survival (158).
A multicenter open-label study performed in 31 patients with pulmonary IA who received 14 days of IV itraconazole followed by 12 weeks of capsules showed that target therapeutic concentrations were obtained within 2 days in 91% of patients and these levels were also maintained after switching to oral therapy. A complete or partial response was seen in 48% (15/31) of patients (156). These two new itraconazole formulations will allow better pharmacokinetics, especially in high-risk patients in whom capsules might be difficult to use because of mucositis, vomiting, or GVHD. While guidelines generally dictate a logical approach to include itraconazole as oral therapy after disease progression is arrested with parenteral AmB (140), in some studies itraconazole is a useful alternative therapy to AmB with comparable response rates (159,160).

E. Voriconazole

Voriconazole (Vfend®, Pfizer Pharmaceuticals, New York, New York, U.S.A.) is a new second generation triazole synthetic derivative of fluconazole. Both fungicidal (161–163) activity and fungistatic (163) activity against Aspergillus have been demonstrated and voriconazole also inhibits 24-methylene dehydrolanosterol demethylation and Aspergillus conidiation and pigmentation (162).

In vitro studies have generally shown greater activity of voriconazole over AmB and itraconazole (164–167), whereas other studies have shown itraconazole had superior activity over voriconazole (168). In an analysis of 413 Aspergillus clinical isolates from phase III clinical trials, voriconazole was generally equivalent in vitro to itraconazole and superior to AmB (169). Additionally, whereas A. terreus is known to be frequently refractory to AmB (170), in vitro testing has shown greatly increased susceptibility to voriconazole (171).

Animal model studies of voriconazole for IA have mirrored the excellent in vitro efficacy against Aspergillus, including superior survival rates over itraconazole and equivalence to AmB (172). In guinea pig models, voriconazole approximated the ability of AmB to reduce tissue burden and showed increased survival over AmB (173) and itraconazole (174). In another guinea pig model, voriconazole performed markedly better than itraconazole in cyclodextrin against Aspergillus endocarditis (175), but in a rabbit model AmB was significantly more effective than voriconazole at decreasing tissue burdens (176).

There are a growing number of anecdotal clinical publications of voriconazole success against IA, often after failing another therapy. The largest prospective clinical trial of voriconazole involved 392 patients at 92 centers in 19 countries over 3 years and compared initial randomized therapy with voriconazole versus AmB followed by other licensed antifungal therapy. Patients who initially received voriconazole had statistically significantly better complete or partial response (53%) vs. those initially receiving AmB (32%). Survival also improved to 71% for voriconazole vs. 58% for those initially receiving AmB (177). Analysis in an open, noncomparative multicenter study of 116 patients treated with voriconazole as primary therapy (60 patients) or salvage therapy (56 patients) also yielded encouraging results as 14% had a complete and 34% had a partial response (178). Additionally, a review of 42 children with IA treated with voriconazole showed the drug was well tolerated and had an overall response rate of 43% (179).

The comparator in the pivotal prospective randomized study was AmB, and this raises the question whether better tolerated therapy, such as a lipid formulation of AmB, might have produced a better result. Although voriconazole has not been
compared in a randomized trial to other modalities, such as itraconazole, echinocandin, or combination therapy, the superiority of voriconazole demonstrated over the reference standard, initial therapy with AmB, makes it for many clinicians the current first choice for primary therapy for IA.

F. Other Triazoles: Posaconazole and Ravuconazole

Posaconazole (Schering-Plough Research Institute, Kenilworth, New Jersey, U.S.A.) is a second-generation triazole and closely related to itraconazole. In vitro testing has shown posaconazole had superior activity over itraconazole and AmB against *Aspergillus* species (180–183) and was also active against itraconazole-resistant isolates (184). In vitro comparison against voriconazole and several of the other second-generation triazoles has shown posaconazole had the greatest activity (185–190), as well as superior activity against echinocandins (191). Posaconazole also had similar activity to itraconazole against an AmB-resistant isolate of *A. fumigatus*, but superior activity against a voriconazole-resistant isolate (188,192).

Animal models have shown efficacy with posaconazole (181), including survival and antifungal efficacy in clearing tissue burden superior (193) or equal to AmB and superior to itraconazole (194). In another rabbit model, posaconazole significantly prolonged survival compared to itraconazole or AmB (195), and in a murine model posaconazole showed a significant reduction in mortality over AmB (196). In a multicenter study including 25 patients with IA to evaluate “salvage” therapy in patients who are refractory to invasive fungal infections, posaconazole was well tolerated and effective in 53% (8/15) at week 4 and 85% (6/7) at week 8 (197).

Ravuconazole (Eisai Medical Research, Inc., Ridgefield Park, New Jersey, U.S.A.) is structurally more similar to fluconazole and voriconazole, containing a thiazole instead of a second triazole. In vitro studies have demonstrated fungicidal activity comparable to other azole compounds as well as general superiority over AmB against various *Aspergillus* species, including activity against *A. terreus* (198,199). Another study found ravuconazole activity slightly less than itraconazole or AmB, but no ravuconazole-resistant isolates were detected (200).

One murine study revealed that both ravuconazole and itraconazole led to decreased lung fungal burden in a dose-dependent fashion (201). Other murine models revealed ravuconazole superior to itraconazole or AmB (202). Using a guinea pig model of disseminated aspergillosis, ravuconazole was also more effective in reducing positive organ cultures compared to AmB or itraconazole (203). A rabbit model showed survival efficacy as well as a decrease in tissue fungal burden comparable to AmB, and only AmB and ravuconazole consistently eliminated *A. fumigatus* from organ tissues (204).

G. Caspofungin

Caspofungin (Cancidas®; Merck, Whitehouse Station, New Jersey, U.S.A.) is a fungistatic water-soluble semisynthetic derivative of the natural product pneumocandin B₁ (205). There is in vitro activity against *Aspergillus* (206), and fluorescent dyes have demonstrated the focus of caspofungin killing is the apical and subapical branching cells (207). Caspofungin activity against *A. fumigatus* was also markedly increased with the in vitro addition of human sera (208). Caspofungin has shown an additive effect with monocytes and monocyte-derived macrophages, but not neutrophils, on *Aspergillus* hyphal growth (209). Animal models have shown that despite
dose-dependent hyphal damage, there was no reduction in residual fungal burden or galactomannan antigenemia, unlike AmB (210). Other animal models have demonstrated equivalent efficacy with caspofungin and AmB (137, 211), and in a murine disseminated aspergillosis model caspofungin showed 50% effective doses (ED$_{50}$), using daily dosing, comparable to AmB (212).

In a pivotal clinical study leading to US approval, 56 patients with acute IA underwent “salvage” therapy after failing primary therapy for more than a week or developing significant nephrotoxicity. Recipients generally tolerated caspofungin well and had better outcome than historic controls; 41% (22/54) had a favorable response with caspofungin (213). A recent update on all 90 patients enrolled in that trial revealed 45% had a complete or partial response, and the drug was generally well tolerated (214). A Spanish study before licensure revealed a 67% (8/12) favorable response rate among patients with proven or probable IA (215).

H. Other Echinocandins: Micafungin and Anidulafungin

Micafungin (Fujisawa Healthcare, Inc., Deerfield, Illinois, U.S.A.) is an echinocandin lipopeptide compound (216–218) and like all echinocandins is fungistatic in vitro vs. Aspergillus (219). In vitro micafungin compared favorably with AmB or itraconazole (219–222), including activity against AmB-resistant isolates (223). Animal models have shown efficacy (224, 225), including a murine pulmonary aspergillosis model where micafungin caused hyphal damage and in a neutropenic rabbit model where micafungin caused dose-dependent damage of hyphal structures in the lung tissues of animals, and a significant improvement in animal survival rates comparable to those treated with liposomal AmB (226). One murine model showed that based on the ED$_{50}$ and survival, micafungin was 1.7–2.3 times inferior to AmB (227), while another murine study yielded a similar survival rate and ED$_{50}$ compared to AmB (228).

In an open-label, multicenter study of micafungin monotherapy that included 10 patients with IA, overall clinical response was 60% with no safety-related issues (229). A recent study of micafungin combined with an existing antifungal agent in pediatric and adult bone marrow transplant patients with IA revealed an overall complete or partial response of 39%, including 40% in allogeneic transplant patients (230).

Anidulafungin (Vicuron, King of Prussia, Pennsylvania, U.S.A.) in some in vitro studies was more active than caspofungin against Aspergillus species (191, 231), and both drugs were considerably more active than AmB (232) or itraconazole (231) but generally less active than posaconazole (191). Favorable antifungal interactions with anidulafungin and neutrophils or monocytes have been demonstrated (233), similar to work with caspofungin (209). Survival in several rabbit models of IA was comparable to AmB; however, tissue fungal burden was not reduced and was actually quite higher compared to untreated controls (204, 234). For instance, while anidulafungin did yield dose-dependent damage of hyphal elements, tissues from the AmB-treated rabbits seldom revealed any hyphal elements (234). In another mouse model, AmB was superior to anidulafungin in reducing renal tissue fungal load, but anidulafungin was effective using an AmB-resistant isolate (235).
I. Combination Therapy

While each individual antifungal agent has limitations, combinations might prove more effective and create a widened spectrum of drug activity, more rapid antifungal effect, synergy, lowered dosing of toxic drugs, or a reduced risk of antifungal resistance (236). An extensive review of combination antifungal therapy for IA is presented elsewhere and showed a two-third clinical improvement rate (237). Before newer antifungals became available, many combinations involved agents not historically viewed as effective anti-Aspergillus drugs, singly. For instance, while 5-fluorocytosine (5-FC) has little inherent anti-Aspergillus activity (238), the fungistatic 5-FC might enhance the antifungal activity of AmB, especially in anatomical sites where AmB penetration is often suboptimal, such as CSF, heart valves, and the vitreous (43). In vitro combination studies for *Aspergillus* demonstrate synergy and antagonism occur with about equal frequency (239). Animal models followed (240–242) and case series indicated clinical improvement (243–245). The only published prospective clinical study of combination therapy for pulmonary IA (246) compared AmB monotherapy to AmB + 5-FC, but the study was terminated early because of poor outcomes in both arms.

Similarly, although rifampin and its analogs alone have no inherent antifungal activity, it is postulated that AmB increases the permeability of the fungal membrane to allow increased penetration of rifampin, which then inhibits the fungal RNA polymerase (247,248). In vitro studies show synergy (249,250) as well as animal models (251) but there are also reports of antagonism (241). However, coadministration of rifampin with azoles, although almost consistently demonstrating enhanced activity in vitro, should be discouraged in humans because of the potent P450 enzyme-inducing properties of rifampin that can result in clinically ineffectiveazole concentrations (140,152,252,253). There are some successful clinical reports with rifampin, but the potential for drug interactions is too high to recommend its combination use.

The most debated combination scheme for treatment of IA is AmB + itraconazole, and the theoretical risks of antagonism with this combination have been reviewed (254). The concern is that the polyene AmB, which functions by binding to ergosterol in the cell membrane, will be antagonized with an azole, which inhibits a late enzyme step in ergosterol synthesis. Therefore, instead of attacking the fungal membrane at two different steps for a synergistic interaction, the concern is the azole will alter the target for the polyene. Pretreatment with itraconazole would be expected to have a much more deleterious effect than concurrent treatment, and this has been demonstrated in vitro (255).

Clinical therapy with AmB and azoles has been extensively reviewed (254). Despite continuously heard concerns of AmB with azoles, AmB + itraconazole for IA is used (5). One retrospective clinical case series of 21 patients examining concurrent therapy of AmB + itraconazole demonstrated no clinical antagonism and a statistically non-significant improvement in mortality over monotherapy (256). Other studies reached the same conclusion (5).

Unfortunately, there is less available information regarding combinations including the newer antifungals and it is clear more testing needs to be done. In vitro testing of caspofungin with various other antifungals showed positive interactions, especially AmB + caspofungin (257,258), and there were also in vitro positive interactions with micafungin and AmB (259,260). In animal models, there was an additive effect with AmB + caspofungin (261), and murine models have shown
significantly higher survival with micafungin + AmB compared to monotherapy with each drug (262,263). Other models showed neither micafungin + itraconazole nor micafungin + AmB resulted in significant improvement over monotherapy with itraconazole or AmB, but no antagonism was seen with any combination (224). In vitro studies with voriconazole and an echinocandin were additive, whereas voriconazole + AmB was indifferent (259).

J. Sequential Therapy

There are reports of various patterns of sequential antifungal therapy, which raises another issue other than concomitant therapy: the appropriate and safe sequence of agents. Confounding matters is the long half-life of AmB; hence, even sequential use has an element of concurrent therapy (264). The most practical experience is with the sequence of AmB followed by itraconazole. The consensus seems to be that there are many instances of initial therapy with AmB followed by itraconazole with generally no harm seen (5,159,160,264). A widely accepted regimen uses AmB to treat a patient’s acute disease until neutropenia recovers, and then itraconazole maintenance antifungal coverage (5,265). While this sequence appears safe and is recommended in recent guidelines (140), the most debated sequence is the reverse, with an azole followed by AmB. Antagonism is once again postulated because of azole inhibition of fungal ergosterol synthesis and subsequent exhaustion of the target for AmB, with loss of antifungal effect of AmB (266).

There has been in vitro antagonism with sequential azole than AmB, whereas concurrent administration showed minimal antagonism (255,267–269). Clinical reports do support this sequential antagonism (270) but also can show clinical improvement with itraconazole then changed to AmB (271,272).

K. Immunomodulatory Therapy

Host defense is paramount as IA generally only develops in certain subsets of severely immunocompromised patients. Few patients with persistent neutropenia and IA survive, and indeed resolution of IA has followed neutrophil recovery in most cases. Immunotherapy is designed to increase the number of phagocytic cells, shorten the duration of neutropenia, modulate the kinetics or actions of those cells at the site of infection, and/or activate the fungicidal activity of phagocytes to kill fungal cells more efficiently (2,273).

Protective immunity is associated with CD4+ Th-1 cells producing interferon-γ (IFN-γ), interleukin (IL)-2, IL-15, macrophages producing IL-12, or those mice treated with antagonism of IL-4 or IL-10. Disease progression is seen in mice producing Th-2 cytokines IL-4, IL-10, IL-13, or mice treated with neutralizing antibody to INF-γ or IL-12 (14,145,274). IL-4 deficient mice were more resistant than wild-type mice to infections (275), and a murine model demonstrated decreased fungal burden and increased survival of IL-10 knockout mice compared to wild type (276). Th-1 resistance was also impaired upon IL-12 neutralization and in IL-12 deficient mice (275); however, administration of recombinant IL-12 failed to increase protective effects in mice (15). One in vitro study found that anti-IL-10 antibody, IFN-γ, and GM-CSF administration counteracted the suppressive host defense effects of IL-10 on phagocyte hyphal damage and oxygen radical production (277).

Prophylaxis with human recombinant granulocyte-stimulating factor (G-CSF) + AmB or itraconazole showed some additive effect in neutropenic animal
models of IA but not in those immunosuppressed with cortisone, which has a greater effect against macrophages. In a neutropenic murine model, human G-CSF alone was ineffective but with AmB showed synergy in survival greater than with itraconazole + G-CSF (278). G-CSF administered to human volunteers increased the fungicidal activity against *Aspergillus* conidia through enhanced respiratory bursts of their PMNs by 4-fold (279). However, there is no clear evidence G-CSF benefits patients with aspergillosis. One review found no significant reduction in fungal infections in acute myelocytic leukemia patients treated with G-CSF (280).

GM-CSF and macrophage colony-stimulating factor (M-CSF) treated human macrophages exhibit enhanced conidial phagocytosis, oxygen radical production, and hyphal damage (281,282). In a murine model, the antifungal activity of bronchoalveolar macrophages treated with dexamethasone was significantly less than macrophages from dexamethasone + GM-CSF treated mice (283–285). Additionally, GM-CSF administered before dexamethasone blocked the deleterious effects, but if given after dexamethasone, GM-CSF could not reverse the effect on macrophages (283). This confirmed earlier in vitro work demonstrating bronchoalveolar macrophage coinoculation with GM-CSF followed by adding dexamethasone significantly prevented the conidiacidal suppression. Also, bronchoalveolar macrophage coinoculation with dexamethasone and subsequent GM-CSF addition removed the deleterious effect if the dexamethasone was discontinued, but if the dexamethasone pretreatment continued despite GM-CSF use, the anticonidiacidal effects persisted (286). In another study, both murine and human GM-CSF can counteract dexamethasone suppression of murine macrophage function (287).

There are case reports of GM-CSF as part of a treatment regimen with success (288–290). GM-CSF has been shown to offer some protection against IA in one clinical trial in patients with acute myelogenous leukemia, decreasing the fungal infection-related mortality from 19% to 2% (291). A small pilot study of GM-CSF in combination with AmB for treatment of proven fungal infection included two patients with refractory aspergillosis, with one showing a partial response and the other failing therapy (292). A neutropenic rabbit model demonstrated that prophylactic administration of M-CSF 3 days prior to inoculation and then throughout neutropenia augmented pulmonary host defenses against IA, leading to increased survival and greater numbers of activated pulmonary alveolar macrophages compared to controls (293). A phase I trial of M-CSF in patients suggested some benefit in patients with *Aspergillus* infections, but an insufficient number of patients were treated to show a statistical benefit (282,294). However, the use of GM-CSF is not without its concerns, as bone marrow recovery may lead to liquefaction of pulmonary foci and to potential erosive bleeding caused by an increased inflammatory response, especially in the first week following cavitation (295,296).

In vitro TNF-α appears to enhance early host defense against *Aspergillus* invasion as well as a late defense with increased PMN hyphal damage by oxygen radical production (297,298). In vitro GM-CSF and TNF-α administration have been shown to counteract dexamethasone-induced immunodeficiency (299). Animal model depletion of TNF-α results in increased fungal burden and mortality (300) and resistance is further impaired in IFN-γ deficient mice (275). Treatment of mice with neutralizing antibodies to TNF-α and GM-CSF reduces the influx of PMNs into the lungs and delays fungal clearance (301). Intratracheal administration of a TNF-α agonist resulted in survival benefits when given 3 days before *A. fumigatus* inoculation but not when given concomitantly with conidia, suggesting that pretreatment may provide macrophage priming (300). However, excessive toxicities in doses...
required to have a biologically useful effect preclude safe administration in humans.

IFN-γ and G-CSF can each enhance the oxidative bursts and fungicidal activity in vitro of human PMNs against *A. fumigatus* hyphae, with the combination of the two cytokines showing an additive effect (303). IFN-γ can also restore the corticosteroid-suppressed fungicidal activity of human PMN and elutriated monocytes (281,299,304), and IFN-γ-treated human monocytes show enhanced oxygen radical production and damage to *A. fumigatus* hyphae (281).

Exogenous administration of IFN-γ and TNF-α has resulted in protective effects in a murine model of IA (305). Conversely, IFN-γ and TNF-α neutralization resulted in increased disease and increased expression of IL-10. Although IFN-γ is better than G-CSF or GM-CSF at enhancing PMN hyphal damage and both IFN-γ and GM-CSF treatment result in enhanced hyphal damage by PMNs in vitro (306), combination treatment does not increase damage (281). In vitro IFN-γ augments PMNs of CGD patients by an undetermined mechanism (307), although previous work demonstrated a myeloperoxidase-dependent oxidative process (308). IFN-γ has been proven to help prevent IA in CGD patients (310), and there are case reports of the successful use of antifungals with IFN-γ for treatment in CGD patients (310,311).

GM-CSF treatment of neutrophils with voriconazole increases activity against hyphae compared to control neutrophils and voriconazole, but no comparable effect was seen on monocytes (264,304). An in vivo additive effect was also found with G-CSF and posaconazole in one study (312), and no antagonism in another in vivo study (313). Anecdotally, granulocyte transfusions have been helpful in treating patients with IA (314–317). G-CSF-primed donor granulocyte transfusion was used to treat 15 patients with neutropenia-related fungal infections refractory to AmB, including seven patients with IA and the favorable responses appeared to be mainly because of the granulocyte transfusion (318). Another study showed infections cleared in five of nine patients with IA (319). A review of granulocyte transfusions in treating fungal infections in neutropenic patients following bone marrow transplantation showed no improvement in infections; however, this was before the use of G-CSF to prime donors (320,321).

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I. INTRODUCTION

The epidemiology patterns of fungal infections are opportunistic and endemic. The opportunistic fungi include Candida, Aspergillus, Fusarium, and Rhizopus species. Histoplasma, Blastomyces, and Coccidioidomyces are the fungi more traditionally characterized as endemic fungi; these endemic mycoses may also be present in an opportunistic fashion in the immunocompromised patient. These three endemic organisms are acquired by inhalation of spores, which then transform into yeast phase at the body temperature and are found in distinctive geographical locales. However, the organisms considered as opportunistic fungi (Aspergillus, Candida, Fusarium, and Rhizopus) do not cause endemic or geographically localized diseases as they are ubiquitous throughout nature and found worldwide.

The epidemiology features, including the risk factors, for the endemic fungal infections tend to fall into geographical patterns. Persons with each of these fungi are likely to live in fairly remote parts of the world with specific ecologic and climatic conditions. However, with the frequency of national and international travel, patients may present with a fungus infection obtained in a remote location of the world; travel history is an essential part of the diagnostic evaluation. Likewise, certain job or recreation related activities might put a person at risk for these endemic fungi. The vast majority of persons infected with these fungi have adequate host defenses. Therefore, many of these infections will cause few or no symptoms at the time of infection, but may later reactivate to cause systemic disease as the person ages or if the human host becomes immune suppressed. The chapter gives a brief summary of the geographical niches for the three common endemic mycoses in the United States and reviews the historical and clinical aspects of these selected endemic fungal infections and two other endemic infections outside the United States.

A. Histoplasmosis

Histoplasma capsulatum is the cause of the endemic mycosis histoplasmosis and was first found as a cause of disease in humans by Darling in 1906 from autopsies in
Panama (1). This is of interest because further cases were not described in Panama for decades. The history of histoplasmosis shifted to the center of the United States where histoplasmosis is now recognized to be very common. As described by Sell (2), Nashville Tennessee became the focus for investigation of this fungus. In 1934, the blood smear from an infant was found have organisms that were similar to the description from Darling’s original case. Cultures of specimen of bone marrow and blood from an autopsy revealed a fungus, *Histoplasma capsulatum*. Over the next several years, 70 or so cases of histoplasmosis were summarized in a review by Meleney (3); all were of the disseminated form and were uniformly fatal. A filtrate of the mycelial form of the fungus was used as a skin test to identify subclinical or asymptomatic cases of infection. This led to a pioneering article by Christie and Peterson in 1945 (4) that allowed greater understanding of interactions of fungi with human hosts. Children with pulmonary calcifications that had been thought to be because of tuberculosis were skin tested with Histoplasmin and found to have been infected with the fungus, even though they never had symptoms. Studies of military recruits and others subsequently confirmed that large numbers of normal persons are infected with *H. capsulatum* early in life, with resultant pulmonary calcifications but little or no clinical illness (5). These studies found that the majority of cases of histoplasmosis occur in the central section of the United States. However, there have been reports of cases throughout the eastern half of the United States and throughout Latin America (6). Infections have also been reported, albeit less commonly, in Asia, including Malaysia, Thailand, India, and Indonesia (6). There is also an African form of histoplasmosis (7). The organisms appear the same as in the mycelial form, but the yeast form is considerably larger. This strain is known as the *H. duboisii* form or large-form African histoplasmosis; a similar pattern of illness is seen in this African histoplasmosis form.

In the United States, there were estimates of 200,000 new cases of histoplasmosis per year in 1968 (8) that accounts for the 80–95 percent positive skin test rates for children in some highly endemic areas. In the presence of a depressed immune system, such as with HIV infection, corticosteroid therapy, organ transplantation, or, sometimes, for no obvious reason, progressive disseminated histoplasmosis may occur (6). In some endemic areas of the country, histoplasmosis is the most frequent opportunistic infection that leads to a diagnosis of AIDS.

The best, and perhaps the only, way to make a diagnosis of acute symptomatic pulmonary histoplasmosis, which is manifest with fever, chills, myalgia, dyspnea, and hypoxia, is to obtain a history of exposure (9). Careful attention should be placed to occupational or recreational exposure to bird droppings or bat guano, since either of these can act as a growth nutrient for *H. capsulatum* (6). Activities such as cutting down trees that had been known to be bird roosts, destroying chicken coops, which had remained unused for long periods of time, or spelunking in caves known to have large bat populations might prompt the consideration of histoplasmosis.

The vast majority of cases of histoplasmosis are asymptomatic and self-limited. The classification system used by Goodwin and Des Prez remains the most useful (6). In the normal host with a moderate to minimal exposure history, usual histoplasmosis was considered to be subclinical or asymptomatic in the vast majority of persons. Infection could either be from primary inoculation or could occur as reinfection with a subsequent exposure to the fungus, particularly, if continuous exposure did not occur in the endemic area. For example, those who left the endemic area for histoplasmosis for a number of years could develop a clinical or subclinical infection again upon return to the geographical areas in which *H. capsulatum* was found (6).
In normal hosts, acute pulmonary histoplasmosis with symptomatic disease manifest by fever, chills, myalgia, dyspnea, and hypoxia was described, but usually in patients with a significant exposure history. As with the asymptomatic form of the infection, the acute pulmonary histoplasmosis could be seen in both primary and reinfection scenarios (6).

Histoplasmosis was also described in the abnormal host. In those with excessive fibrotic response to the fungus, mediastinal fibrosis and retroperitoneal fibrosis were described (6). A solitary pulmonary nodule called a histoplasmoma can also be caused by excessive fibrosis, but with less severe manifestations of excessive scarring and collagen production than mediastinal fibrosis (6). In these cases, growth of the fungus is not considered to be the primary factor but the assumption is that too much fibrosis occur in response to the fungal antigens.

In other abnormal hosts, \textit{H. capsulatum} can act as an opportunistic pathogen. In those with structural defects of the lung due to centrilobular or bullous emphysema, chronic pulmonary histoplasmosis may occur (6). Usually found in middle aged to older cigarette smokers, this condition consists of cavitory lesions on chest radiographs, symptoms of fever, and copious sputum production. Antifungal treatment can reduce symptoms and signs, but the relapse rate is high (6,9). It is thought that growth of the fungus is not as much of the problem as is the inability of host defenses to clear the organism because of the structural defects of the emphysema. Finally, in the abnormal host with cellular immune deficiency, progressive disseminated histoplasmosis may occur. Currently, the usual clinical setting for this severe and often fatal condition is HIV infection with depression of the \textit{CD4} lymphocyte counts below 150–200/mm$^3$ (9). The clinical manifestations of histoplasmosis in AIDS patients have been extensively reviewed (10). Prior to the AIDS epidemic, patients with lymphoreticular neoplasms (e.g., Hodgkins disease), immunosuppressant chemotherapy (e.g., for organ transplantation or rheumatic diseases), sarcoidosis, or steroid-treated patients were the more commonly diagnosed with progressive disseminated histoplasmosis (6). Infection of the reticuloendothelial system with focal destructive granulomatous lesions in reaction to the hematogenously disseminated fungi accounts for the majority of signs in progressive disseminated histoplasmosis rather than findings in the lung (6). Bone marrow involvement is very common with thrombocytopenia, anemia, and/or leukopenia frequently found. Because of this localization, samples of bone marrow for culture and histology are appropriate methods of diagnosis of this condition. Symptoms and signs of fever, hepatosplenomegaly, and oropharyngeal or intestinal ulceration are commonly seen. Gastrointestinal bleeding in AIDS patients who reside or have resided in the \textit{Histoplasma} endemic area should prompt a search for this fungus (9). Less commonly, Addison’s disease, meningitis, or endocarditis may be diagnosed secondary to histoplasmosis (6).

The diagnosis of histoplasmosis has been reviewed extensively by Wheat (11). For cases of acute pulmonary histoplasmosis or the mild clinical cases of usual histoplasmosis, a history of exposure is essential to make the diagnosis. In the other manifestations, culture of clinical specimens is helpful and will generally take 2–3 weeks. Histopathology also allows a secure diagnosis with either Wright staining of bone marrow, peripheral blood smears, or Gomori methenamine silver stains of tissue specimens. Wheat has reported success in diagnosis of primary histoplasmosis, as well as relapse, with detection of \textit{Histoplasma} polysaccharide antigen in the urine of infected individuals (12); commercial testing for this antigen is available. Even though skin testing with histoplasmin allowed understanding of the
epidemiology of this infection, the usefulness of skin testing for diagnosis is low (11). In the endemic areas, skin tests simply mean prior infection and do not indicate if the histoplasmosis is responsible for the clinical signs and symptoms of the patient. The same is the case for *Histoplasma* serology. Both complement fixation and immunodiffusion precipitin bands for antibodies have sufficiently high rates of both false-positivity and false-negativity to limit their usefulness (11). Positive serologic studies should prompt even greater efforts to locate tissue material for culture and histopathology.

Antifungal treatment of histoplasmosis has been summarized in guidelines from the Infectious Diseases Society of America (13). In asymptomatic primary (usual histoplasmosis) cases of histoplasmosis, treatment is not necessary. In acute pulmonary histoplasmosis, supportive care with supplemental oxygen and even ventilatory support may be more important than the specific antifungal therapy. Typically, in a severely ill patient with this diagnosis, amphotericin B is administered to a total of 500 mg dose over a period of 2–3 weeks in the adult patient; some recommend concomitant corticosteroids (6). Lipid formulations of Amphotericin B can be used in this setting as clinically indicated. After discharge from the hospital, itraconazole, 200 mg twice daily, should be used to complete a 6 months course. For patients who are not sufficiently ill to require hospitalization, itraconazole alone, 200 mg once or twice daily for 6 months could be used (13).

In progressive disseminated histoplasmosis, amphotericin B is considered the drug of choice for life-threatening infection, and after stabilization, followed by itraconazole 200 mg twice daily for 6–18 months would be appropriate according to the immune status of the host and the speed of response to therapy. The first oral agent, ketoconazole, was associated with successful outcomes in those without AIDS (13) but ketoconazole was found to have an unacceptably high failure rate, both, for initial therapy and chronic suppressive therapy of this fungus in AIDS patients (9). Itraconazole has been effective in primary treatment of histoplasmosis in AIDS patients (13) as well as chronic suppression (13). In addition, in non-AIDS associated progressive disseminated histoplasmosis, itraconazole has been shown to be effective (13).

In chronic pulmonary histoplasmosis, itraconazole and amphotericin B have been shown to be effective although both have a relatively high relapse rate (13). An initial course of amphotericin B (0.7 mg/kg/d), followed by itraconazole 200 mg, twice daily for 12 to 24 months is suggested. Fluconazole has not been well studied in chronic pulmonary histoplasmosis but it does not appear to be as effective as itraconazole in AIDS patients; this agent or voriconazole might be considered in a patient who is unable to tolerate itraconazole (13).

For central nervous system disease, amphotericin B or liposomal amphotericin B ((Ambisome) 3 mg/kg/d) are recommended until stabilization, followed by fluconazole (800 mg/d) or voriconazole. Ambisome may achieve higher brain tissue levels than amphotericin B and it appears to have a better safety profile.

For granulomatous mediastinitis with obstruction, the treatment of choice is amphotericin B followed by itraconazole for 6–12 months for more severe disease. Although clinical trials have not been done, itraconazole alone is typically effective in moderate to mild disease.

1. **Treatment of Special Populations**

For pregnant women, amphotericin B is the drug of choice because of the potential of embryotoxicity of azoles. AIDS patients need suppressive therapy for life or until
immune reconstitution after HAART (CD4 count sustained $\geq 200/\mu\text{L}$). Because of limited trials with azoles, children with severe disease might be best treated with amphotericin B (1 mg/kg/d for 40 days); itraconazole 200 mg/d for 6 months might be expected to be effective.

B. Blastomycosis

The first reported case of blastomycosis was by Gilchrist in 1894 with subsequent isolation of a fungus, *Blastomyces dermatitidis* (14). This dimorphic fungus has a characteristic geographic niche in a similar area as the endemic area for histoplasmosis, but perhaps a bit more restricted, and includes states surrounding the Mississippi and Ohio Rivers in the United States (15). The majority of cases have been reported from Arkansas, Kentucky, Mississippi, North Carolina, Tennessee, Louisiana, Illinois, Minnesota, and Wisconsin (15). Most are endemic or isolated infections, but a few epidemics of infection from point sources have also been described. Cases have also been reported in Canada in the provinces of Manitoba, Ontario, Alberta, and Saskatchewan (15). Blastomycosis has been described in Africa and in India, and extremely rarely from Israel, Lebanon, Saudi Arabia, and Mexico. There had been previous reports of blastomycosis in South America and Central America; most likely, these cases are of paracoccidioidomycosis, which were once known as South America blastomycosis. This terminology has been abandoned since it is preferable to use the term that identifies the infecting fungus, *Paracoccidioides brasiliensis*, and since the infection from *B. dermatitidis* has been documented to occur outside of North America (16).

The epidemiology of blastomycosis is not as fully characterized as that of histoplasmosis because there are no reliable skin test or in vitro markers of prior asymptomatic infection. Records of infection are dependent on clinical diagnosis. Some epidemiologic clues are noted. Occupational or recreational exposures that have been important in blastomycosis are ones that lead to contact with soil (16). Specifically, this includes fishing, hunting, farming, construction work, or other activities that involve disturbances of moist earth (15). In several of the epidemics of infection, soil near bodies of water was thought to be responsible (17–19). Therefore, exposure to outdoor activities is a frequent historical cause in patients with blastomycosis.

Infections with this fungus begin with inhalation of spores into the lung. If the organism escapes nonspecific host defense mechanisms, the fungus undergoes phase transition from mycelia to yeast cells with increased numbers in the parenchyma of the lung and spread to other organs through the bloodstream. With the development of immunity, inflammatory pyogranulomatous reactions occur at the initial pulmonary site and at the widespread foci of infection. The initial response to the fungus is suppurative followed by granuloma formation. This mixed neutrophilic and mononuclear cell response is distinctive of blastomycosis, although necrosis or fibrosis may also be found (16). Typically, the granuloma of blastomycosis does not caseate, as found in tuberculosis. Despite spontaneous resolution of the pneumonia in some cases, endogenous reactivation may occur at either pulmonary or extrapulmonary sites with or without previous therapy (15).

The clinical manifestations of blastomycosis are variable. Weight loss, fever, malaise, fatigue, and other nonspecific complaints are fairly common but offer little diagnostic help in blastomycosis. The typical patient is a young to middle-aged male who works or recreates outdoors. Apart from an epidemic, it is very rare for children
to be diagnosed with blastomycosis (16). The male-to-female ratio has been reported from 4:1 to 15:1 in series of endemic cases (15). However, some of these studies were from Veterans Administration medical centers, which conspicuously add bias to the ratio.

Many patients with blastomycosis will have a delay in diagnosis since it is uncommon even in endemic areas and because the illness can mimic other disease processes. The different types of clinical presentations in pulmonary blastomycosis include acute pneumonia and chronic pneumonia. Patients with acute pneumonia may appear to have acute bacterial pneumonia with fever, chills, and a productive purulent cough with or without hemoptysis. Chronic pneumonia because blastomycosis exists 2–6 months prior to diagnosis with weight loss, night sweats, fever, cough with sputum production, and chest pain. Many such patients are thought to have malignancy or tuberculosis. A pulmonary infiltrate is the most common presentation of clinical blastomycosis with the majority of patients showing either an alveolar or mass-like infiltrate. Miliary or reticulonodular patterns are the next most frequent pattern. Although cavitary disease may occur, this pattern is not found commonly as in chronic pulmonary histoplasmosis or tuberculosis. Because of the mass lesions on chest roentenograms, many blastomycosis patients are initially thought to have lung cancer.

Skin lesions are the most common manifestation of extrapulmonary blastomycosis and almost always originate from dissemination from a lung focus, rather than from cutaneous inoculation. The skin typically has either verrucous or ulcerative lesions. The verrucous or fungating form is raised with a sharp but irregular border, that may suggest a diagnosis of cancer, unless specific examination for fungus is made with stains, such as Gomori’s methenamine silver stain. Ulcers because of blastomycosis have the same histologic changes as the verrucous, form but are different in that the subcutaneous abscess has drained to the surface. The borders are heaped-up, and the base usually contains exudate. Osteomyelitis due to B. dermatitidis infection is reported in the vertebrae, pelvis, sacrum, skull, ribs, or long bones more commonly, but almost every bone has had involvement reported (16). The genitourinary system is next most likely in frequency of involvement, and because males are more likely to have extrapulmonary manifestations than females, prostatitis and epididymo-orchitis have been reported most commonly (15). Urine collected after prostatic massage will improve the detection of genitourinary involvement. Typically, as with skin or bone infection, the organism will cause concurrent presentations in the lung as well as the prostate or testicle; chest radiographs should be performed in every case of this infection to aid the diagnosis. Meningitis, or even more commonly, epidural or cranial abscesses are the manifestations of neurologic involvement of blastomycosis (15). Both may be difficult to diagnose, and require biopsy or evaluation of ventricular fluid for higher positive culture rates than lumbar spinal fluid. Lesions of blastomycosis have been reported to cause disease in virtually every organ. Widely disseminated or miliary blastomycosis may occur with adult respiratory distress syndrome (ARDS) as the presenting feature (20,21). The majority of patients, but not all, with this pattern of diffuse infiltrates, noncardiac pulmonary edema, and refractory hypoxemia die very quickly.

Immunocompromised patients have been reported with blastomycosis, including patients with AIDS and sarcoidosis, transplantation patients, and those being treated with corticosteroids. This infection has not been frequently diagnosed in the cancer patient with neutropenia; cellular immunity is a more important host defense than leukocytes. A number of immunosuppressed patients with blastomycosis have been
reported with an increased percentage of cases in the patient population from 1978 through 1991 as compared to the cases from 1956 through 1977 (22). Although this could have been from a bias in referral patterns of patients, the speculation was that this more likely reflected the continually enlarging population of patients with complicated immune compromising illnesses, who have lived in the endemic area of this fungus (22). A number of cases of adult respiratory distress syndrome were described in these immunocompromised hosts, as has been described with blastomycosis in AIDS patients (23). Although blastomycosis may cause infections in immunocompromised patients, other fungal infections such as progressive disseminated histoplasmosis or cryptococcal meningitis are more likely to be opportunistic than blastomycosis. Immunosuppressed patients are thought to develop infection following exposure in the environment or through subsequent reactivation, just like immunocompetent patients. Unlike similar fungi, B. dermatitidis has been reported a significant pathogen following infection with human immunodeficiency virus (HIV) in only a relatively small number of cases (23,24).

The diagnosis of blastomycosis is made by either seeing the fungus in tissue, exudates, or by growing the organism by culture. As with H. capsulatum, B. dermatitidis is relatively easy to detect in both smears and cultures and, since colonization does not occur, this discovery is reliable for a secure diagnosis (16). In addition to examinations of sputum by potassium hydroxide preparations, cytology preparations can be used for a dependable diagnosis. Cytology is commonly performed since blastomycosis often looks radiographically like carcinoma of the lung. Many cases will be diagnosed only after one or more invasive procedures such as bronchoscopy or open biopsy. Other diagnostic tests including complement fixation (CF) antibodies, immunodiffusion precipitin bands, and delayed hypersensitivity skin testing with blastomycin are, unfortunately, unreliable for diagnosis. Therefore, a serodiagnosis of blastomycosis is problematic owing to potential low sensitivity and low specificity rates. In the future, newer antibody detection systems may be useful, as might newly described antigens such as a surface protein of B. dermatitidis, which may allow more consistent detection of antibody in patients (15,25). Skin testing with blastomycin is, unfortunately, no better than serology as a diagnostic procedure. This mycelial phase antigen does not provide suitable specificity or sensitivity for reliable patient assessment, and blastomycin is no longer obtainable clinically. Until antigen testing is available for B. dermatitidis, as has been described for H. capsulatum (11), the diagnosis of blastomycosis will depend on visualization of the fungus on smear, in tissue, or in culture.

Antifungal therapy for blastomycosis has been summarized by the Infectious Diseases Society of America (26). The first consideration for the patient diagnosed with blastomycosis is whether or not to use an antifungal agent. Subclinical disease occurs with this infection just as with H. capsulatum and Coccidioides immitis. This approach of observation should be limited to mild pulmonary blastomycosis in the normal host. If the patient has deterioration or progression of the pneumonia, antifungal therapy should begin. The presence of pleural disease or any extrapulmonary manifestations during the course of illness means that antifungal treatment should be given.

IV amphotericin B in a dosage of at least 1.0 g resulted in cure without relapse in up to 90% of treated patients in various series (15) and a dosage of 2 g has been associated with cure rates of up to 97% (27). However, this high degree of antifungal activity of amphotericin B is associated with a relatively large amount of toxicity. In a group of patients with blastomycosis reported by Abernathy (27), almost
three-fourths experienced a decline in renal function and a number of other toxicities were also reported including anemia, anorexia and nausea, fever, hypokalemia, and thrombophlebitis. Interruption of therapy during some point of the course was required in 41% of patients and termination of therapy with amphotericin B before reaching the desired amount was mandatory because of toxicity in 14% of the patients (27).

Azole antifungal agents have been used in blastomycosis; orally absorbed agents of ketoconazole, itraconazole, and fluconazole are generally well tolerated with itraconazole being the most useful for blastomycosis. Itraconazole is preferred to ketoconazole because of a high cure rate and lower rates of toxicity (26), particularly with regards to endocrine abnormalities associated with ketoconazole. Itraconazole is regarded as the primary oral agent (26).

There has been some experience in *B. dermatitidis* infections treated with fluconazole at doses of 200–800 mg per day (28). This agent, which is approved by the FDA only for cryptococcal infection and *Candida* infections, should not be considered equivalent to itraconazole for blastomycosis; it might be considered so when itraconazole is not tolerated, or in the case of central nervous system involvement when amphotericin B cannot be utilized. There have been no clinical trials of voriconazole in blastomycosis.

In the very ill and in the immunocompromised patient, amphotericin B remains the treatment of choice (26). For life-threatening disease, a cumulative dose of 1.5–2.5 g, is recommended. Lipid formulations of amphotericin B can be used in patients who cannot tolerate conventional amphotericin B. Itraconazole, at a dose of 200 mg twice daily for 6 months, should replace amphotericin B as therapy in compliant patients who do not have overwhelming or life-threatening blastomycosis, and to those who have had rapid response over 1–2 months to amphotericin B treatment (26). However, for a person with life-threatening manifestation of infection, such as the appearance of adult respiratory distress syndrome (ARDS), or the person with central nervous system involvement with blastomycosis, amphotericin B remains the treatment of choice.

In addition to antifungal therapy, surgery is indicated in some patients with large abscesses, empyema, bronchopleural fistula, bone debridement, or in cases of osteomyelitis that are poorly responsive to azole antifungal agents.

C. *Coccidioidomycosis*

Like histoplasmosis, coccidioidomycosis was described first in Latin America in the Southern Hemisphere. In 1891, a medical student named Alejandro Posadas working in the pathology laboratory of Robert Wernicke diagnosed a patient with an unusual skin tumor in Buenos Aires. In 1892, the patient’s illness was described in Argentina (29) with Wernicke reporting the same case in Germany (30). Four years later, Rixford and Gilchrist reported the first North American case from a Portuguese immigrant patient in California (31,32). There is a rich history of the mycology and ecology of this fungus in the first three decades of the 20th century (33).

With a disease pattern like histoplasmosis, there are probably similar numbers of cases of coccidioidomycosis as histoplasmosis diagnosed annually in the United States (33). The majority of patients have minimal to mild disease with only 1% of infected persons developing progressive disease (33,34). Persons of Filipino or other Asian descent and African-Americans have a much greater risk of disseminated coccidioidomycosis (33,34). In addition, any immunosuppression, including the mild
form of immunosuppression associated with pregnancy, will lead to an increased risk of dissemination (33).

Coccidioidomycosis occurs in the Lower Sonoran Life Zone (33). This corresponds to central California, Arizona, Nevada, Utah, Texas, and New Mexico. In Central America, Guatemala, Honduras, and Mexico can possibly be endemic foci for this fungus. In South America, cases are diagnosed in Argentina, Paraguay, Bolivia, Venezuela, Uruguay, and Ecuador (33). These endemic areas for coccidioidomycosis have hot summers and mild winters, alkaline soil and little rainfall.

Exposure to dust, dirt, or disturbed soil in the endemic area raises the potential for infection; hence recreational or occupational risks exist for coccidioidomycosis. Construction workers, excavation workers, or military personnel have an increased propensity for infection (33,35). An earthquake in the late 1990s in Los Angeles led to increases in the cases (36). The potential of even larger numbers of new cases of coccidioidomycosis with increasing population movement into coccidioidomycosis endemic areas has been noted (37).

Like histoplasmosis, many patients infected with *C. immitis* have no clinical symptoms. The pneumonia that occurs may be associated with immunologic manifestations of erythema nodosa as well as fever, sweats, cough, and sputum production. Disseminated disease of coccidioidomycosis involves the skin and soft tissues, bones and joints, and the meninges. The disease is also more likely to be severe in immunocompromised patients. Typically, this would include those with altered T-lymphocyte function such as renal transplants or AIDS patients.

Diagnosis is made with either isolation of the fungus or by serology. Of the endemic fungi, coccidioidomycosis has the most reliable antibody testing for diagnosis. As with histoplasmosis, positive skin tests for fungal antigens of coccidioidomycosis are frequently found in children and adults. In the San Joaquin Valley, prevalence rates of 50–70% have been documented. Long-term exposure in these areas is not required for infection; however, there are reports of infection far outside the endemic area after having been exposed to dust from the coccidioidomycosis region (33). Persons may have only briefly visited an endemic area before returning home with their incubating infection (34).

Antifungal therapy for coccidioidomycosis has been summarized by the Infectious Diseases Society of America (38). This therapy is recommended for patients with severe forms of the disease and for patients with immunosuppression. More than 90% of acute episodes of coccidioidomycosis resolve without antifungal therapy. However, some authors recommend antifungal therapy for patients with symptomatic uncomplicated acute respiratory infection. A follow up of 1–2 years of these patients is recommended for the early identification of chronic pulmonary or extrapulmonary disease. The length of therapy ranges from 1 year to lifelong suppression in immunocompromised patients.

In the absence of immunosuppression, asymptomatic solitary pulmonary nodules do not require antifungal therapy or surgical resection. Asymptomatic pulmonary cavities may resolve with time. Resection may be indicated if the cavity is still detectable after 2 years, if it shows enlargement or if it is located adjacent to pleura, with risk of pyopneumothorax. Symptomatic or ruptured cavities usually will require surgical intervention and antifungal therapy.

Progressive or chronic pulmonary, disseminated and extrapulmonary disease require antifungal therapy and in, diagnosis of coccidioidomycosis during the 3rd trimester of pregnancy or immediately during the postpartum period. Antifungal agents for coccidioidomycosis include IV amphotericin B (0.5–0.7 mg/kg/d, fluconazole
(400–800 mg/d po or IV once daily), and itraconazole (200 mg po twice daily). Amphotericin B is the drug of choice for pregnant women and for patients with rapidly progressive or life-threatening disease, while azoles are used in cases of sub-acute or chronic presentations or after stabilization of more severe disease. In a recent randomized, double-blind trial that included 198 patients, fluconazole (400 mg/d) and itraconazole (200 mg twice daily) were comparable in efficacy for the treatment of progressive nonmeningeal coccidioidomycosis, although a trend toward greater efficacy with itraconazole was observed (39). Despite the availability of antifungal agents, relapse is frequent after discontinuation of therapy. In the above-mentioned trial, relapse rates occurred among 28% and 18% of patients randomized to fluconazole and itraconazole, respectively.

For the treatment of meningeal disease, fluconazole is currently preferred in doses from 400–1000 mg/d. Itraconazole (400–600 mg/d) may also be effective. Patients who do not respond to azoles are candidates for intrathecal amphotericin B (0.01–1.5 mg, from daily to weekly dosing). Patients who respond to azoles should continue this treatment indefinitely. Hydrocephalus may develop regardless of the type therapy and does not necessarily indicate failure of antifungal therapy.

Newly available antifungal agents appear to be promising for the treatment of coccidioidomycosis. Voriconazole has been shown to be effective in a patient with meningeal disease refractory to fluconazole (40). Caspofungin and posaconzole appear to be active in experimental infections but no clinical data are available yet (41,42). Liposomal amphotericin B (Ambisome) was effective in a patient with disseminated disease who could not receive conventional amphotericin B (43). AmBisome has been shown to be more effective than conventional amphotericin B and fluconazole in an experimental model of coccidoidal meningeal disease in rabbits (44), and might be an attractive option for the treatment of meningeal disease when patients cannot tolerate conventional amphotericin B.

D. Paracoccidioidomycosis

Paracoccidioidomycosis is due to the fungus *Paracoccidioides brasiliensis* and is the only fungal infection geographically restricted to Latin America (45). Humans and armadillos are the only known susceptible hosts to natural infection (46). The pattern of infection is similar to that of histoplasmosis or coccidioidomycosis in that primary infection is thought to be pulmonary and is most commonly asymptomatic. Later in life, the previously asymptomatic infection can reactivate into systemic disease. In paracoccidioidomycosis, this usually occurs in middle-aged to older-aged adult males who present with either pneumonia, mucocutaneous lesions, or skin lesions (47).

Paracoccidioidomycosis has been diagnosed in patients in the United States, Canada, Europe, and Asia. However, some of these patients are individuals who had lived in Latin America at some point before the diagnosis (45). The endemic area for paracoccidioidomycosis ranges from Mexico to Argentina with the largest number of cases being reported from Brazil, followed by Venezuela, Colombia, and Ecuador (45). As with histoplasmosis and blastomycosis, there are probably hyperendemic sub-regions within these countries. The infection remains relatively rare even in endemic areas and why one individual develops disease from the infection while the next person does not is not clear.

There are gender differences in this infection. Children and young adults are unlikely to develop systemic disease. Skin tests with an antigen of *P. brasiliensis*
(paracoccidioidin) have similar positive results of around 60–70% in normal healthy children and adults of both sexes (45). However, clinical disease is found almost exclusively in men. This may be secondary to hormonal differences relating to fungal growth, but the explanation is not fully understood (45).

Severe and progressive disease is known as subacute infection or juvenile form. The progressive adult form is more likely to be seen in older men with chronic disease (45). This is the only fungal infection that responds to sulfonamide therapy, although azole or amphotericin therapy is more reliable (45).

Since the infection may remain dormant with later activation and subsequent disease up to three to four decades after primary infection, the major way to make the diagnosis outside of Latin America is a history of travel or residence previously in Central or South America (45,47). Diagnosis is confirmed by observations of the characteristic numerous buds with the refractile cell wall of the fungal elements on KOH examination of sputum or pus, by culture, or by histologic examination of tissue.

Paracoccidioidomycosis can be a fatal disease if left untreated. Effective agents include the sulfa drugs, amphotericin B, and the azoles. Treatment is usually very long and relapses are frequent. Sulfa drugs include sulfadiazine (4 g/d in 4 doses for adults) or trimethoprima-sulfamethoxazole (1 double strength tablet twice a day) among others. However, sulfa treatments require maintenance of 3–5 years to avoid relapse.

Currently, the drug of choice is itraconazole (100–200 mg/d) for 6 months. This therapy is associated with a response rate of 98% and a relapse rate of < 5% (48,49). Of note, although itraconazole can effectively control active disease, the pulmonary fibrosis present at the onset of treatment may not clear, and could even worsen, as fibrosis correlates with the severity of pulmonary infiltrates at diagnosis (50). Amphotericin b (0.7–1.5 mg/kg) is reserved for severe refractory disease (total dose 1.5–3 g). Initial amphotericin B treatment should be followed by maintenance with azoles or sulfas. This sequential therapy has been associated with a 75% response and 15–25% of relapse (48). Ketoconazole (200–400 mg/d) for 6 months is also ineffective, but is associated with a higher toxicity and relapse rate (11%) than itraconazole. Fluconazole is not recommended because of a very high relapse rate (50%).

One randomized study compared itraconazole (50–100 mg/d) to ketoconazole (200–400 mg/d) and sulfadiazine (100–150 mg/kg/d up to 6g) for 4–6 months for the treatment of active paracoccidioidomycosis in 42 patients and failed to show superiority of any of these agents (51). However, the small sample size, the low dose of itraconazole use, and the lack of follow up represent serious limitations for this study.

Voriconazole is active in vitro against P. brasiliensis (52), but no clinical data exist to support its use. Terbinafine is an allylamine active in vitro against P. brasiliensis (53) and was effective, at a dose of 250 mg twice a day for 6 months, in a patient refractory to trimethoprim-sulphamethoxazol (54).

**E. Penicilliosis**

The only dimorphic fungus of the genus *Penicillium* is *Penicillium marneffei*. This fungus has been described as a cause of systemic illness in HIV-infected individuals who resided in Southeast Asia and residents of southern China (55). The organism was first isolated in 1956 from bamboo rats in Vietnam (56). The first case in a
human was described in 1959 after the author had accidentally inoculated the organ-
ism into his finger; he treated himself with oral nystatin successfully (56), but this
agent has not been associated with cure subsequently. The next case was described
by DiSalvo et al. (57) in a minister who had worked in Vietnam and later developed
Hodgkin’s disease requiring a splenectomy. The spleen grew *P. marneffei*. Prior to
the surge in the HIV epidemic in Southeast Asia, penicilliosis was extremely uncom-
mon. However, since 1988, the infection has been diagnosed much more frequently;
Supparatpinyo et al. (58) report that 15–20% of all AIDS-related illnesses are due to
this fungal infection. It is the third most common opportunistic infection in this
patient group in Thailand following tuberculosis and cryptococcosis (60).

The organism grows as a mold at 25°C and as yeast at 37°C. Unlike *Histoplasma*,
which divides by budding, *P. marneffei* divides by fission. The histology is
similar to that seen in blastomycosis in that both suppuration and granulomatous
changes in the tissue may be found in response to the fungus.

The majority of cases of penicilliosis in Thailand have been in males with the
vast majority being immunosuppressed by HIV infection (55,59). In contrast, cases
in southern China were reported in persons with normal immune systems (55). A
handful of cases have been in persons from the USA or Europe, but all had exposure
to Southeast Asia or China.

The manifestations of *P. marneffei* have been systemic illness with skin lesions,
cough, lymphadenopathy, and weight loss (55,59). The disease has been considered
to be very similar to the manifestations of histoplasmosis in HIV-infected indivi-
duals. Amphotericin has been associated with improvement, but relapse is common
once the antibiotic is stopped; itraconazole as maintenance therapy has been success-
ful in preventing relapse (61).

As with paracoccidioidomycosis, it is unlikely that a diagnosis of *P. marneffei*
will be made in the Western world unless a careful history of exposure is obtained.
Culture and/or histology would identify the organism once the diagnosis has been
considered.

Disseminated penicilliosis is usually fatal if left untreated and the outcome is
very poor when treatment is delayed. By contrast, timely and appropriate therapy
are associated with a high survival rate. Relapses after the end of treatment are
frequent among immunocompromised patients. Amphotericin B, itraconazole, and
fluconazole have all been used for treatment of penicilliosis.

The current treatment for severe disease consists of amphotericin B (0.6 mg/
kg/d) for 2 weeks, followed by itraconazole 200 mg twice a day for 10 weeks. This
strategy was effective in 97% of 74 HIV positive patients with disseminated disease
(61). For mild to moderate disease, itraconazole or ketoconazole is recommended.

Continued suppressive therapy is recommended in HIV-infected patients to
prevent relapses (62). Primary prophylaxis with itraconazole 200 mg/d should also
be considered for HIV infected patients, who live in endemic areas of penicilliosis
especially if their CD4 is < 200 cells/μL.

II. SUMMARY

There are a number of systemic fungal infections that has a specific and characteristic
geographic niche. Careful historical questioning may be the only means to make the
diagnosis. Many of these endemic fungi will cause asymptomatic primary infection in
a portion of the population that lives in the endemic area. Either at the time of
primary infection or much later, the disease may progress with lymphohematogeneous dissemination to various organs. Skin, nodes, bone marrow, lungs, or the central nervous system are the most common sites of progressive infection. Culture and histologic examination of tissue will confirm the diagnosis of fungal infection and treatment with either amphotericin B or an azole, such as itraconazole or fluconazole, may well cure the infection.

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Emerging Fungal Infections

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I. INTRODUCTION

An increased number of fungal pathogens are arising as a result of novel therapies in the field of oncology and transplantation. *Candida* spp. constitutes the fourth most common cause of nosocomial bloodstream infection (1). *Candida albicans* is by far the most common cause of candidemia, however, emerging antifungal resistance in non-*albicans* species has become a common problem in high-risk population. Parallel to the emergence of non-*albicans* *Candida* infections, increasingly recognized pathogenic yeasts have emerged as a cause of invasive infections, which include *Trichosporon* species, *Malassezia* species, *Hansenula anomala*, and *Wangiella dermatitidis*. *Aspergillus fumigatus* is the leading cause of pneumonia in hematopoietic transplant recipients. However, non-*Aspergillus* filamentous fungi now account for 27% of mold infections in transplant recipients (2). Characteristically, filamentous fungi including *Fusarium* spp., *Scedosporium* spp., *Acremonium* spp., and *Paecilomyces* species cause clinical infection that is indistinguishable from that of invasive aspergillosis and are associated with poorer outcome (3).

The increasing importance of these emerging pathogens mandates familiarity with the pathogenesis and options for therapeutic and preventive measures. The epidemiology, microbiology, clinical presentation, and treatment of these uncommon fungi is discussed in this review.

II. EMERGING YEASTS

A. *Trichosporon* species

1. Epidemiology

*Trichosporon* species are pathogenic yeasts that are known to colonize the normal human skin, as well as respiratory, gastrointestinal, and urinary tracts. *Trichosporon*
infections are rare but have been associated with a wide spectrum of clinical manifestations, ranging from superficial involvement in immunocompetent patients to deep invasive and disseminated disease in immunosuppressed individuals (4,5). Superficial infection of hair shafts caused by *Trichosporon* is known as white piedra (6). White piedra presents as a soft white nodule on hairs in the axillae, scalp, and genital region. In contrast, a deep localized or disseminated infection is seen in immunocompromised patients. *Trichosporon* is also responsible for the summer-type hypersensitivity pneumonitis described in Japan (7–9). Risk factors for *Trichosporon* fungemia include acute leukemia, neutropenia, itraconazole prophylaxis, and the presence of a central venous catheter (10). The three most common portals of entry for *Trichosporon* infections are the respiratory tract, gastrointestinal tract, and skin (4,5,11).

2. **Microbiology**

*Trichosporon* is a yeast-like, rapid growing, and characteristically a producer of urease enzyme. On cornmeal tween agar, *Trichosporon* species are distinguished by the presence of hyphae, pseudohyphae, blastoconidia, and arthroconidia. All the clinical manifestations were previously attributed to *Trichosporon beigelii*; however, recent taxonomic revisions of the genus *Trichosporon* suggested that *T. beigelii* consists of 17 species with five varieties (12–15). The new taxonomy suggests that *T. asahii* and *T. mucoides* are associated with deep invasive infections, *T. asteroids* and *T. cutaneum* cause superficial infections, and *T. ovoides* and *T. inkin* cause white piedra of the scalp and pubic hair, respectively. *Trichosporon pullulans* has also been associated with invasive infection in immunocompromised hosts (10,16,17). *Trichosporon asahii*, a major cause of deep-seated trichosporonosis, is also associated with summer-type hypersensitivity pneumonitis (18).

3. **Pathogenesis and Clinical Manifestations**

*Trichosporon* expresses glucuronoxylomannan (GXM) in its cell wall that is antigenically and biochemically similar to GXM in *Cryptococcus neoformans* (19,20). This antigen may be detected in sera from patients with disseminated *Trichosporon* infection by the cryptococcal latex agglutination assay (21,22). The GXM-like polysaccharide antigen of *Trichosporon* was shown to inhibit phagocytosis by monocytes (20). Reduced phagocytic response and microbicidal activity may be attenuated by GXM-mediated immunosuppression (23). The clinical manifestation of invasive trichosporonosis is similar to that of disseminated candidiasis. Widespread infection occurs because of hematogenous dissemination resulting in azotemia, pulmonary infiltrates, characteristic chorioretinitis, and fungemia with septic shock. Multiple cutaneous lesions occur in 30% of patients with trichosporonosis (4). Characteristically, the skin lesions are described as purpuric papules and nodules with central necrosis or ulceration. Fungal cultures of cutaneous lesions yield *Trichosporon* in 90% of the cases (24). A chronic hepatic trichosporonosis following recovery from neutropenia is also described with *Trichosporon* (4,5,25).

4. **Treatment**

Disseminated trichosporonosis in neutropenic patients carries an unfavorable outcome, because *Trichosporon* is known to be resistant to the fungicidal effects of amphotericin B (11). Previous attempts to treat invasive trichosporonosis with
amphotericin B were unsuccessful, reporting a mortality rate approaching 80% in cancer patients (5,10). A neutropenic rabbit model of disseminated trichosporonosis at the National Cancer Institute suggested better activity of the triazoles superior to that of amphotericin B against *Trichosporon* (11). Antifungal triazoles have demonstrated the best activity in clearing *Trichosporon* infection (26). In vitro data confirm superiority of triazoles compared with amphotericin B against *Trichosporon* infections (27). Fluconazole alone has been considered the best first line of therapy. The new triazoles voriconazole, posaconazole, and ravuconazole exhibit more activity in vitro against *Trichosporon* than fluconazole (27). Treatment relies on rapid diagnosis of trichosporonosis and differentiation from more common *Candida* species. Echinocandins have poor activity against *Trichosporon* (28,29) and should not be recommended for treatment.

**B. *Wangiella dermatitidis***

1. **Epidemiology**

*Wangiella dermatitidis* (also known as *Exophiala dermatitidis*) is a black yeast that inhabits the soil and plants and has been mainly associated with infection of skin and subcutaneous tissue. *Wangiella dermatitidis* is an occasional agent of mycetoma and phaeohyphomycosis in humans; however, it has also been reported as the etiologic agent in cases of keratitis, otitis externa, peritonitis, endophthalmitis, and fungemia associated with central venous catheters. *Wangiella* is a neurotropic fungus and infections of the central nervous system have been reported predominantly in Asia (30,31). These infections occur in both immunocompetent and immunosuppressed patients; however, disseminated infections tend to occur in immunosuppressed patients. Environmental factors also play a role in the transmission of *Wangiella*. Epidemiologic data from an outbreak of *Wangiella* meningitis in patients receiving injectable steroids have recently been reported (32). In this outbreak, five patients receiving epidural or intra-articular injections of methylprednisolone that were prepared by an index pharmacy developed meningitis. Microbiologic cultures from unopened vials yielded isolates of *W. dermatitidis*.

2. **Microbiology**

*Wangiella dermatitidis* grows slowly on potato dextrose agar and displays black colonies from the front and reverse. Colonies are initially mucoid but after 3–4 weeks develop aerial hyphae. *Wangiella* is characterized by septate, brown hyphae, conidiophores, and yeast cells. Unlike *Exophiala* species, *W. dermatitidis* produces phialides but not annelids.

3. **Pathogenesis and Clinical Manifestations**

*Wangiella* is darkly pigmented because of melanin. The fungal pigments dihydroxyphenylalanine melanin and dihydroxynaphthalene melanin have recently been linked to virulence in some human pathogenic fungi. Although the function of melanin in human pathogenic fungi is not clearly defined, its role in protecting fungal cells has clearly been shown (33,34). In a murine model of disseminated *W. dermatitidis* infection, longer survival was demonstrated in the melanin-deficient mutant (35). The most common clinical manifestation of *Wangiella* is subcutaneous phaeohyphomycosis. The infection develops after traumatic implantation of the fungus through the skin. Dissemination is more commonly seen in immunocompromised patients.
4. **Treatment**

Treatment of *Wangiella* infections commonly involves amphotericin B and response to therapy is variable. Limited data are available on treatment, however, in vitro data suggest activity of amphotericin B, miconazole, terbinafine, itraconazole, posaconazole, voriconazole, and anidulafungin against *Wangiella* (36–38). Voriconazole exhibited higher antifungal activity in vitro when compared with itraconazole (39). In a murine model of disseminated *Wangiella* infections, posaconazole prolonged survival and significantly reduced brain *W. dermatitidis* counts (38). In vitro data suggest modest activity of the echinocandin anidulafungin against *W. dermatitidis* (37). Response to antifungal therapy in *Wangiella* infections depends on the extent of the infection. For localized infections, the treatment is variable, but generally a combination of both medical and surgical intervention is recommended (40). In cases of fungemia, removal of central venous access, along with systemic antifungal therapy, remains the mainstay of treatment.

C. **Malassezia species**

1. **Epidemiology**

*Malassezia* are lipophilic yeasts that colonize the human skin and body surfaces. It has been shown that *Malassezia* colonize the skin as early as the neonatal period (41). Factors that predispose for *Malassezia* colonization in neonates differ from those factors that predispose to invasive infection. Pathogens of the genus *Malassezia* comprise seven known species including *Malassezia furfur* and *M. pachydermatis*. *Malassezia furfur* is the causative agent of *Pityriasis versicolor* and has also been implicated in seborrheic dermatitis and dandruff. It has also been recovered in blood cultures from newborns and adult patients receiving lipid-replacement therapy through central venous catheters (42,43). These infections are not associated with neutropenia. *Malassezia pachydermatis* is an emerging pathogen increasingly reported to cause infections in neonates, is also a recognized cause of canine, feline, and equine dermatitis, and may lead to zoonosis. *Malassezia pachydermatis* has been reported to spread from dogs to humans via the unwashed hands of a healthcare worker causing a nosocomial outbreak in a neonatal intensive care unit (44).

2. **Microbiology**

*Malassezia* species are lipophilic yeasts that comprise seven species involved in pathogenic disease, which include *M. furfur* and *M. pachydermatis*. Other lipid-requiring species, known as *M. furfur complex*, include *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. restricta*, and *M. slooffiae*. Lipid dependency differentiates among *Malassezia* species; *M. furfur* is obligately lipophilic and requires long chain fatty acids for growth, but *M. pachydermatis* does not. Culture of *M. furfur* from blood is best achieved with isolator tubes and planting onto a solid medium supplemented with a lipid source (45). The laboratory diagnosis of *M. pachydermatis* can be complicated by the fact that this organism can be misidentified as *Candida lipolytica* by some conventional systems (46).

3. **Pathogenesis and Clinical Manifestations**

Exposure of *Malassezia* to lipid emulsions in parenteral hyperalimentation solution enhances growth of this organism. Hematogenous infection with multiple tissue
invasions has been described in neonates. Massive lung involvement is characteristic. Fever is the predominant feature of Malassezia infection, but bradycardia, respiratory failure, thrombocytopenia, and catheter blockage have been described in several cases (47). In immunocompromised patients, the infection presents as folliculitis and catheter-related fungemia. Isolated folliculitis in neutropenic patients resembles the lesions of acute disseminated candidiasis. IV lipid infusion was not shown to be a risk factor for M. pachydermatis infection. The overall outcome for this infection is more favorable when compared with the infections of other uncommon fungi.

4. Treatment
Treatment of Malassezia fungemia requires prompt removal of IV central catheters and discontinuation of the fat emulsion therapy followed by systemic antifungal therapy (48). Malassezia species are usually susceptible to antifungal azoles but exhibit variable activity to amphotericin B and resistance to flucytosine (49). Recent reports suggest better in vitro activity of voriconazole compared with that of itraconazole and amphotericin B against Malassezia spp. (50). In vitro data demonstrate better activity of the new azoles voriconazole and albiconazole (ABC) against Malassezia species (51,52). The same reports demonstrated variable susceptibilities to terbinafine against Malassezia species. Strains of M. furfur, M. globosa, and M. obtusa were more tolerant to terbinafine.

D. Pichia species
1. Epidemiology
Pichia species belong to the ascomycetous class and are increasingly recognized as human pathogens. The genus Pichia has several species, but clinically important species include P. anomala, P. guillermondii, P. norvegensis, and P. ohmeri. Infections caused by Pichia are rare but have been increasingly reported among children and preterm neonates (53,54). Several outbreaks of P. anomala were reported especially from intensive care unit settings (54–57). Cross-transmission from healthcare worker hands was thought to contribute to the spread of the organism in one outbreak (54). Other risk factors identified included acute leukemia, use of broad-spectrum antibiotics, use of central venous catheters, endotracheal intubation, and high colonization rate with P. anomala (56,58).

2. Microbiology
Pichia anomala (formerly H. anomala) is a free-living environmental yeast, which grows well in a high-sugar containing medium. In the majority of cases, the organism presents in its teleomorphic form and less commonly in the anamorphic form of C. pelliculosa. Colony morphology of Pichia is similar to that of Candida species. Ascospores production is the distinctive feature that differentiates Pichia from Candida species.

3. Clinical Manifestations
The spectrum of disease ranges from asymptomatic fungemia to severe disseminated life-threatening infection. Fungemia is the most common manifestation of this infection, however, ventriculitis, endocarditis, pneumonia, lymphadenitis, and enteritis have also been described (53,55,56).
4. Treatment

*Pichia anomala* is susceptible to all currently available antifungal agents, however, two cases of breakthrough fungemia have been described in patients receiving fluconazole (59). One study demonstrated comparable or higher activity of voriconazole against *P. anomala* compared with that of amphotericin B, fluconazole, and itraconazole (60). In contrast, other in vitro data have demonstrated resistance of *P. ohmeri* to fluconazole, itraconazole, and ketoconazole (61). There is a paucity of data regarding in vitro antifungal susceptibility of *Pichia* species to newly developed antifungals. Response rate because of *Pichia* fungemia is high when early therapy is instituted and central venous catheter is removed.

III. EMERGING FILAMENTOUS FUNGI

Hyalohyphomycosis is the term used for infections caused by colorless septate fungal hyphae (nondematiaceous) in infected tissue (Table 1). *Non-Aspergillus* filamentous fungi account now for 27% of mold infections in organ-transplant recipients (2). A similar increase was also reported among recipients of hematopoietic stem-cell transplants (62). These molds are likely to be associated with disseminated infection and poorer outcome, than is aspergillosis. These filamentous fungi including species of *Fusarium*, *Scedosporium*, *Acremonium*, and *Paecilomyces* cause clinical infection indistinguishable from that of invasive aspergillosis. A remarkable feature of some of these hyaline molds is the ability to cause fungemia and disseminate hematogenously causing numerous embolic skin lesions. In histologic sections, they appear as hyaline, septate, branching filamentous organisms that can mimic aspergillosis. Definitive identification in hyalohyphomycosis requires isolation of the fungal organism. Although hyaline hyphae represent the distinctive feature of the hyalohyphomycosis, it is the identification of conidia and phialides that makes the distinction of non-*Aspergillus* hyalohyphomycosis.

A. *Fusarium* species

1. Epidemiology

*Fusarium* species recently emerged as a cause of disseminated infections in neutropenic patients and those undergoing transplantation (62–65). *Fusarium* represents the second most common fungal pathogen, after *Aspergillus*, as the cause of life-threatening invasive infection in recipients of hematopoietic transplant. *Fusarium* is a ubiquitous fungus commonly found in soil, water, and plants, and is a well-recognized plant pathogen that may cause extensive crop destruction and contamination. *Fusarium* species are causative agents of superficial and localized infections in immunocompetent hosts and deep invasive or disseminated infections in severely immunocompromised hosts (64). Risk factors for invasive fusariosis include acute leukemia, hematopoietic transplant, neutropenia, and use of corticosteroids (63,66). Specific portals of entry are the respiratory tract and skin. Trauma constitutes the major predisposing factor for development of cutaneous infections caused by *Fusarium* in immunocompetent hosts (65). Similarly, the skin is predominantly the primary source of infection, especially in patients with onychomycosis.
### Table 1  Overall Characteristics of Emerging Hyaline Fungi

<table>
<thead>
<tr>
<th>Genus</th>
<th>Portal entry</th>
<th>Clinical presentation</th>
<th>Diagnosis</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fusarium</em> spp.</td>
<td>Skin, respiratory</td>
<td>Skin lesion, disseminated</td>
<td>Culture of blood, tissue</td>
<td>VRC</td>
</tr>
<tr>
<td><em>Acremonium</em> spp.</td>
<td>Cutaneous, respiratory, gastrointestinal</td>
<td>Endophthalmitis, keratitis, disseminated</td>
<td>Culture of blood, tissue</td>
<td>VRC, surgery</td>
</tr>
<tr>
<td><em>Scedosporium</em> spp.</td>
<td>Trauma, respiratory tract</td>
<td>CNS*, subcutaneous, bone, disseminated</td>
<td>Culture of blood, tissue</td>
<td>VRC: <em>S. apiospermum</em> Surgery: <em>S. prolificans</em></td>
</tr>
<tr>
<td><em>Paecilomyces</em> spp.</td>
<td>Skin, respiratory tract</td>
<td>Localized and disseminated Peritonitis in CAPD</td>
<td>Culture of blood, tissue, sterile fluids</td>
<td>VRC: <em>P. lilacinus</em> AmB, VRC: <em>P. variotii</em> Surgery, catheter removal</td>
</tr>
<tr>
<td><em>Trichoderma</em> spp.</td>
<td>Respiratory and gastrointestinal tract</td>
<td>Peritonitis in CAPD, localized, disseminated</td>
<td>Culture of blood, tissue, sterile fluids</td>
<td>Catheter removal Optimal anti fungal unclear</td>
</tr>
<tr>
<td><em>Zygomycetes</em></td>
<td>Percutaneous, respiratory tract</td>
<td>Rhinocerebral in DM, localized, disseminated</td>
<td>Tissue culture</td>
<td>Medical and surgical High-dose AmB</td>
</tr>
</tbody>
</table>

*Scedosporium in CNS seen in both immunocompetent and immunosuppressed hosts.

**Abbreviations:** VRC, voriconazole; AmB, amphotericin B; CNS, central nervous system; CAPD, continuous ambulatory peritoneal dialysis; DM, diabetes mellitus.
2. Microbiology

*Fusarium* species are septate filamentous fungi that produce conidiophores, phialides, macroconidia, and microconidia. In the early phase of growth where only microconidia are apparent, some *Fusarium* spp. may resemble *Acremonium* spp. In the later phases of growth, the more characteristic sickle-shaped multisepitate macroconidia are used in identifying the genus and species of *Fusarium*. Three species causing human disease are more commonly seen in the genera *Fusarium*: *F. solani*, *F. oxysporum*, and *F. moniliforme*. Molecular methods such as 28S rRNA gene sequencing may also be used for rapid identification of *Fusarium* to the species level (67).

3. Pathogenesis and Clinical Manifestations

Human fusariosis in immunocompetent patients results as a consequence of trauma, burn, or foreign body and include keratitis, onychomycosis, and occasionally cellulitis (68). In immunocompromised patients, inhalation of conidia of *Fusarium* through the respiratory tract after high-dose chemotherapy or transplantation may lead to sinopulmonary infection with subsequent hematogenous dissemination. Breakdown of the skin or trauma appears to be a common portal of entry, especially in the setting of onychomycosis with associated cellulitis (65). Similar to *Aspergillus*, this organism is highly angioinvasive and leads to tissue infarction in severely immunocompromised patients. In contrast, *Fusarium* continuously releases spores into the bloodstream and is frequently isolated from blood in disseminated infections. The pathogenesis of this phenomenon is caused by the occurrence in vivo of intravascular adventitious forms (69). The clinical picture resembles invasive aspergillosis characterized by fever unresponsive to broad-spectrum antibiotics, and nodular cutaneous lesions. Skin lesions occur in 70% of the infections and are commonly seen in extremities and occasionally in trunk and face (65). Evolution from painful subcutaneous lesions to erythematous induration followed by ecthyma gangrenosum-like necrotic lesion surrounded by a rim of erythema is characteristic of fusarial infection (64,65).

4. Treatment

The outcome of disseminated fusariosis in neutropenic patients remains poor despite aggressive antifungal therapy. Mortality rates for disseminated infection are variable and approach 100% in the absence of neutrophil recovery. A combined approach of surgical debridement, excision of localized infections (sinuses, eye, soft tissue, bone), and removal of infected intravascular catheters is required for patients with localized infections. Treatment options have been limited by the lack of activity of available antifungal agents against *Fusarium* spp., and higher doses of amphotericin B (1.0–1.5 mg/kg) or its lipid formulations (5 mg/kg/day) were required (70,71). The antifungal agents fluconazole and itraconazole are not active against *Fusarium* species. The new azoles voriconazole and posaconazole introduce a treatment option that demonstrates both in vitro and in vivo activity against some *Fusarium* spp. and represent a less toxic alternative to amphotericin B (72). New antifungal agents exhibit variable activity against *Fusarium* isolates depending on the species and rapid identification at the species level is required (50,60,73). There is no reported clinical or in vitro activity of caspofungin against *Fusarium* species.
B. *Acremonium* species

1. Epidemiology

*Acremonium* species are saprophytic filamentous fungi commonly isolated from the environment. Unlike other filamentous fungi, many cases of human disease occur in immunocompetent hosts. A reported outbreak involving four cases of *Acremonium* endophthalmitis was reported from an ambulatory surgery center where colonization of humidifier water in the ventilator system was thought to be the source of the infection (74). *Acremonium* is one of the causative agents of white mycetoma and usually presents after trauma. Keratomycosis caused by *Acremonium* usually develops in contact lens wearers. Invasive disease, however, is almost exclusively seen in immunocompromised patients with neutropenia and transplantation (75,76).

2. Microbiology

The genus *Acremonium* (also known as *Cephalosporium*) contains 100 species, however, species implicated in human infections include *A. falcifome*, *A. kiliense*, *A. strictum*, and *A. recifei*. Recently, it was demonstrated by DNA sequence analysis that *A. strictum* displays a broad genetic polymorphism (77). Like other hyaline molds, septate colorless hyphae are found in tissue. Variation in the diameter of the hyphae and both 45° and 90° branching are usually present (69).

3. Pathogenesis and Clinical Manifestations

The respiratory and gastrointestinal tracts are considered portals of entry of deep infection caused by *Acremonium* species. Similar to *Fusarium* and *Paecilomyces* species, *Acremonium* can invade vascular structures resulting in thrombosis, tissue infarction, and necrosis. In vivo sporulation can occur facilitating dissemination and may explain the high rate of hematogenous disseminated cutaneous lesions, as well as positive blood cultures. The spectrum of invasive disease ranges from sinusitis, endophthalmitis, osteomyelitis, arthritis, peritonitis, pneumonia, meningitis, esophagitis, subcutaneous infections, and disseminated infections (75,76,78,79).

4. Treatment

Optimal treatment of invasive infections caused by *Acremonium* has not been established given the rarity of this disease. *Acremonium* species have little susceptibility to available antifungal agents; however, recent reports suggest higher in vitro activities of the new azoles and caspofungin against *Acremonium* (28,80). In vitro activity of amphotericin B against *Acremonium* is variable (75,81). Anecdotal cases of invasive infections suggested some benefit with the use of amphotericin B and surgical excision of the infected tissue. Successful treatment of *Acremonium strictum* pneumonia with posaconazole has recently been reported in a patient with leukemia who failed amphotericin B treatment (82), suggesting that the second-generation triazoles may be effective antifungals against *Acremonium* infections.

C. *Scedosporium* species

1. Epidemiology

*Scedosporium* species are common fungi found in soil and stagnant or polluted water that were previously associated with asymptomatic colonization and now have
emerged as an important cause of deep infection in immunocompromised patients and accidentally injured people. Two medically significant species of Scedosporium include *S. apiospermum* (teleomorph: *Pseudallescheria boydii*) and *S. prolificans*. *Scedosporium apiospermum* is associated with three distinct clinical entities: mycetoma, deeply invasive infection known as pseudallescheriasis, and saprophytic colonization. Mycetoma, previously termed “Madura foot,” is the most common manifestation in normal hosts, usually occurs after penetrating injury, and presents as tumor-like swelling with draining sinuses. It most commonly involves the lower extremities resulting in arthritis and osteomyelitis. Other manifestations include mycotic keratitis and nonmycetoma-like cutaneous and subcutaneous infections. Deeply invasive infection caused by *S. apiospermum* is usually seen in immunocompromised patients with the lung being the most frequent site of infection. Central nervous system infection is seen in both immunosuppressed and healthy population. Noninvasive colonization of the lower respiratory tract in patients with cystic fibrosis and bronchiectasis, as well as fungus-ball formation in preformed cavities, is similar to those seen with *Aspergillus* (83,84). *Scedosporium prolificans* causes localized infection usually restricted to bone and soft tissue in immunocompetent patients and causes deeply invasive infection in immunocompromised hosts (85,86). *Scedosporium prolificans* has been documented to cause disseminated infection exclusively in immunocompromised patients with neutropenia and after hematopoietic transplantation (87,88). The portal of entry in disseminated disease is by inhalation of conidia through the lungs and further hematogenous spread. Recent reports of nosocomial outbreaks of *S. prolificans* in hematology–oncology units have been reported (88,89). The organism was thought to be aerially transmitted in both outbreaks. Invasive deep infection caused by *S. prolificans* has also been reported among AIDS patients (90,91).

### 2. Microbiology

The anamorph genus *Scedosporium* contains two medically important pathogens: *S. apiospermum*, the asexual state of *P. boydii*, and *S. prolificans* (formerly *S. inflatum*). The species are distinguished by their terminal annelloconidia with an inflated base in *S. prolificans*, whereas those of *S. apiospermum* are cylindrical. In histologic sections, the genus *Scedosporium* appears as a septate hyaline mold that cannot be reliably distinguished from *Aspergillus* species or *Fusarium* species unless conidia are present (92). The sexual stage of *S. prolificans* is *Petriella* species.

### 3. Pathogenesis and Clinical Manifestations

Phagocytic host defenses against conidia of *P. boydii* depend upon monocytes and macrophages, whereas the defense against hyphae depends upon PMNs. In healthy individuals, localized infection result from penetrating trauma and dissemination is rarely seen. Only one fatal report of a healthy man with *S. apiospermum* osteomyelitis of the foot developed CNS infection and died (93). Mycetoma develops after trauma causing destruction of the muscle, tendons, and bone. A chronic infection with draining sinus tract is characteristic of mycetoma. Similar to invasive aspergillosis, the route of entry of *Scedosporium* spp. is through inhalation of conidia leading to sinopulmonary infection and subsequent hematogenous dissemination. The clinical hallmark of disseminated infection includes multiple skin lesions, characterized by cutaneous nodules with a tender eschar and surrounding erythema accompanied by neurologic symptoms suggestive of CNS involvement (83,87). Compared to
Aspergillus, Scedosporium maybe isolated from bloodstream with a reported rate of fungemia of 75% (87). Fever is a prominent feature that should prompt the performance of a CT scan of the chest that may demonstrate nonspecific bronchopneumonia, nodular densities, wedge-shape infiltrates, or halo sign. Like Aspergillus, a crescent sign may be evident in patients recovering from neutropenia. Disseminated infection is seen in both species of Scedosporium. CNS infection caused by S. apiospermum is clinically and pathologically indistinguishable from CNS aspergillosis. Frequently, it manifests as either multiple or solitary parenchymal brain abscesses; the majority of cases are fatal even when early and aggressive therapy is instituted. A recent review of CNS scedosporiosis identified medical immunosuppression and near-drowning as risk factors for the acquisition of CNS infection (94).

4. Treatment

Disseminated infections caused by Scedosporium spp. in immunocompromised patients often carry a poor outcome. Until recently, administration of high-dose amphotericin B has been the initial approach to the treatment of invasive pseudallescheriasis; however, response rate has been dismal in immunocompromised hosts. In vitro studies have shown amphotericin B and its lipid formulations to have poor activity on either S. apiospermum or S. prolificans (95). The echinocandins may have inhibitory in vitro activity against S. apiospermum (80,96), but fluconazole has no activity against Scedosporium species. Recent in vitro studies demonstrated superiority of voriconazole compared with other conventional antifungal agents, against Scedosporium spp.(97–99). The same studies demonstrated fungistatic but not fungicidal activity of voriconazole and itraconazole against S. apiospermum. The combination of the azoles voriconazole, itraconazole, and posaconazole with human PMN leukocytes exhibited synergy or additive effects against hyphae of S. apiospermum and S. prolificans in vitro (100). These data suggest that recovery of host defense is essential for treatment response. In vitro, S. prolificans is more resistant to treatment compared with S. apiospermum (97); this observation was confirmed with clinical experience. The overall response of voriconazole to S. apiospermum infection in pediatric patients with invasive scedosporiosis was 83%; however, patients with S. prolificans infection remained refractory to voriconazole monotherapy (101). In an immunocompetent rabbit model of invasive S. prolificans infection, the investigational azole ABC was superior to amphotericin B to reduce the tissue burden (102). Recent in vitro studies demonstrated that the combination of the antifungal allylamine terbinafine with azoles was synergistic against S. prolificans (103,104). In addition, there are increased number of anecdotal successes with the combination of voriconazole plus terbinafine as therapy against invasive S. prolificans (105,106). Surgical resection remains the only definite therapy for infection caused by S. prolificans.

D. Paecilomyces species

1. Epidemiology

Paecilomyces spp are saprophytic fungi that are distributed worldwide in soil, decaying plants, and food products. Paecilomyces spp. have been shown to survive well on commonly used fabrics and plastics and is frequently found as airborne contaminant in clinical specimens and resistant to most sterilizing techniques. Human infections caused by Paecilomyces are rare but devastating in immunocompromised patients with neutropenia. Human cases include cutaneous disease, onychomycosis,
catheter-related fungemia, pneumonia, peritonitis, osteomyelitis, and prosthetic-valve endocarditis. Other infections reported in immunocompetent hosts include keratitis in contact lens wearers, skin infection, sinusitis, pneumonia, and rarely vaginitis. Several outbreaks have been attributed to Paecilomyces in the last two decades. The first surgical outbreak reported 13 cases of P. lilacinus endophthalmitis after insertion of intraocular lens that were manipulated with the same neutralizing solution (107). A nosocomial outbreak caused by P. lilacinus was also reported in two hematology–oncology units after administration of a contaminated skin lotion (108,109). Risk factors for invasive infection are neutropenia, use of corticosteroids, diabetes, and transplantation.

2. Microbiology
Paecilomyces is a genus of hyaline filamentous fungi closely related to Penicillium, from which it is distinguished by the characteristic of the phialides. There are two medically significant species of the genus Paecilomyces responsible for human disease: P. variotii and P. lilacinus. Both species differ morphologically, clinically, and in their in vitro susceptibility to antifungal therapy.

3. Pathogenesis and Clinical Manifestations
The portal of entry for this fungus includes respiratory tract, indwelling catheters, and the skin resulting in hematogenous dissemination. Similar to Fusarium and Acremonium species, the development of adventitious forms in tissue may explain its propensity for dissemination (69). Clinical manifestations of this infection are variable; in immunocompetent hosts, infections caused by Paecilomyces have been documented as keratitis associated with the use of contact lens, sporothrichosis-like skin infection, sinusitis, and lung abscess. In immunocompromised patients, disseminated disease has been reported, but other manifestations include onychomycosis, catheter-related fungemia, pneumonia, peritonitis, osteomyelitis, and prosthetic-valve endocarditis.

4. Treatment
All cases of infection caused by Paecilomyces should be identified to the species level. Previous studies have demonstrated that P. variotii and P. lilacinus show significant differences in their in vitro susceptibilities to the commonly used antifungals. Paecilomyces variotii is susceptible to amphotericin B and the azole antifungals including itraconazole and voriconazole (110), whereas P. lilacinus often shows variable resistance to amphotericin B, flucytosine, and the azole antifungals (111). However, voriconazole has recently been shown to have in vitro activity against P. lilacinus (112). Despite predicted resistance, high dosage of amphotericin B deoxycholate (1 mg/kg/day) with surgical intervention for localized disease has been recommended and remains a therapeutic option for P. lilacinus infection. The use of lipid formulations of amphotericin B (5 mg/kg/day) or voriconazole may allow for less toxic alternatives in primary therapy.

E. Trichoderma species
1. Epidemiology
Members of the genus Trichoderma are the main components of the soil micro flora, but they are also encountered in air. Trichoderma is a filamentous fungus that was
previously regarded as nonpathogenic to humans and has emerged as an important fungal pathogen in immunocompromised patients and peritoneal dialysis patients. Twenty cases of *Trichoderma* infections in humans have been reported so far. The first report describes a case of *T. viride* isolated from a pulmonary mycetoma in a patient with chronic lung disease (113). Eight cases of peritonitis were documented in patients undergoing continuous ambulatory peritoneal dialysis by different species of *Trichoderma* (114–121). Four cases of disseminated infection by *Trichoderma* were reported in two patients receiving bone marrow transplant (122,123), one in a renal transplant (124), and one in a neutropenic patient with lymphoma (125). Other infections caused by *Trichoderma* include endocarditis, sinusitis, liver abscess, pneumonia, infected perihepatic hematoma, and skin infection (126–130).

2. Microbiology

*Trichoderma* is a filamentous fungus species, member of the class Hyphomycetes. *Trichoderma* species are rapid-growing organisms and form colonies that are initially smooth or translucent and later become floccose, forming concentric white and green rings. A characteristic sweet or “coconut” odor is produced by some species. Microscopically, it is characterized by smooth-walled hyaline, septate, and branched hyphae. Five species of the genus *Trichoderma* have been identified as human pathogens: *T. longibrachiatum*, *T. harzianum*, *T. koningii*, *T. pseudokoningii*, and *T. viride*. Teleomorphs of *Trichoderma* are species of the ascomycete genus *Hypocrea*.

3. Pathogenesis and Clinical Manifestations

The lack of pathogenicity of *Trichoderma* species in immunocompetent hosts was evident after the report of an inadvertent infusion of *T. viride* in a contaminated IV solution. This patient received a single dose of amphotericin B and remained well (131). Suggested portals of entry for *Trichoderma* infection include the respiratory and gastrointestinal tract, and skin. *Trichoderma* infections appear mainly in immunocompromised patients as nodular pulmonary infiltrates, peritonitis complicating peritoneal dialysis, localized cutaneous lesions, endocarditis, and disseminated infection.

4. Treatment

Most isolates of *Trichoderma* demonstrate resistance to fluconazole and fluucytosine but found susceptible to and intermediate to amphotericin B, itraconazole, ketoconazole, and miconazole (124,130). In vitro, voriconazole showed better activity against *T. longibrachiatum* than amphotericin B and itraconazole (112). Likewise, good in vitro activity against species of *Trichoderma* was demonstrated with terbinafine and posaconazole (110,132). Although data are limited, the activity of two echinocandins’ derivatives anidulafungin and caspofungin appears promising in vitro (80). Mortality associated with disseminated infection approaches 100%. Previous reports of *Trichoderma* infections associated favorable outcome when the administration of amphotericin B was coupled with surgical resection (127,130,133). Complete response after surgical drainage alone was reported in a liver-transplant recipient with *T. pseudokoningii* liver abscess (128). Favorable outcome of *Trichoderma* spp. peritonitis was associated with catheter removal (115,118,119). Surgical resection of localized infection is recommended whenever feasible.
F. Zygomycetes

There are two orders of Zygomycetes containing organisms that cause human disease: the Mucorales and Entomophthorales. The majority of human infections are caused by the Mucorales. *Rhizopus* spp. is the most common genera to cause human infection; however, other genera associated with human infection include *Mucor, Rhizomucor, Absidia, Apophysomyces, Saksenaea, Cunninghamella, Cokeromyces,* and *Syncephalastrum* species. Described herein are the most relevant characteristics of these organisms in immunocompromised hosts.

1. Epidemiology

Members of the class Zygomycetes are filamentous fungi found in soil, decaying fruit and vegetables, and old bread. Commonly, zygomycosis develops in patients with diabetic ketoacidosis, immunocompromised patients with neutropenia, and recipients of solid-organ or hematopoietic stem-cell transplant (134,135). Other populations at risk include high-risk newborns, burned patients, trauma, and patients receiving deferoxamine therapy. The major mode of transmission is presumed to be the inhalation route, however, percutaneous route of infection plays a significant role, particularly in surgery, trauma, burns, needle sticks, and tattooing. Apart from the risk incurred by cutaneous breakdown, burned wounds have the additional risk for zygomycosis because of the administration of sulfamylon cream and broad-spectrum antibiotics to prevent infections caused by *Pseudomonas aeruginosa*. The development of zygomycosis in areas of skin breakdown has been associated with a variety of environmental factors such as contaminated adhesive products, elastic bandages, and tongue depressors used in the hospital setting. Gastrointestinal zygomycosis occurs in the setting of diabetes mellitus or organ transplant.

2. Microbiology

The *Mucorales* grow rapidly and well on both fungal selective and nonselective media. Characteristically, in tissue sections, the Zygomycetes display wide, hyaline, aseptate hyphae in a setting of extensive necrosis. The width of the hyphae varies substantially and generally branches at 90°. Better recovery in culture media is obtained in minimally manipulated tissue placed onto the culture medium or baited with breads to promote mycelial growth (136).

3. Pathogenesis and Clinical Manifestations

Neutrophils constitute the major host defense mechanism against zygomycosis. Alteration in the number or quality of neutrophils, monocytes, or macrophages results in increased risk for invasive zygomycosis. Ketoacidosis plays an important role in predisposing diabetic patients to zygomycosis. Low serum pH impairs the phagocytic and chemotactic ability of neutrophils (137). The interaction between transferrin, iron molecules, and fungus has been associated as promoting fungal growth. Resembling invasive aspergillosis, Zygomycetes are angioinvasive resulting in thrombosed vessels and tissue necrosis. The clinical manifestations of disease have evolved from primarily rhinocerebral infection in diabetic patients to pulmonary and disseminated disease including gastrointestinal, subcutaneous, cutaneous, allergic disease, and even asymptomatic colonization.

Rhinocerebral infection represents one-third to one-half of all cases of zygomycosis. Following inhalation of fungal spores, the infection originates in the sinuses,
resulting in sinus pain, drainage, and soft-tissue swelling. The disease becomes rapidly progressive with local extension including periorbital tissues. Extension into the mouth is manifested by necrotic ulceration in the hard palate. Involvement of the ethmoid sinuses may be associated with cavernous sinus thrombosis (138).

Pulmonary involvement occurs in severely neutropenic patients and manifests as solitary nodular, lobar or segmental, cavitary, and bronchopneumonic lesions. Angioinvasion may result in thrombosis of pulmonary vessels and subsequent pulmonary infarction. Cutaneous infection may result from primary inoculation or secondary to disseminated disease. The lesions appear red and indurated that often develop in black eschars. Extension to the subjacent bone and development of necrotizing fasciitis may result from cutaneous or subcutaneous zygomycosis.

4. Treatment
Disseminated zygomycosis carries a high-mortality rate despite antifungal therapy. A combined approach with medical and surgical treatment is required to obtain a favorable outcome. Medical therapy consists in correction of the immune status and antifungal therapy. High doses of amphotericin B remain the first-line therapy for most cases of zygomycosis. Lipid formulations of amphotericin B with or without the use of cytokines demonstrated activity in patients’ refractory to therapy with amphotericin B (139,140). The currently available triazoles including voriconazole and the echinocandins are considered inactive when given as monotherapy. The combination of voriconazole and terbinafine demonstrated in vitro synergy against Zygomycetes in 44% of the isolates (141). The experimentalazole posaconazole has shown in vitro activity in animal models and in some patients with refractory zygomycosis (142–144).

Surgical debridement is considered an integral part of treatment for localized disease. Surgical resection for localized pulmonary lesions improved survival compared with medical therapy alone (145).

IV. DEMATIACEOUS MOLDS

Dematiaceous fungi represent a group of heterogeneous fungal organisms characterized by the presence of pale brown to dark melanine-like pigment in their cell wall. Clinical entities related to dematiaceous fungi are chromoblastomycosis and phaeohyphomycosis. Black-grained mycetoma is also associated with dematiaceous fungi. The description of localized, subcutaneous infections caused by dematiaceous fungi is not described in this chapter. Focus is on the salient aspects of invasive disease in immunocompromised hosts.

1. Epidemiology
Darkly pigmented fungi are widely distributed in the environment and occasionally cause human infections. In the past two decades, there has been an increasing report of dematiaceous fungi as causative of invasive infection in patients with cancer and hematopoietic or organ transplant (2,146). Invasive disease has also been reported in patients with AIDS, diabetes, and chronic granulomatous disease and is increasingly reported in patients undergoing peritoneal dialysis. The number of dematiaceous molds that have been documented as agent of phaeohyphomycosis continues to increase. A recognized group of these organisms seems to be neurotropic, where they
 localize in the CNS causing one or multiple brain lesions. Dematiaceous fungi that are known to be neurotropic include *Cladophialophora bantiana*, *Wangiella* (*Exophiala*) *dermatitidis*, *Ramichloridium obovoideum*, *Xylohypha* (*Cladosporium*), *Fonsecaea pedrosii*, *Chaetomium atrobrunneum*, and *Dactylaria* (*Ochrochonis*) *gallipavum*. In addition, *Bipolaris* spp. and *Exserohilum rostratum* most commonly cause sinusitis and invade the CNS via extension from the paranasal sinuses. Other fungal pathogens, known as etiologic agents of sinusitis, include *Curvularia* and *Alternaria* spp. In addition to causing invasive disease, these dark molds are also etiologic agents of allergic reactions manifested by sinusitis and pulmonary disease. In a recent prospective multicenter study of mycelial non-*Aspergillus* infections in organ transplant, phaeohyphomycosis represented 9.4% (5/53) of all the infections (2). Suggested portals of entry for phaeohyphomycosis include not only the direct inoculation by penetrating injury but also the inhalation route, ingestion of contaminated food or water, and breakdown of skin barrier with the use of vascular catheters. Although most of the pathogens have a worldwide distribution, *R. obovoideum* is a well-known cause of sinusitis and CNS infection in the Middle East.

### 2. Microbiology

Phaeohyphomycosis is characterized by dark-walled fungal elements consisting of yeast-like cells, pseudohyphae, and hyphae. Despite the dematiaceous nature of these fungi, the brown pigment is not always present (147). Some of them produce various amounts of melanin pigment in their cell walls under different conditions. In vivo, melanin production may be minimal and fungal elements appear colorless; however, in culture, increasing melanin production leads to darker pigmentation. In histologic sections, confirmation of the presence of dematiaceous mold can be achieved by using a melanin-specific stain as the Fontana–Mason stain (148).

### 3. Pathogenesis and Clinical Manifestations

Melanin plays an important role in evasion of host defense by dematiaceous molds. Melanins have attracted interest as virulence factors in fungi. Among the mechanisms proposed is quenching of oxidative metabolites, which reduces susceptibility to antifungal and enzymatic degradation (149,150). Unlike chromoblastomycosis and mycetoma, phaeohyphomycosis invades deep structures and elicits a variety of inflammatory responses. Subcutaneous phaeohyphomycosis usually presents as a single lesion, but multiple lesions have been occasionally described. The clinical manifestations of CNS disease commonly include headache, low-grade fever, and eventual development of focal neurologic signs. Patient may have no history of mold exposure, no obvious portals of entry, or distant dissemination. The clinical spectrum also includes sinusitis, pneumonia, ocular disease, arthritis, osteomyelitis, fungemia, endocarditis, peritonitis, and gastrointestinal disease. In the spectrum of sinus infection, *Bipolaris* or *Exserohilum* may occur as an allergic sinusitis or asthma. *Bipolaris hawaiienensis* is associated with a more aggressive behavior, frequently leading to bone erosion or tissue necrosis (151,152).

### 4. Treatment

Cerebral phaeohyphomycosis has a high degree of mortality requiring early and aggressive therapy. There are no trials that assess and compare the different strategies for the treatment of phaeohyphomycosis. Historically, early treatment with
amphotericin B and complete surgical resection has been recommended until new alternatives are found. Treatment depends on the extent and location of the infection. Surgical excision is the first choice of treatment for subcutaneous and cutaneous phaeohyphomycosis resulting in cure in the majority of cases. The in vitro activity of amphotericin B, itraconazole, and voriconazole was demonstrated against 25 strains of dematiaceous fungi. Overall, the fungicidal activity of the three agents was similar, with the exception of decreased activity of the azoles against *B. spicifera* and *Dactyliaria constricta* var. *gallopava* (112). Few clinical data are available with the use of echinocandins in the treatment of phaeohyphomycosis; however, micafungin demonstrated in vitro activity against the dematiaceous fungi *Cladosporium trichoides*, *E. spinifera*, *F. pedrosoi*, and *E. dermatitidis* (153). As a class, the antifungal triazoles may have superior activity against many dematiaceous molds and offer less toxic alternatives to amphotericin B. The duration of treatment has not been established; however, long-term therapy may prevent recurrences.

Whenever possible, surgical resection is recommended for lesions in other organs, coupled with systemic antifungal therapy. Reduction in immunosuppressive agents may be attempted if feasible.

V. **CRYPTOCOCCUS** SPP.

1. **Epidemiology**

Cryptococcosis is an infection caused by the encapsulated yeast-like fungus *C. neoformans*. Cryptococcal infections occur worldwide without any defined endemic areas, but the environmental distribution of the serotypes shows some differences. Antigenic specificity of the capsular polysaccharide defines four different serotypes of *C. neoformans*: A, B, C, and D. Serotype D constitutes the majority of clinical isolates of *C. neoformans* var. *neoformans*. In contrast, serotype A describes the newly recognized *C. neoformans* var. *grubii* (154). The overwhelming majority of isolates from serotypes A and D are recovered from AIDS patients. The less common serotypes B and C are classified as *C. neoformans* var. *gattii*. Accompanying the increase in cryptococcosis after the HIV epidemic is the recognition that non-*neoformans* Cryptococcus strains are now reported with increasing frequency. Focus in this section, is on the less common strains of Cryptococcus that cause human disease.

*Cryptococcus neoformans* var. *gattii* is largely restricted to tropical and subtropical areas where the infection occurs in otherwise healthy individuals. The only known environmental source of *C. neoformans* var. *gattii* is the river gum tree (*Eucalyptus camaldulensis*), as well as the forest red gum (*Eucalyptus tereticornis*), which grows in rural Australia. The role of Eucalyptus tree and the occurrence of human infections caused by *Cryptococcus* cannot be established. In a surveillance study of cryptococcosis in Australia, all the *C. neoformans* var. *gattii* infections occurred in healthy hosts (155). In the same study, meningitis was the commonest manifestation, and focal CNS and pulmonary lesions were found primarily in patients with *C. neoformans* var. *gattii*. Another study conducted in Taiwan (156) identified 21 (35.6%) cryptococcal infections caused by *C. neoformans* var. *gattii*. Infection tended to occur predominantly during the months of July and August.

Infections with cryptococci other than *C. neoformans* are rare; however, several cases of infections with non-*neoformans* species have been reported (Table 2). *Cryptococcus laurentii* has emerged as an important cause of fungemia in neutropenic patients and peritonitis in a setting of continuous ambulatory peritoneal dialysis.
<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Patients</th>
<th>Clinical diagnosis</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Reference</th>
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<td>Ganglioneuroblastoma</td>
<td>Fungemia</td>
<td>AmB</td>
<td>Response</td>
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<td>VLBW</td>
<td>Fungemia</td>
<td>AmB</td>
<td>Favorable</td>
<td>165</td>
</tr>
<tr>
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<td>1999</td>
<td>Cancer</td>
<td>Fungemia</td>
<td>FLC</td>
<td>Favorable</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>Cancer</td>
<td>Fungemia</td>
<td>AmB</td>
<td>Favorable</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>1999</td>
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<td>Fungemia</td>
<td>Not stated</td>
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<td>166</td>
</tr>
<tr>
<td></td>
<td>1998</td>
<td>AIDS</td>
<td>Meningitis</td>
<td>AmB</td>
<td>Favorable</td>
<td>167</td>
</tr>
<tr>
<td></td>
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<td>VLBW</td>
<td>Fungemia</td>
<td>AmB + FC</td>
<td>Favorable</td>
<td>168</td>
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<tr>
<td></td>
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<td>Fungemia</td>
<td>FLU</td>
<td>Favorable</td>
<td>168</td>
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<tr>
<td></td>
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<td>DM</td>
<td>Keratitis</td>
<td>AmB + FC</td>
<td>Enucleation</td>
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<td>1997</td>
<td>BMT</td>
<td>Fungemia</td>
<td>FLC</td>
<td>Favorable</td>
<td>170</td>
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<td>Uveitis</td>
<td>FLC</td>
<td>Favorable</td>
<td>171</td>
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<tr>
<td></td>
<td>1989</td>
<td>CAPD</td>
<td>Peritonitis</td>
<td>Cath removal</td>
<td>Favorable</td>
<td>172</td>
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<tr>
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<td>1989</td>
<td>CAPD</td>
<td>Peritonitis</td>
<td>AmB</td>
<td>Favorable</td>
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<td>Cutaneous</td>
<td>AmB</td>
<td>Favorable</td>
<td>175</td>
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<td><em>Cryptococcus albidus</em></td>
<td>2004</td>
<td>HSCT, neutropenia</td>
<td>Fungemia</td>
<td>AmB, ITC</td>
<td>Favorable</td>
<td>176</td>
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<tr>
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<td>2004</td>
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<td>AIDS</td>
<td>Fungemia</td>
<td>AmB + FC</td>
<td>Death</td>
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<tr>
<td></td>
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<td>ALL, neutropenia</td>
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<td>AmB</td>
<td>Favorable</td>
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<td>Fungemia</td>
<td>FLC</td>
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<td>Fungemia</td>
<td>AmB + FC</td>
<td>Death</td>
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<td>RA</td>
<td>Meningitis</td>
<td>AmB</td>
<td>Death</td>
<td>183</td>
</tr>
<tr>
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<td>1973</td>
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<td>Meningitis</td>
<td>AmB</td>
<td>Favorable</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>1972</td>
<td>Immunocompetent</td>
<td>Pneumonia</td>
<td>AmB</td>
<td>Favorable</td>
<td>185</td>
</tr>
<tr>
<td><em>Cryptococcus curvatus</em></td>
<td>1995</td>
<td>AIDS</td>
<td>Myeloradiculitis</td>
<td>AmB</td>
<td>Favorable</td>
<td>186</td>
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<tr>
<td><em>Cryptococcus luteolus</em></td>
<td>1955</td>
<td>Measles</td>
<td>Pneumonia</td>
<td>None</td>
<td>Favorable</td>
<td>187</td>
</tr>
<tr>
<td><em>Cryptococcus uniguttulatus</em></td>
<td>2001</td>
<td>SHA</td>
<td>Ventriculitis</td>
<td>AmB</td>
<td>Death</td>
<td>188</td>
</tr>
</tbody>
</table>

**Abbreviations:** ALL, acute lymphocytic leukemia; AmB, amphotericin B; BMT, bone marrow transplant; CAPD, continuous ambulatory peritoneal dialysis; DM, diabetes mellitus; FC, flucytosine; FLC, fluconazole; HIV, human immunodeficiency virus; HSCT, hematopoietic stem-cell transplant; IVDU, intravenous drug use; RA, rheumatoid arthritis; SHA, subarachnoid hemorrhage; VLBW, very low birth weight.
There are also reports on *C. albidus* sepsis and meningitis, *C. curvatus* myeloradiculitis, and *C. humicola* meningitis, all reported in immunosuppressed patients with AIDS or cancer.

2. **Pathogenesis and Clinical Manifestations**

The major virulence factors identified in *C. neoformans* and non-*neoformans* are capsule formation and melanin synthesis. However, those factors are less expressed in the non-*neoformans* species (157). There is a distinct characteristic between infections and *C. neoformans* variety. *Cryptococcus neoformans* var. *gattii* is common in immunocompetent hosts. Early manifestations of meningoencephalitis for both varieties include headache, nausea, unsteady gait, dementia, irritability, confusion, and blurred vision. When compared with *C. neoformans* var. *neoformans*, infections caused by var. *gattii* are associated with cerebral or pulmonary cryptococcomas, papilledema, high CSF, and serum cryptococcal antigen titers (158). Hypodense lesions and hydrocephalus are common radiologic findings in cranial CT in cases of meningitis caused by *C. neoformans* var. *gattii*, and CSF analysis consists of mild pleocytosis with elevated protein level (159).

3. **Treatment**

Most isolates of *C. neoformans* remain susceptible to amphotericin B, flucytosine, and fluconazole (160). The standard regimen of amphotericin B (0.7 mg/kg/day) in combination with flucytosine (100 mg/kg/day) for 2 weeks of induction followed by fluconazole (400 mg/d) for 8 weeks is recommended for both HIV-infected and healthy hosts with cryptococcal meningitis (161,162). Infections caused by *C. neoformans* var. *gattii* demonstrate slower response to antifungal therapy. Anecdotal reports suggested longer induction with amphotericin B combined with flucytosine for 2–7 months (159). Worse prognosis is usually associated with var. *gattii*. Neurologic sequela is often present despite prolonged amphotericin B therapy and intraventricular shunt. Ocular complications are common. Visual loss accompanies var. *gattii* infections in 50% of the cases (159,163) and is associated with optic atrophy following optic disc swelling. Limited data on in vitro susceptibilities of non-*neoformans* cryptococci demonstrate higher resistance to fluconazole and flucytosine of these species compared with *C. neoformans* var. *neoformans*.

VI. **CONCLUSION**

Infections caused by these newly recognized pathogens represent an increasing problem in the immunocompromised host. These infections are difficult to diagnose and can present to the clinicians caring for high-risk patients with a serious challenge. Because of the uncommon occurrence of these infections, clinical trials focusing on optimal antifungal therapy are almost impossible to perform, further complicating the choice of antifungal agent and the complete understanding of the role of adjunctive measures such as surgery, cytokines, etc. The recent availability of several new antifungal agents, some with promising in vitro and experimental activity against these pathogens, may result in an improved outcome.
REFERENCES


162. van der Horst CM, Saag MS, Cloud GA, Hamill RJ, Graybill JR, Sobel JD, Johnson PC, Tuazon CU, Kerkering T, Moskovitz BL, Powderly WG, Dismukes WE. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome.


Conventional Methods for the Laboratory Diagnosis of Fungal Infections in the Immunocompromised Host

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I. INTRODUCTION

The frequency of invasive fungal disease due to systemic and opportunistic pathogens has increased dramatically over the past two decades (1–7). This increase in infections is associated with considerable morbidity and mortality (8–10) and is largely because of expanding patient populations at risk for the development of life-threatening fungal infections, which include individuals undergoing solid-organ and blood and marrow transplantation (BMT), major surgery, those with AIDS, neoplastic disease, immunosuppressive therapy, and premature birth (2,4,6,10–13). Serious infections are being reported with an ever-increasing spectrum of pathogens including the well-known opportunistic fungal pathogens Candida albicans, Cryptococcus neoformans, and Aspergillus fumigatus (2,3,14,15). New and emerging pathogens include species of Candida and Aspergillus other than C. albicans and A. fumigatus, yeasts such as Trichosporon, Rhodotorula, and Malassezia species, hyaline hyphomycetes including Fusarium, Acremonium, and Paecilomyces species, and a wide variety of dematiaceous fungi (Table 1) (6,14–19). Modern medical mycology has become an extremely challenging study of infections caused by a broad range of taxonomically diverse opportunistic fungi (16). It is now quite clear that there are no nonpathogenic fungi; virtually any fungus can cause a lethal mycosis in an immunocompromised host.
<table>
<thead>
<tr>
<th>Organism group</th>
<th>Examples of specific pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Candida</strong></td>
<td>C. albicans</td>
</tr>
<tr>
<td></td>
<td>C. glabrata</td>
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<tr>
<td></td>
<td>C. parapsilosis</td>
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<td>C. tropicalis</td>
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<td></td>
<td>C. dubliniensis</td>
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<td></td>
<td>C. krusei</td>
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<tr>
<td></td>
<td>C. lasthaniae</td>
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<tr>
<td></td>
<td>C. guillermondii</td>
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<td></td>
<td>C. rugosa</td>
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<tr>
<td><strong>Other yeasts</strong></td>
<td></td>
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<tr>
<td></td>
<td>Cryptococcus neoformans</td>
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<tr>
<td></td>
<td>Trichosporon</td>
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<tr>
<td></td>
<td>Blastoschizomyces</td>
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<td></td>
<td>Rhodotorula</td>
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<td></td>
<td>Malassezia</td>
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<td></td>
<td>Saccharomyces</td>
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<td></td>
<td>Hansenula</td>
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<tr>
<td><strong>Aspergillus</strong></td>
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<tr>
<td></td>
<td>A. fumigatus</td>
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<tr>
<td></td>
<td>A. flavus</td>
</tr>
<tr>
<td></td>
<td>A. niger</td>
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<tr>
<td></td>
<td>A. versicolor</td>
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<tr>
<td></td>
<td>A. terreus</td>
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<tr>
<td></td>
<td>A. nidulans</td>
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<td><strong>Zygomycetes</strong></td>
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<td>Rhizopus</td>
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<td></td>
<td>Rhizomucor</td>
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<tr>
<td></td>
<td>Mucor</td>
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<tr>
<td></td>
<td>Absidia</td>
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<tr>
<td></td>
<td>Apophysomyces</td>
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<tr>
<td></td>
<td>Cunninghamella</td>
</tr>
<tr>
<td></td>
<td>Saksenaea</td>
</tr>
<tr>
<td></td>
<td>Cokeromyces</td>
</tr>
<tr>
<td><strong>Other hyaline molds</strong></td>
<td></td>
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<tr>
<td></td>
<td>Fusarium</td>
</tr>
<tr>
<td></td>
<td>Acremonium</td>
</tr>
<tr>
<td></td>
<td>Scedosporium apiospermum</td>
</tr>
<tr>
<td></td>
<td>S. prolificans</td>
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<tr>
<td></td>
<td>Trichoderma</td>
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<td>Paecilomyces</td>
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<tr>
<td><strong>Dematiaceous molds</strong></td>
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<tr>
<td></td>
<td>Alternaria</td>
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<td></td>
<td>Bipolaris</td>
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<tr>
<td></td>
<td>Curvularia</td>
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<tr>
<td></td>
<td>Exophiala</td>
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<tr>
<td></td>
<td>Cladophialophora</td>
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<td>Phialophora</td>
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<tr>
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<td>Dactylaria</td>
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<tr>
<td></td>
<td>Wangiella</td>
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<tr>
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<td></td>
<td>Histoplasma</td>
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<tr>
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<td></td>
<td>Blastomyces</td>
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<tr>
<td></td>
<td>Paracoccioides</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>Penicillium marneffei</td>
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<tr>
<td><strong>Other</strong></td>
<td>Pneumocystis jiroveci</td>
</tr>
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</table>

*aList not all inclusive.*
Owing to the complexity of the various patient groups at risk for infection and the increasing array of fungal pathogens, opportunistic mycoses pose a considerable diagnostic challenge to both clinicians and microbiologists (16,20,21). Despite recognition of the importance of the invasive mycoses, these infections remain difficult to diagnose. The current diagnostic approach includes clinical suspicion, culture of appropriate sites, histopathologic examination of tissue biopsies, and diagnostic imaging techniques (Table 2) (16,22–27). Although these approaches may suffer from a lack of diagnostic sensitivity and specificity, it is absolutely essential that individuals and institutions caring for high-risk immunocompromised patients place a high priority on maximizing their diagnostic capabilities for early diagnosis of invasive opportunistic fungal infections (16,21,23). Optimal diagnosis and treatment of mycotic infections in the compromised host are directly dependent upon a team approach involving clinicians, microbiologists, and pathologists (16,22,27).

In this chapter, the more conventional diagnostic approaches including direct microscopy, culture, and histopathology are addressed (Table 2). Diagnostic imaging and the nonculture-based methods of serology and molecular diagnostics are covered in subsequent chapters.

II. CLINICAL RECOGNITION OF FUNGAL INFECTION

The increased frequency of invasive mycoses requires that clinicians caring for immunocompromised individuals have an enhanced index of clinical suspicion and a greater appreciation of the major risk factors that predispose patients to fungal infections (2,6,11). Specific fungal infections may be associated with well-known clinical scenarios such as endophthalmitis and macronodular skin lesions (candidiasis), onychomycosis in
a neutropenic patient (fusariosis), sinus infection in diabetic ketoacidosis (zygomycosis), or myalgias and myositis (candidemia caused by \textit{C. tropicalis}). Other clinical signs and symptoms that may be associated with fungal infections include suppurative thrombophlebitis (\textit{Candida}), cholestasis (chronic disseminated or hepatosplenic candidiasis), purpura fulminans and bullous dermatitis (\textit{Candida}, \textit{Aspergillus}, and \textit{Fusarium} spp.), and osteomyelitis (\textit{Candida}) (26,28). Although these are useful clinical clues, they are not entirely specific and often are observed rather late in the course of the infectious process. The newer serologic and molecular diagnostic tests promise to enhance the diagnostic approach to fungal infections; however, at present conventional microbiologic and histologic methods serve as the cornerstone for definitive diagnosis of the mycoses. Unfortunately, these methods may require invasive procedures (biopsy), may not exhibit optimal sensitivity or specificity, and often are too slow (culture) to provide a diagnosis in a clinically meaningful time frame.

### III. SPECTRUM OF OPPORTUNISTIC FUNGAL PATHOGENS

As noted earlier, the spectrum of possible opportunistic fungal pathogens is quite broad (Table 1). However, \textit{C. albicans} remains the most common fungal pathogen (3) and \textit{A. fumigatus} is the commonest mold pathogen (29). Likewise, \textit{Candida} spp., \textit{Aspergillus} spp., and \textit{C. neoformans} account for more than 80\% of all fungal infections in BMT, solid-organ transplant, and other immunosuppressed patient populations (30).

The frequency of fungal infections, predilection for specific fungal pathogens, and the time of onset of various fungal infections differ for different patient groups (Table 3) (13). Unique risk factors, exposures, and the degree and duration of immunosuppression account likely for this variability (13). Thus, invasive aspergillosis (IA) exerts a major impact on patients following allogeneic BMT, lung and liver transplant, whereas infections caused by \textit{Candida} spp. are most problematic among neonates, postsurgical intensive care unit (ICU) patients, and postpancreas and small-bowel transplant patients (Table 3) (2,11–13,21,31,32). Aside from \textit{Candida} and \textit{Aspergillus} spp., the less common and “emerging” fungal pathogens are often found in the environment and tend to cause infection in those individuals with long-term immunosuppression (6). The greater the degree and duration of immunosuppression, the broader the spectrum of possible opportunistic fungal pathogens (20,28).

### IV. CONVENTIONAL METHODS FOR THE LABORATORY DIAGNOSIS OF FUNGAL INFECTIONS

The laboratory diagnosis of fungal infections begins with the proper collection and prompt transport of clinical material to the microbiology laboratory. Direct microscopic examination is useful to provide a rapid presumptive diagnosis and an aid in directing culture efforts. Culture on appropriate medium, while not rapid, provides important information regarding the identity of the etiologic agent and is necessary to provide an isolate for subsequent antifungal susceptibility testing. Histopathologic examination of biopsy material using special stains is usually done outside of the mycology laboratory but is often essential in defining the type and extent of the infection. Every step of this process is enhanced by direct communication between the clinician, the mycologist, and the pathologist. Information regarding a
Table 3  Relative Frequency of Opportunistic Mycoses Among Different Patient Groups

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Asp</th>
<th>Can</th>
<th>Cryp</th>
<th>Tri</th>
<th>PCP</th>
<th>Hyal</th>
<th>Phae</th>
<th>Blas&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Hist&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Cocci&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Pmar&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Zygo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transplant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Allo. BMT</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
<td>++</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Liver</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Lung</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
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<td>(+)</td>
</tr>
<tr>
<td>Kidney</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Heart</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
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<td>(+)</td>
</tr>
<tr>
<td>Sm. Bowel</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
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<td>(+)</td>
</tr>
<tr>
<td>Malignancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
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</tr>
<tr>
<td>Heme</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Solid</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(++)</td>
<td>(+++)</td>
<td>(+++)</td>
<td>(+++)</td>
<td>(+)</td>
</tr>
<tr>
<td>Critical care</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(++)</td>
<td>(+++)</td>
<td>(+++)</td>
<td>(+++)</td>
<td>(+)</td>
</tr>
<tr>
<td>Adult</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neonate</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Relative frequency of mycoses within each patient group indicated as ++++ (most frequent) to + (least frequent).

<sup>b</sup>Frequency of endemic mycoses indicated by (++) to (++++) within endemic regions only.

Abbreviations: Allo BMT, allogeneic blood and marrow transplant; Sm. bowel, small bowel; Heme, hematologic malignancy; Solid, solid tumor malignancy; Asp., aspergillosis; Can., candidiasis; Tri, trichosporonosis; Cryp., cryptococcosis; PCP, *Pneumocystis jiroveci* pneumonia; Hyal., hyalohyphomycosis; Phae., phaeohyphomycosis; Blas., blastomycosis; Hist., histoplasmosis; Cocci., coccidioidomycosis; Pmar., *Penicillium marneffei*; Zygo., zygomycosis.

For mycosis abbreviations, see Table 1 for specific examples within each group.
clinical suspicion for a specific fungus will alert the laboratory to modify routine processing (e.g., addition of olive oil to the culture to enhance detection of *Malassezia*) or to be on the alert for a biohazardous organism (e.g., *Coccidioides immitis*).

A. Specimen Collection and Processing

As in the case with all infectious diseases, the diagnosis of fungal infection is directly dependent upon the proper collection of the appropriate clinical specimen and prompt transport of specimens to the clinical laboratory (16,24,33,34). Clinical and radiographic examination, as well as consideration of the most likely fungal pathogen that may cause a specific type of infection in a given patient should drive the selection of the appropriate specimen for culture and microscopic examination (Tables 3 and 4). Specimens should be collected after proper cleaning and decontamination of the collection site and under aseptic conditions if possible. An adequate amount of suitable clinical material should be promptly submitted for culture and examination (Table 5). Unfortunately, many specimens submitted to the laboratory are either of insufficient amount or of poor quality and are inadequate to make a diagnosis. As a general rule, swab specimens are inappropriate for mycologic and microbiologic culture and microscopic examination (33).

Specimens should be placed in a sterile, leak proof container, and be properly labeled and accompanied by a relevant clinical history. As mentioned earlier, the clinical information is very important in directing specimen processing and as an aid in interpreting the culture results. The clinical information is especially important when the specimen is from a nonsterile site such as the respiratory, genitourinary, (GU) or gastrointestinal (GI) tract, and the skin.

Whenever possible, specimens should be delivered to the laboratory within 2 hr of collection to prevent overgrowth by bacteria or other commensal flora. Most fungi can be recovered from specimens submitted in bacteriologic transport medium, although direct microscopic examination may be obscured by the transport medium components. In general, most specimens for fungal culture may be safely stored at 4°C for a short time.

As with any infectious disease, there are specimens that are better than others for diagnosis of mycotic infections (Table 4). Cultures of blood, cerebrospinal fluid (CSF), and other normally sterile body fluids are usually quite useful and should be performed if clinical signs and symptoms are suggestive of hematogenous dissemination. Biopsies of skin lesions or other focal sites of infection often yield a diagnosis and should be obtained for culture and histopathologic examination whenever possible. Diagnosis of oral or vaginal thrush is often best established by clinical presentation and direct microscopic [wet mount or potassium hydroxide (KOH) preparation] examination of scrapings or secretions because culture often represents simple colonization or contamination. Similarly, fungal infections of the GI tract are better diagnosed by biopsy and histopathologic examination of the involved tissue than by culture alone. Culture of lower respiratory tract and urine specimens may be confounded by contamination with normal oral and periurethral flora, respectively, and care should be taken to minimize the possibility of such contamination when obtaining these specimens (Table 5). Twenty-four-hour collections of sputum or urine are not appropriate for mycologic examination because of overgrowth of bacterial and fungal contaminants (Table 5).
### Table 4  Selection of Clinical Specimens for Detection and Isolation of Opportunistic Fungal Pathogens

<table>
<thead>
<tr>
<th>Suspected pathogen</th>
<th>Blood</th>
<th>Bone marrow fluid</th>
<th>Joint fluid</th>
<th>Eye</th>
<th>Urine</th>
<th>Respiratory</th>
<th>Skin and mucous membranes</th>
<th>Multiple systemic sites</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yeast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida</em> spp.</td>
<td>++++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>+++</td>
<td>+</td>
<td>++++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Trichosporon</em> spp.</td>
<td>++++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td><em>Malassezia</em> spp.</td>
<td>++++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rhodotorula</em> spp.</td>
<td>++++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Molds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus</em> spp.</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><em>Zygomycetes</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fusarium</em> spp.</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><em>Scedosporium apiospermum</em></td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Scedosporium prolificans</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Dematiaceous</em> molds</td>
<td>++++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><strong>Dimorphic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Histoplasma capsulatum</em></td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><em>Blastomyces dermatitidis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>+</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td><em>Coccidioides immitis</em></td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td><em>Paracoccidioides brasiiliensis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><em>Penicillium marneffei</em></td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td><em>Sporothrix schenckii</em></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pneumocystis jiroveci</em></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+++</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

*a* Aspergillus terreus only.

*Source:* Adapted from Ref. 16.

*Note:* Predominant sites for recovery are ranked in order of importance and frequency (i.e., ++++, most important or most frequent; +, Less important or less frequent) based on the most common clinical presentation.
### Table 5  Collection and Processing of Clinical Specimens for Recovery of Fungi

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Collection guidelines</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>20–30mL blood</td>
<td>Avoid catheter draws if possible</td>
</tr>
<tr>
<td></td>
<td>A variety of broth-based systems (Bact/Alert, BACTEC, ESP, Septi-Check), as well as lysis centrifugation systems</td>
<td>Lysis centrifugation (Isolator) preferred for dimorphics, filamentous fungi, and C. neoformans</td>
</tr>
<tr>
<td></td>
<td>Transport at room temperature within 24 hr</td>
<td>Subculture broth systems to blood, chocolate, and BHI agar</td>
</tr>
<tr>
<td></td>
<td>Do not refrigerate</td>
<td>Plate lysate (Isolator) onto blood, chocolate, and BHI agar</td>
</tr>
<tr>
<td></td>
<td>Isolator should be processed within 16 hr</td>
<td></td>
</tr>
<tr>
<td>Sterile fluids (CSF, pleural, pericardial, peritoneal, or joint fluid)</td>
<td>Collect in sterile container</td>
<td>May need ~30mL CSF for chronic meningitis</td>
</tr>
<tr>
<td></td>
<td>At least 3–5mL fluid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transport immediately to laboratory</td>
<td>May inoculate all but CSF into blood-culture bottles for yeasts</td>
</tr>
<tr>
<td></td>
<td>Concentrate by centrifugation, use sediment for culture and supernatant for antigen testing</td>
<td></td>
</tr>
<tr>
<td>Tissue biopsy</td>
<td>Place in sterile container with small amount of nonbacteriostatic saline</td>
<td>Tissue should be minced rather than ground to optimize recovery of fungi</td>
</tr>
<tr>
<td></td>
<td>Transport immediately to laboratory</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Sterile collection using a lysis centrifugation pediatric tube (Isolator) or a heparin tube</td>
<td>Useful when disseminated histoplasmosis or coccidioidomycosis is suspected</td>
</tr>
<tr>
<td></td>
<td>Transport at room temperature within 4 hr.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Do not refrigerate</td>
<td></td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>Early morning sputum collected in sterile container</td>
<td>24 hr sputum collections not acceptable</td>
</tr>
<tr>
<td></td>
<td>Minimum volume, 1 mL</td>
<td>Induced sputum or BAL best for P. jiroveci detection by microscopy</td>
</tr>
<tr>
<td></td>
<td>Induced sputum, BAL, or tissue biopsy is preferred for diagnosis of invasive fungal infection</td>
<td>Isolation of Candida spp. usually represents oral contamination</td>
</tr>
<tr>
<td></td>
<td>Transport to laboratory within 2 hr</td>
<td>Significance of culture positive for Aspergillus spp. depends on patient risk group</td>
</tr>
<tr>
<td></td>
<td>May refrigerate for up to 24 hr</td>
<td></td>
</tr>
<tr>
<td>Specimen Collection Site</td>
<td>Instructions</td>
<td>Notes</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Intravascular catheter tip</td>
<td>Approximately 5 cm of the distal end should be sent in a sterile dry container</td>
<td>Any number of fungal colonies may be significant</td>
</tr>
<tr>
<td></td>
<td>Transport within 2 hr at room temperature</td>
<td>Skin contamination is problematic</td>
</tr>
<tr>
<td></td>
<td>May refrigerate for up to 24 hr</td>
<td>Blood cultures should also be obtained</td>
</tr>
<tr>
<td>Corneal scrapings</td>
<td>Direct inoculation on fungal media (e.g., BHI) is optimal</td>
<td>Consultation with laboratory personnel regarding media selection and inoculation procedures is helpful</td>
</tr>
<tr>
<td></td>
<td>May use aerobic swab transport system for conjunctival infection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intraocular fluid should be collected in a sterile container</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transport immediately to laboratory</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>First AM specimen collected in a sterile container</td>
<td>24 hr collections not acceptable</td>
</tr>
<tr>
<td></td>
<td>Volume &gt; 1 mL (50–200 mL best)</td>
<td>Candiduria of uncertain clinical significance. May represent colonization or contamination</td>
</tr>
<tr>
<td></td>
<td>Transport at room temperature within 2 hr May refrigerate for up to 24 hr</td>
<td></td>
</tr>
<tr>
<td>Wounds and abscess</td>
<td>Aspirates or biopsies preferable to swabs</td>
<td>Avoid swabs whenever possible</td>
</tr>
<tr>
<td></td>
<td>Place material into sterile anaerobic transport system</td>
<td>If surgical drainage of an abscess is performed, send both abscess material and a portion of the abscess wall</td>
</tr>
<tr>
<td></td>
<td>Transport to laboratory within 2 hr</td>
<td></td>
</tr>
</tbody>
</table>

*aSpecimen collection sites are not all inclusive.

*Source:* Adapted from Refs. 23, 34.
B. Direct Microscopy

Direct microscopic examination of specimens submitted to the microbiology laboratory is performed routinely as a means of providing a rapid diagnosis and to guide specimen processing and culture. In contrast with histopathologic examination (see below), direct microscopy, as performed in the microbiology laboratory, does not utilize fixed tissue and relies instead on rapid examination of wet mounts, zGram, Giemsa, or Calcofluor-stained material (Table 6). Such direct microscopy is perhaps the most rapid, useful, and cost-effective means of diagnosing fungal infections (24).

Detection of fungal elements microscopically may provide a presumptive diagnosis in < 1 hr compared to days or weeks with culture. In some instances, a definitive diagnosis may be possible by direct microscopy based on the distinctive morphology of the infecting fungus (Table 7). For example, if distinctive yeast cells, spherules, or other structures are observed microscopically, an etiologic diagnosis can be made for infections caused by *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *C. immitis*, *Pneumocystis jiroveci* (*carinii*), and *Penicillium marneffei* (Table 7). In other infections, microscopy can usually yield preliminary information that a yeast or a mold is present and, in some instances, the morphologic appearance may provide a presumptive diagnosis of the type of infection (e.g., aspergillosis, fusariosis, candidiasis, and trichosporonosis) but not the actual species identification of the etiologic agent (Table 7).

All direct examinations should be confirmed by culture. Detection of fungal elements on direct examination often serves to guide the laboratory in selecting the most appropriate means to culture the clinical material. Similarly, the direct microscopic results may aid in the interpretation of the culture results (Figs. 1 and 2). Direct examination is less sensitive than culture for most mycotic infections, and a negative microscopic examination does not rule out a fungal infection.

Direct microscopy, as performed in the microbiology laboratory, most commonly relies on the use of 10–20% KOH containing the fluorophore Calcofluor white, or staining of smears, or touch preparations with either Gram or Giemsa stains (Table 6). The Calcofluor white binds to the chitin in the fungal cell wall and fluoresces blue-white or green, providing rapid and sensitive means of detecting fungi in clinical material (24) (Fig. 3). The Gram stain is useful for detection of *Candida* and *Cryptococcus* spp. (Figs. 4–6) and also stains hyphal elements of molds such as *Aspergillus* (Fig. 7), *Fusarium*, and the Zygomycetes (Figs. 8 and 9). Fungi are typically Gram-positive but may appear speckled or Gram-negative (Figs. 4–10). The capsular material of *C. neoformans* often appears as an orange-red precipitate around the cells (Fig. 6). Many fungi will stain blue with the Giemsa stain but this stain is especially useful in detecting *H. capsulatum* intracellularly in bone marrow, peripheral blood, bronchoalveolar lavage (BAL) specimens, or touch preparations of lymph nodes or other tissues (Fig. 11).

The morphologic characteristics of fungi seen on direct microscopic examination include budding yeasts, hyphae, and pseudohyphae. The combination of budding yeast cells and pseudohyphae is characteristic of *Candida* (Figs. 1–5); however, these structures may also be seen with *Trichosporon* and *Geotrichum* (Table 7).

Among the molds, *Aspergillus* spp. typically shows hyaline dichotomous acute angle branching septate hyphae (Fig. 12); however, this appearance is also typical of other hyaline molds such as *Fusarium*, *Acremonium*, *Paecilomyces*, *Trichoderma*, and *Scedosporium* (Table 7). In contrast, the Zygomycetes (e.g., *Rhizopus*, *Mucor*)
Table 6 Methods and Stains Available for Direct Microscopic Detection of Fungal Elements

<table>
<thead>
<tr>
<th>Method/stain</th>
<th>Use</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcian blue stain</td>
<td>Detection of <em>C. neoformans</em> in CSF</td>
<td>Rapid (2 min); insensitive and not commonly used</td>
</tr>
<tr>
<td>Calcofluor white stain</td>
<td>Detection of all fungi including <em>P. jiroveci</em></td>
<td>Rapid (1–2 min); detects fungal cell wall chitin by bright fluorescence. Used in combination with KOH. Requires fluorescent microscope and proper filters. Background fluorescence may make examination of some specimens difficult</td>
</tr>
<tr>
<td>Fluorescent monoclonal antibody treatment</td>
<td>Examination of respiratory specimens for <em>P. jiroveci</em></td>
<td>Sensitive and specific method for detecting the cysts of <em>P. jiroveci</em>. Does not stain the extracystic (trophozoite) forms</td>
</tr>
<tr>
<td>Fontana–Masson Stain</td>
<td>Melanin stain for histologic sections</td>
<td>Confirms the presence of melanin in lightly pigmented cells of dematiaceous fungi when present in tissue sections. Useful for distinguishing <em>C. neoformans</em> (positive) from most other yeasts (e.g., <em>Candida</em> spp. are negative for melanin).</td>
</tr>
<tr>
<td>Giemsa stain</td>
<td>Examination of bone marrow, peripheral smears, touch preparations, and respiratory specimens</td>
<td>Detect intracellular <em>H. capsulatum</em> and both intracystic and extracystic (trophozoite) forms of <em>P. jiroveci</em>. Does not stain cysts of <em>P. jiroveci</em>. Does stain organisms other than <em>H. capsulatum</em> and <em>P. jiroveci</em></td>
</tr>
<tr>
<td>Gram stain</td>
<td>Detection of bacteria and fungi</td>
<td>Rapid (2–3 min); commonly performed on clinical specimens. Will stain most yeasts and hyphal elements. Most fungi stain Gram-positive, but some, such as <em>C. neoformans</em>, exhibit stippling or appear Gram-negative</td>
</tr>
<tr>
<td>Hematoxylin and eosin stain</td>
<td>General purpose histologic stain</td>
<td>Best stain to demonstrate host reaction in infected tissue. Stains most fungi, but small numbers of organisms may be difficult to differentiate from background. Useful in demonstrating natural pigment in dematiaceous fungi</td>
</tr>
<tr>
<td>India ink treatment</td>
<td>Detection of encapsulated yeasts</td>
<td>Rapid (1 min); insensitive (40%) means of detecting <em>C. neoformans</em> in CSF</td>
</tr>
<tr>
<td>KOH treatment</td>
<td>Clearing specimens of cellular debris to make fungi more visible</td>
<td>Rapid (5 min); some specimens may be difficult to clear and require an additional 5–10 min. May produce confusing artifacts. Most useful when combined with Calcofluor white</td>
</tr>
<tr>
<td>Methenamine silver stain (GMS)</td>
<td>Detection of fungi in histologic sections and <em>P. jiroveci</em> cysts in respiratory specimens</td>
<td>Staining of tissue may take up to 1 hr. Respiratory specimens more rapid (5–10 min). Best stain for detection of all fungi. Usually performed in cytopathology laboratory</td>
</tr>
<tr>
<td>Mucicarmine stain</td>
<td>Histopathologic stain for mucin</td>
<td>Useful for demonstrating capsular material of <em>C. neoformans</em>. May also stain the cell walls of <em>B. dermatitidis</em> and <em>Rhinosporidium sieberi</em>.</td>
</tr>
<tr>
<td>Papanicolaou stain (PAP)</td>
<td>Cytologic stain used primarily to detect malignant cells</td>
<td>Stains most fungal elements; yeasts &gt; hyphae. Allows cytologist to detect fungal elements</td>
</tr>
<tr>
<td>PAS stain</td>
<td>Histologic stain for detection of fungi</td>
<td>Stains both yeasts and hyphae in tissue. <em>Blastomyces dermatitidis</em> may appear pleomorphic. PAS-positive artifacts may resemble yeast cells</td>
</tr>
<tr>
<td>Toluidine blue stain</td>
<td>Examination of respiratory specimens for <em>P. jiroveci</em></td>
<td>Stains <em>P. jiroveci</em> cysts a purple color. Does stains other fungi. Largely displaced by fluorescent antibody and Calcofluor white treatments</td>
</tr>
<tr>
<td>Wright stain</td>
<td>Examination of bone marrow, peripheral smears, and touch preparations</td>
<td>Similar to Giemsa stain. Detects intracellular <em>H. capsulatum</em></td>
</tr>
</tbody>
</table>

*Source*: Adapted from Refs. 16, 24.
<table>
<thead>
<tr>
<th>Fungus</th>
<th>Microscopic morphologic features in clinical specimens</th>
<th>Macroscopic</th>
<th>Microscopic</th>
<th>Additional tests for identification</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida</em> spp.</td>
<td>Oval, budding yeasts 2–6 μm in diameter. Pseudohyphae and hyphae may be present. (Figs. 1–5)</td>
<td>Variable morphology. Colonies usually pasty, white to tan, and opaque. May have smooth or wrinkled morphology. Some colonies produce fringes of pseudohyphae at periphery.</td>
<td>Clusters of blastoconidia, pseudohyphae, and/or terminal chlamydospores in some species</td>
<td>Germ-tube production by <em>C. albicans</em>, <em>C. dubliniensis</em>, and <em>C. stellatoidea</em>. Carbohydrate assimilation. Morphology on cornmeal agar. Colony color on CHROMagar</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>Spherical budding yeasts of variable size, 2–15 μm. Capsule may be present. No pseudohyphae or hyphae (Figs. 6 and 22)</td>
<td>Colonies are shiny, mucoid, dome shaped, and cream to tan in color</td>
<td>Budding spherical cells of varying size. Capsule present. No pseudohyphae. Cells may have multiple narrow-based buds</td>
<td>Tests for urease (+), phenoloxidase (+), and nitrate reductase (−). latex agglutination or EIA test for polysaccharide antigen. Carbohydrate assimilation. Mucicarmine and melanin stains in tissue.</td>
</tr>
<tr>
<td><em>Trichosporon</em> spp.</td>
<td>Hyaline arthroconidia, blastoconidia, and pseudohyphae 2–4 by 8 μm</td>
<td>Colonies are variably smooth and shiny to membranous, dry, and cerebriform</td>
<td>Hyphae, pseudohyphae, blastoconidia, and arthroconidia. No chlamydospores</td>
<td>Carbohydrate assimilation and biochemical tests</td>
</tr>
<tr>
<td><em>Malassezia</em> spp.</td>
<td>Small oval budding yeasts. “Bowling pin” appearance with collarette (Fig. 23). Both hyphal and yeast forms may be seen in skin scrapings</td>
<td>Slow-growing colonies. May require fatty acid source (olive oil) for growth</td>
<td>Small oval budding cells with collarette. Rudimentary hyphae</td>
<td>Species may be differentiated by lipid requirement: <em>M. furfur</em> and <em>M. sympodialis</em>, positive; <em>M. pachydermatis</em>, negative. <em>Malassezia furfur</em> will grow in 10% Tween 20, whereas <em>M. sympodialis</em> will not</td>
</tr>
<tr>
<td><em>Aspergillus</em> spp.</td>
<td>Septate, dichotomously branched hyphae of</td>
<td>Varies with species. Colonies of <em>A. fumigatus</em> usually blue-green to black in color</td>
<td>Varies with species. Conidiophores with</td>
<td>Identification based on microscopic and colonial morphology</td>
</tr>
<tr>
<td>Zygomycetes</td>
<td>Fusarium spp.</td>
<td>Scedosporium apiospermum (anamorph, asexual stage; Pseudallescheria boydii is the teleomorph or sexual stage)</td>
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<tr>
<td>uniform width (3–6 μm). Conidial heads may be seen in cavitary lesions (Figs. 7 and 12)</td>
<td>Colonicis rapid growing, wooly, and gray-brown to gray-black in color</td>
<td>Hyaline, branching septate hyphae. Angioinvasion is common.</td>
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<tr>
<td>Conidia heads may be seen in cavitary lesions (Figs. 7 and 12)</td>
<td>Colonies are purple, lavender, or rose-red with rare yellow variants</td>
<td>Both macro- and microconidia may be present. Macroconidia are multicelled and sickle- or boat-shaped</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gray-green; A. flavus yellow-green; A. niger black; other species vary widely</td>
<td>Colonies are rapid growing, wooly, and gray-brown to gray-black in color</td>
<td>Single-celled brownish conidia produced at the tips of annellides (S. apiospermum). Cleistothecia containing ascospores may be produced (P. boydii)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyphae are hyaline and septate</td>
<td>Identification based on microscopic morphologic features.</td>
<td>Identification based on microscopic and colonial morphology. May be confused with Aspergillus spp. in tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enlarged vesicles covered with flask-shaped metulae or phialids. Hyphae are hyaline and septate</td>
<td>Broad, ribbon-like hyphae with rare septa and irregular sides. Sporangium or sporangiola produced from sporangiophore. Rhizopus spp.: rhizoids at base of sporangiophore (Fig. 9)</td>
<td>Identification based on microscopic and colonial morphology. May be confused with Aspergillus spp. in tissue</td>
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<tr>
<td><em>Scedosporium prolificans</em></td>
<td>Hyaline, branching septate hyphae</td>
<td>Wooly, gray to dark brown. Does not grow on cycloheximide-containing medium</td>
<td>Inflated conidiophores</td>
<td>Based on morphologic appearance. <em>Scedosporium prolificans</em> does not have a known sexual stage</td>
</tr>
<tr>
<td><em>Dematiaceous fungi</em> (e.g., <em>Alternaria, Cladosporium, Curvularia</em>) (Table 1)</td>
<td>Pigmented (brown, tan, or black) hyphae, 2–6 (\mu m) wide. May be branched or unbranched. Often constricted at the point of septation</td>
<td>Colonies are usually rapidly growing, wooly, and gray, olive, black, or brown in color</td>
<td>Varies considerably depending on genus and species. Hyphae are pigmented. Conidia may be single or in chains, smooth or rough and dematiaceous</td>
<td>Identification based on microscopic and colonial morphology</td>
</tr>
<tr>
<td><em>Histoplasma capsulatum</em></td>
<td>Small (2–4 (\mu m)) budding yeasts within macrophages (Figs. 11 and 18)</td>
<td>Colonies are slow growing and white or buff-brown in color (25°C). Yeast phase colonies [37°C] are smooth, white, and pasty.</td>
<td>Thin septate hyphae that produce tuberculate macroconidia and smooth-walled micro conidia (25°C). Small oval budding yeasts produced at 37°C</td>
<td>Demonstration of temperature-regulated dimorphism by conversion from mold to yeast phase at 37°C. Exoantigen and DNA probe tests</td>
</tr>
<tr>
<td><em>Coccidioides immitis</em></td>
<td>Spherical, thick-walled spherules, 20–200 (\mu m). Mature spherules contain small, 2–5 (\mu m) endo spores. Arthroconidia and hyphae may form in cavitary lesions. (Fig. 17)</td>
<td>Colonies initially appear moist and glabrous, rapidly becoming downy and gray-white with a tan or brown reverse</td>
<td>Hyaline hyphae with rectangular arthroconidia separated by empty disjunctor cells</td>
<td>Exoantigen and nucleic acid probe tests</td>
</tr>
</tbody>
</table>
**Blastomyces dermatitidis**

- Large (8–15 μm) thick-walled budding yeast cells. The junction between mother and daughter cells is typically broad-based. Cells may appear multinucleate (Figs. 10 and 19).
- Colonies vary from membranous yeast-like colonies to cottony white mold-like colonies at 25°C. When grown at 37°C, yeast phase colonies are wrinkled, folded and glabrous.
- Hyaline, septate hyphae with one-celled smooth conidia (25°C). Large thick-walled budding yeast at 37°C
- Demonstration of temperature-regulated dimorphism; exoantigen and DNA probe tests.

**Sporothrix schenckii**

- Yeast-like cells of varying sizes. Some may appear elongated or “cigar-shaped”. Tissue reaction forms asteroid bodies (Fig. 21).
- Colonies initially smooth, moist, and yeast-like becoming velvety as aerial hyphae develop (25°C). Tan to brown pasty colonies at 37°C.
- Thin branching septate hyphae Conidia borne in rosette-shaped clusters at the end of the conidiophore (25°C). Variable sized budding yeast at 37°C.
- Demonstration of thermal dimorphism; exoantigen and DNA probe.

**Penicillium marneffei**

- Oval, intracellular yeast cells with septum.
- Colonies produce diffusible red pigment at 25°C.
- Septate hyphae with metulae, phialides with chains of conidia in a “paint-brush” distribution (25°C). Yeast cells divide by fission (37°C).
- Demonstration of thermal dimorphism

**Pneumocystis jiroveci**

- Cysts are round, collapsed, or crescent shaped. Trophozoites seen on special stains. (Figs. 14–16).
- Not applicable
- Not applicable
- Immunofluorescent stain, GMS, Giemsa, Toluidine blue stains (Table 6)
Figure 1  *Candida albicans* demonstrating budding yeasts and pseudohyphae. GMS stain. Magnification, ×1000.

Figure 2  *Candida glabrata* demonstrating small budding yeasts without pseudohyphae. GMS stain. Magnification, ×1000.
Figure 3  *Candida tropicalis* in CSF stained with Calcofluor white. Magnification, ×1000.

Figure 4  *Candida parapsilosis* from a central nervous system shunt. Budding yeasts and pseudohyphae visualized on Gram stain. Magnification, ×1000.
Figure 5  *Candida tropicalis* from a blood culture. Budding yeasts and hyphae seen on Gram stain. Magnification, ×1000.

Figure 6  *Cryptococcus neoformans* in CSF. Variable-sized encapsulated budding yeasts seen on Gram stain. Note stippling because of uneven retention of crystal violet stain. Magnification, ×1000.
Figure 7  *Aspergillus versicolor* detected by Gram stain in a tracheal aspirate. Although often Gram-positive, this specimen did not retain the crystal violet and appears Gram-negative. Magnification, ×1000.

Figure 8  Hyphal fragment of *Rhizopus* spp. in pleural fluid demonstrating characteristic broad aseptate hypha that folds back on itself. Gram stain. Magnification, ×1000.
Figure 9  Hyphal fragment of *Rhizopus* spp. demonstrating root-like rhizoids. Gram stain. Magnification, ×1000.

Figure 10  Biopsy specimen demonstrating budding yeast cells of *B. dermatitidis*. H&E stain. Magnification, ×1000.
Figure 11  Macrophages containing numerous intracellular yeast forms of *H. capsulatum*. Giemsa stain. Magnification, ×1000.

Figure 12  (A) GMS stain showing dichotomously branching septate hyphae characteristic of *Aspergillus* spp. Magnification, ×1000. (B) GMS stain showing the branching septate hyphae of *Trichoderma longibrachiatum* that are indistinguishable from that of *Aspergillus* spp. Magnification, ×1000.
characteristically show broad ribbon-like sparsely septate or aseptate hyphae (Figs. 8 and 13). Finally, the dematiaceous fungi often present as darkly pigmented yeast-like and hyphal forms that may be visualized on unstained material and further characterized by the Fontana–Masson stain for melanin (Tables 6 and 7).

Although the cysts and/or intracystic bodies of *P. jiroveci* may be detected in induced sputum or BAL fluid following staining with Giemsa (Figs. 14 and 15), Gomori's methenamine silver (GMS) (Fig. 16), or toluidine blue stains, the commercial availability of fluorescent monoclonal antibody-based conjugates has enhanced the detection of this organism and provides a sensitive and highly specific diagnosis (35).

C. Histopathology

Histopathology and cytopathologic examination of tissue, fine-needle aspirates, or cytologic preparations employ a variety of stains designed to illustrate the cellular morphology of the host and any potential infecting organism invading the cells or tissues (Table 6). Among the more useful stains for detecting fungi in tissues are GMS stain and the Periodic Acid-Schiff (PAS) stain. In addition, fungi may be detected with hematoxylin and eosin (H&E) and Papanicolaou stains and further characterized using mucicarmine and Fontana–Masson stains (Table 6) (27,36–38). The H&E, GMS, and PAS stains can detect fungi such as *B. dermatitidis*, *H. capsulatum*, *C. immitis*, *Candida* spp., *Fusarium*, *Sporothrix*, *Cryptococcus*, and the hyphae of Zygomycetes (e.g., *Rhizopus, Mucor*), as well as *Aspergillus* spp. (Table 6) (Figs. 17–22). Although most fungi may be visualized in tissue stained with H&E when present in large numbers, *Candida* and *Aspergillus* species do not stain well and may be missed in H&E-stained tissue sections. In order to detect small numbers of organisms and to clearly define the morphologic features of the infecting organism, special stains such as GMS and PAS must be used. The Fontana–Masson stain is used to stain for melanin in the fungal cell wall. It is useful in highlighting lightly pigmented dematiaceous fungi and for differentiating capsule-negative strains of *C. neoformans* (positive for melanin) from other yeasts (negative for melanin) in tissue (27). The mucicarmine stain is also used to identify *C. neoformans* in tissue by staining the polysaccharide capsule of the fungus. When available, specific immunofluorescent stains may be quite helpful not only in detection of fungal elements, but also in confirming a presumptive histologic identification of fungi, such as *Aspergillus*, *Candida*, *Cryptococcus*, the dimorphic fungi, and others (39). In situ hybridization with specific molecular probes may also be used more in the future (40). Finally, histologic examination of fixed tissue serves as an aid in determining infection (deep penetration into viable tissue) vs. colonization (superficial involvement of nonviable tissue) (27). A description of the microscopic morphologic features of several of the etiologic agents is presented in Table 7.

D. Culture

The most-sensitive means of diagnosing a fungal infection is generally considered the isolation of the infecting agent on culture media. In most instances, culture is necessary to specifically identify the etiologic agent and, if indicated, to determine the susceptibility to various antifungal agents.

Multiple factors must be considered in order to optimize the recovery of fungi from clinical material. In addition to adequate specimen procurement, a combination of culture media should be used to ensure the isolation of a broad array of medi-
Figure 13  Tissue biopsy showing *Rhizopus* spp. stained with H&E. The variation in shape and size of hyphae is evident. Magnification, ×1000.

Figure 14  Giemsa-stained BAL cytopreparation. Mature *P. jiroveci* cysts containing intra-cystic forms with purple nuclei and light blue cytoplasm are seen. The cyst wall is unstained and appears as a clear rim. Magnification, ×1000.
Figure 15  Clump of *P. jiroveci* stained with Giemsa. Both extracystic and intracystic forms are evident. Magnification, ×1000.

Figure 16  GMS stain of BAL fluid demonstrating cysts of *P. jiroveci (carinii)*. Both intact and collapsed cysts are present. Extracystic forms (clump) are not stained. Magnification, ×1000.
Figure 17  A mature spherule of *C. immitis* in lung tissue. PAS stain. Magnification, ×500.

Figure 18  Silver stain demonstrating small budding yeast forms of *H. capsulatum*. Note the similarity in size and shape to *C. glabrata* shown in Figure 2.
**Figure 19**  Broad-based budding yeasts of *B. dermatitidis* in a cytological preparation. PAS stain. Magnification, ×1000.

**Figure 20**  Tissue biopsy showing the branching septate hyphae of *Fusarium* spp. GMS stain. Magnification, ×1000.
Figure 21  PAS stain of tissue demonstrating the variable size and shape of *Sporothrix schenckii* yeast cells. Magnification, ×1000.

Figure 22  *Cryptococcus neoformans* in skin biopsy stained with PAS. Note variation in size and capsular artifact surrounding yeast cells. Magnification, ×1000.
cally important fungi. Generally, both selective and nonselective media are used for primary recovery of fungi from clinical specimens. Nonselective media such as brain heart infusion (BHI) agar or Sabouraud glucose plus BHI (SABHI) agar will permit the recovery of both rapidly growing molds and yeasts, as well as the more slowly growing, or fastidious fungi. Sabouraud glucose agar is generally considered inferior to these media for primary isolation and should not be used. Routine bacteriologic media, such as sheep blood agar and chocolate agar will support the growth of most fungi; however, growth may be slow and not detectable in the short time period (3–5 days) allowed for the incubation of most bacterial cultures. A blood-containing medium such as BHI, with 5–10% sheep blood, may be used in addition to the nonselective primary isolation media as an aid in recovering fastidious dimorphic fungi such as \textit{H. capsulatum} and \textit{B. dermatitidis}. Although cycloheximide is often added to BHI-blood agar to inhibit the growth of rapidly growing yeasts and molds that may “contaminate” the specimen, it is important to realize that this agent can inhibit the growth of many opportunistic pathogens that may also be the etiologic agent of the infection. For this reason, media supplemented with cycloheximide must always be complemented by the same media without the inhibitory agent. Specimens that may be contaminated with bacteria should also be cultured on a selective medium such as inhibitory mold agar, SABHI, or BHI plus antibacterial agents (e.g., gentamicin, chloramphenicol, or ciprofloxacin plus streptomycin). CHROMagar (Hardy Diagnostics, Santa Maria, California, U.S.A.) is a recently developed medium that is both selective for fungi and differential for certain \textit{Candida} species (41,42). This medium inhibits bacterial growth and because different species of \textit{Candida} appear as different colored colonies, it is useful in detecting mixed cultures of \textit{Candida} and other fungi (42). In certain situations, it is necessary to utilize specialized media for recovery of specific fungi. For example, medium supplemented with olive oil is necessary for recovery of \textit{Malassezia furfur} (Fig. 23), and the use of malt agar, or even sterile bread without preservatives, will enhance the isolation of the Zygomycetes.

Although not all serious fungal infections are marked by hematogenous dissemination and fungemia, detection of fungemia is useful in diagnosing opportunistic infection caused by \textit{Candida} spp., \textit{C. neoformans}, \textit{Trichosporon} spp., \textit{Malassezia} spp., \textit{Fusarium} spp., and occasionally \textit{Acremonium} spp. (23,26,43). Blood cultures may be negative in the face of disseminated disease; however, advances in blood culture technology have markedly improved the ability of laboratories to detect fungemia (43,44). The lysis centrifugation method (Isolator, Wampole, Dayton, New Jersey, U.S.A.) and the continuous monitoring automated blood culture systems are all sensitive methods for the detection of fungemia caused by \textit{Candida} spp. (43). The development of specialized broth media containing lytic agents, resins, charcoal, or diatomaceous earth coupled with continuous agitation has contributed to the improved performance of the broth-based systems (43). However, recovery of \textit{C. neoformans}, \textit{H. capsulatum}, \textit{M. furfur}, and \textit{Fusarium} spp. may be inferior to broth-based methods compared with results of the lysis centrifugation method (43–45). Optimal detection of fungemia requires the collection of adequate volumes (20–30 mL) of blood and the use of both a broth-(vented, agitated) and an agar-based (lysis centrifugation) blood culture method (16,26).

Interpretation of the results of fungal cultures may be difficult because of the issues of colonization of certain body sites (e.g., respiratory, GI, and GU tracts) and contamination of specimens or cultures by environmental organisms many of which can also serve as etiologic agents of opportunistic mycoses. While most isolates of \textit{Candida}, \textit{C. neoformans}, \textit{H. capsulatum}, and \textit{Fusarium} obtained from blood
cultures are clinically significant (26,43,44), others such as *Aspergillus* spp. (not *A. terreus*) and *Penicillium* spp. (not *P. marneffei*) are most probably contaminants (46). Direct visualization of the organism in tissue helps to confirm the clinical importance of the isolation of *Aspergillus* from cultures obtained from the respiratory tract (25). However, for high-risk patients, such as allogeneic BMT recipients, individuals with hematologic malignancies, and those with neutropenia or malnutrition, a positive culture alone that yields *Aspergillus* spp. is associated with invasive disease (25) (Table 8). Specific identification of fungi isolated from culture can often

### Table 8  Risk of IA Among Patients in Different Risk Categories Whose Cultures were Positive for *Aspergillus* spp.

<table>
<thead>
<tr>
<th>Risk category</th>
<th>% Risk of IA (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57 (50–64)</td>
</tr>
<tr>
<td>Intermediate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15 (8–28)</td>
</tr>
<tr>
<td>Low&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;1 (0–1)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Includes allogeneic BMT, neutropenia, and hematologic cancer patients.

<sup>b</sup>Includes autologous BMT, malnutrition, corticosteroids, HIV, solid-organ transplant, diabetes, underlying pulmonary disease, and solid-organ cancer patients.

<sup>c</sup>Includes cystic fibrosis and connective tissue disease patients.

*Source:* Adapted from Ref. 25.
help in determining clinical significance: *A. niger* is rarely a pathogen, whereas *A. terreus* and *A. flavus* have been shown to be statistically associated with IA when isolated from respiratory tract cultures (25). Likewise, *H. capsulatum, B. dermatitidis*, and *C. immitis* are virtually always considered to be pathogens.

E. Identifying Characteristics of Fungi

Identification of fungi to genus and species is increasingly important as the spectrum of opportunistic pathogens continues to expand. Although the clinical presentation of many fungal infections may be indistinguishable, specific identification of the etiologic agent may have a direct bearing on the prognosis and therapeutic considerations. Now, more than ever it is clear that a single approach (e.g., using amphotericin B) for the management of all fungal infections is inadequate for many of the invasive mycoses (47,48). Furthermore, the identification of fungal pathogens may have additional diagnostic and epidemiologic implications. In the case of the more unusual mycoses, specific identification of the etiologic agent may provide access to the literature and the experience of others regarding the clinical course of infection and response to therapy.

The first and most basic step in the identification process is to differentiate yeasts from molds. Visual inspection of colonies growing on agar provides the first clue; yeasts usually produce pasty opaque colonies, whereas molds form large filamentous or “hairy” colonies with variations in texture, color, and topography. Microscopic examination with visualization of budding cells and pseudohyphae (yeasts) or filamentous hyphae and fruiting bodies (molds) further delineates these two large groups. Depending on the fungus, identification to the level of genus and species usually requires more detailed morphologic characterization supplemented by specific biochemical and physiologic tests (49) (Table 7). Increasingly, definitive identification of fungi is accomplished by immunologic and molecular characterization (50,51).

Yeasts are usually characterized morphologically as single cells that reproduce by simple budding; however, under certain conditions some yeasts may form true hyphae, pseudohyphae, capsules, arthroconidia, and other reproductive structures (49). Colonies form on most agar media within a few (2–5) days and are usually round, opaque, moist or mucoid, and white or cream colored. Because *C. albicans* constitutes the vast majority of yeasts recovered from clinical specimens, several rapid and simple tests have been devised to distinguish it from other yeasts (52). The most widely used test for the identification of *C. albicans* is the germ tube test. *Candida albicans* forms germ tubes in 3 hr when incubated in serum or plasma at 35°C. Other *Candida* species are capable of germ-tube formation but require extended incubation. *Candida dubliniensis* and *C. stellatoidea* are capable of forming germ tubes within 3 hr and may be difficult or impossible to differentiate from *C. albicans* without performing additional physiologic, immunologic, or nucleic acid-based testing (53–55). More recently, a rapid colorimetric test based on the detection of *C. albicans*-specific enzymes (l-proline aminopeptidase and β-galactosaminidase) or the use of agar medium containing chromogenic substrates (CHROMagar Candida, Hardy Diagnostics, Santa Maria, California, U.S.A.) has proven to be useful in the rapid presumptive identification of yeasts (41,42,56,57). Although a single presumptive identification test is not sufficient for identifying most yeasts, a positive germ tube, colorimetric test, characteristic green colony on CHROMagar medium is generally considered to be acceptable for the identification of *C. albicans*.
Importantly, although the appearance of small hyphal projections or “feet” from the edge of a colony has been cited as characteristic of *C. albicans* (52,58,59), further investigation has shown that both *C. tropicalis* and *C. krusei* are also capable of producing this phenotype (60).

Although more than 100 species of *Candida* have been identified, 95–98% of invasive candidal infections are caused by only five species: *C. albicans* (56%), *C. glabrata* (16%), *C. parapsilosis* (13%), *C. tropicalis* (10%), and *C. krusei* (3%) (3,61,62). Recent reports suggest that shifts have occurred in the distribution of non-*albicans* species with the emergence of *C. glabrata*, *C. krusei*, *C. lusitaniae*, and other less common species (16,61,63). Infections with these various species may require different therapeutic considerations (64,65a), and so further identification of all germ tube-negative or colorimetric test-negative yeasts is mandatory for isolates obtained from blood and other normally sterile body fluids (64,65a). Due to the pathogenic potential of *C. neoformans*, all encapsulated yeasts from any body site should also be identified. There are several rapid screening tests that may be used for the presumptive identification of *C. neoformans* including the urease test (positive), nitrate test (negative), and production of phenol oxidase (positive) (49).

Further identification of germ tube-negative yeasts to species requires the determination of biochemical and physiologic profiles and an assessment of their morphology when grown on a medium such as cornmeal agar or yeast morphology agar (49). In addition to the identification of *C. albicans*, colony morphology on CHROMagar allows the presumptive identification of *C. tropicalis* and *C. krusei* (41,42). Likewise, *C. glabrata* may be identified by a rapid trehalose test (49) or by differential growth on blood (no growth or slow growth) vs. eosin methylene blue (rapid growth) agar (66). Carbohydrate assimilation tests provide definitive identification of most of these species and may be performed by using one of the several commercial identification systems (55). Differentiation of yeasts with similar biochemical profiles can be accomplished by observing their microscopic characteristics on cornmeal agar (Table 7).

In contrast to yeasts, the identification of molds is largely based upon morphologic features such as gross colony appearance and microscopic morphology (Table 7). Visible growth on agar media may be obtained within 1–5 days for the Zygomycetes, most hyaline (light colored hyphae and conidia) hyphomycetes, and some, but not all, dematiaceous (dark pigmented hyphae and conidia) fungi. In contrast, the dimorphic fungi (*H. capsulatum*, *B. dermatitidis*, *C. immitis*, *Sporothrix schenckii*, and *Paracoccidioides brasiliensis*) grow much more slowly and may require 2–4 weeks of incubation. Furthermore, the dimorphic fungi are not inhibited by cycloheximide, an agent that inhibits the growth of most of the more rapidly growing molds that may represent either clinically unimportant “contaminants” or opportunistic pathogens causing infections in immunocompromised hosts.

The macroscopic appearance of many filamentous fungi when growing on solid media may provide clues as to the identification of the fungus. Variations in colonial morphology that may be medium- or strain-specific preclude the use of this feature as the sole criterion for identification. Surface texture, topography, color, reverse pigmentation, growth at 37°C, and requirements for specific vitamins are all useful characteristics. Potato glucose agar and cornmeal agar are considered two of the more reliable media for assessment of gross colonial morphology. Color development may be dependent on exposure to light.

Definitive identification of most molds is dependent upon visualization of the microscopic morphology of the fungus. Key morphologic features include the size,
the shape, the method of production, and the arrangement of the conidia or spores, as well as the size and appearance of the hyphal structures. Material must be prepared for microscopic examination in such a way as to minimize any disruption of the relationship of the conidia or spores to their respective reproductive structures. This is usually best accomplished by the use of slide cultures whereby a cover slip is placed on the agar in such a way that the fungus spreads over the glass surface. The cover slip is then removed and placed on a slide for examination under the microscope. Determination of melanin and temperature-regulated dimorphism are also important characteristics. The dimorphic pathogens may also be characterized by immunologic- or nucleic acid probe–based methods in addition to morphology and thermal dimorphism. The typical features of selected filamentous and dimorphic pathogens are listed in Table 7.

F. Role of Surveillance Cultures

Fungal surveillance cultures of immunocompromised patients have been studied as potential predictors of invasive or disseminated mycoses (67–71). Although active surveillance of high-risk patients may enhance case detection of invasive candidiasis and aspergillosis, the data are quite variable (11,69,70,72–80). In the light of this problem, guidelines for the development of microbiologic surveillance protocols have been published and include (67,71,81) consideration of (i) the probability and severity of a specific infection in the patient population; (ii) the time period in which the risk of infection exists relative to the onset of immunosuppression; (iii) the accuracy, timeliness, and the cost of the tests used for surveillance; (iv) the sensitivity, specificity, positive (PPVs) and negative predictive values of a test result in an asymptomatic patient; (v) the efficacy of prophylactic therapy and the risk of development of resistance; and (vi) the expected impact on the clinical outcome.

It is generally acknowledged that the primary reservoir for hematogenously disseminated candidiasis is the GI tract and, to a lesser extent, the GU system (82). As such, one might expect that those immunocompromised patients with demonstrated colonization of several anatomic sites by Candida spp. to be at increased risk of invasive candidiasis compared with similar patients without colonization (69,78,83–85). Although several studies have shown this to be the case, it is clear that colonization without infection is very common, with the result that the PPV of a positive surveillance culture for Candida is quite low (11,69,71,75). Conversely, negative surveillance cultures may be helpful because individuals without colonization by Candida spp. rarely develop candidiasis (11,69,70,75).

The PPV of surveillance cultures for Candida may improve when species such as C. tropicalis, C. glabrata, or C. krusei are found colonizing high-risk patients (69,70,86). Detection of C. glabrata and C. krusei as colonizing agents may be of additional importance given that these species are typically less susceptible to azoles and other systemically active antifungal agents than C. albicans (3,63,86), thus prompting a change in empirical antifungal coverage for patients colonized by those species (64).

The poor sensitivity of routine nasal and respiratory cultures for Aspergillus spp. limits the usefulness of surveillance cultures for early detection of IA (25,67). The isolation of Aspergillus from respiratory specimens is often considered to represent contamination or colonization (67); however, it is now well established that a positive respiratory culture for Aspergillus in a host at high risk for IA is very
suggestive of invasive disease and is an indication for more aggressive attempts at
diagnosis and early empiric antifungal therapy (25,73,74,76,80).

The performance of surveillance cultures for Candida and Aspergillus may have
some value in highly selected patient groups. However, the use of routine surveillance
cultures of asymptomatic immunocompromised patients remains of questionable
value and generally should be discouraged (71).

Environmental surveillance for Aspergillus and other molds is another contro-
versial area (71). Clearly, environmental monitoring should be performed in an out-
break setting in an effort to detect and eliminate the source of infection (68,71,87,88).
Beyond that specific situation, there are only a few additional indications for environ-
mental surveillance (68,87). Specifically, air-quality monitoring to detect Aspergil-
lus spores in air should be performed: (i) when clinical surveillance demonstrates a
possible increase in mold infections; (ii) during periods of construction or renovation
that take place near high-risk areas; (iii) before patients enter a new protected area or
after renovation of the protected area; and (iv) when there is suspicion of dysfunc-
tion in the quality of air systems (68,87). Many centers also perform routine periodic
air cultures (in addition to particulate counts) in protected environments to monitor
the effectiveness of high-efficiency particulate air filters.

Unfortunately, specific standards for air-quality monitoring have not been
established. A variety of air-sampling techniques exist. Although each may have
its own specific merits, the preferred method for use in the hospital setting is based
on filtration and the impacting of particles (spores) from an air stream onto an agar
surface. Once the air has been sampled, the number of colony forming units (CFUs)
per cubic millimeter can be determined; however, interpretation of this number is
also problematic because of a lack of agreement over the specific threshold of
CFU per cubic millimeter above which the risk of IA is increased (89). Counts of
< 0.1–1 CFU/mm³ are desirable; however, most experts recommend counts of
< 5 CFU/mm³ in protected isolation units and selected operating rooms (68). It
should be emphasized that such air sampling is just an aspect of a more comprehen-
sive program of prevention (87). Routine air sampling in the absence of a specific
problem generally should be discouraged (71).

V. ANTIFUNGAL SUSCEPTIBILITY TESTING

The field of antifungal susceptibility testing has progressed and matured over the
past 15–20 years. In vitro susceptibility tests with antifungal agents are performed
for the same reasons that tests with antibacterial agents are performed (65a,90–
92): (i) to provide a reliable estimate of the relative activities of two or more agents;
(ii) to correlate with in vivo activity and predict the likely outcome of therapy; (iii) to
provide means by which to monitor the development of resistance among a normally
susceptible population of organisms; and (iv) to predict the therapeutic potential of
newly discovered investigational agents.

At the present time, the state of the art for susceptibility testing of yeasts is
comparable to that of bacteria (65a,90–92). Standardized methods for performing
antifungal susceptibility testing are reproducible, accurate, and available for use in
clinical laboratories (93–95). The establishment of quality-control guidelines and
interpretative criteria for a limited number of antifungal agents (Table 9) provides
the basis for the application of this testing in the clinical laboratory (65a,90–92).
Establishing a clinical correlation between in vitro susceptibility tests and clinical outcome has been difficult; however, it is now clear that antifungal susceptibility testing can predict outcome in several situations including candidemia and mucosal infections (Table 10)(65a,91). The National Committee for Clinical Laboratory Standards (NCCLS) Sub-Committee on Antifungal Susceptibility Tests has

<table>
<thead>
<tr>
<th>Antifungal Agent</th>
<th>Interpretive breakpoints (μg/ml)</th>
<th>Comments (ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluconazole</td>
<td>$\leq 8$ $16-32$ $\geq 64$</td>
<td>NCCLS M27-A2, follows 90-60 rule of clinical response (65a)$^b$</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>$\leq 0.12$ $0.25-0.05$ $\geq 1$</td>
<td>NCCLS M27-A2, follows 90-60 rule of clinical response (65a)</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>$\leq 4$ $8-16^c$ $\geq 32$</td>
<td>NCCLS M27-A2 follows 90-60 rule of clinical response (65a)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>$\leq 1$ $&gt;1$</td>
<td>Use Etest or antibiotic medium 3</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>NA NA NA 96% $\leq 2 \mu g/ml$ by NCCLS M27-A2(65b)</td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>$\leq 1$ $2$ $\geq 4$</td>
<td>98% $\leq 1 \mu g/ml$ by NCCLS M27-A2 (62)</td>
</tr>
</tbody>
</table>

$^a$Pertains to Candida spp. only.
$^b$90-60 rule: infections caused by susceptible isolates respond to appropriate therapy 90% of the time, whereas infections caused by resistant isolates (or infections treated with inappropriate antimicrobials) respond 60% of the time.

Establishing a clinical correlation between in vitro susceptibility tests and clinical outcome has been difficult; however, it is now clear that antifungal susceptibility testing can predict outcome in several situations including candidemia and mucosal infections (Table 10)(65a,91). The National Committee for Clinical Laboratory Standards (NCCLS) Sub-Committee on Antifungal Susceptibility Tests has

<table>
<thead>
<tr>
<th>Organism group</th>
<th>Number of studies</th>
<th>Number of patients</th>
<th>Cases with successful outcome % (no. of cases/total) by susceptibility class$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi$^d$</td>
<td>13</td>
<td>1,197</td>
<td>$S$ $R$ $p$ Value</td>
</tr>
<tr>
<td>Bacteria$^c$</td>
<td>12</td>
<td>5,447</td>
<td>$S$ $R$ $p$ Value</td>
</tr>
</tbody>
</table>

$^a$Antifungal testing performed according to NCCLS M27-A2.
$^b$Susceptibility to antibacterial agents determined by MIC, zone diameter, AUC/MIC ratio, or peak/MIC ratio.
$^c$Outcome measurement varied from clinical and/or microbiologic response to therapy.
$^d$Includes mucosal, fungemia, meningitis, and disseminated infections treated with fluconazole, itraconazole, or ketoconazole.
$^e$Includes bacteremia, otitis, and severe infections treated with various agents including cephalosporins, $\beta$-lactamase inhibitor combinations, aminoglycosides, and fluoroquinolones.

Source: Adapted from Ref. 65a.
<table>
<thead>
<tr>
<th>Clinical setting</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine</td>
<td>Species level identification of all <em>Candida</em> isolates from deep sites</td>
</tr>
<tr>
<td></td>
<td>Genus level identification of molds (species level preferred for <em>Aspergillus</em>)</td>
</tr>
<tr>
<td></td>
<td>Routine antifungal testing of fluconazole and flucytosine against <em>Candida</em> isolated from blood and normally sterile body fluids and tissue</td>
</tr>
<tr>
<td>Oropharyngeal candidiasis</td>
<td>Determination of susceptibility to fluconazole and itraconazole may be helpful but not routinely necessary</td>
</tr>
<tr>
<td></td>
<td>Susceptibility testing may be useful for patients unresponsive to azole therapy</td>
</tr>
<tr>
<td>Invasive disease with clinical failure of initial therapy</td>
<td>Consider susceptibility testing as an adjunct</td>
</tr>
<tr>
<td></td>
<td><em>Candida</em> species and amphotericin B</td>
</tr>
<tr>
<td></td>
<td><em>Cryptococcus neoformans</em> and fluconazole, flucytosine, or amphotericin B</td>
</tr>
<tr>
<td></td>
<td><em>Histoplasma capsulatum</em> and fluconazole</td>
</tr>
<tr>
<td></td>
<td>Consultation with an experienced microbiologist recommended</td>
</tr>
<tr>
<td>Infection with species with high rates of intrinsic or acquired resistance</td>
<td>Susceptibility testing not necessary when intrinsic resistance is known</td>
</tr>
<tr>
<td></td>
<td>Select therapy based on literature. When high rates of acquired resistance (e.g., <em>C. glabrata</em> and fluconazole) monitor closely for signs of failure and perform susceptibility testing</td>
</tr>
<tr>
<td>New treatment options (e.g., caspofungin, voriconazole) or unusual organisms</td>
<td>Select therapy based on published consensus guidelines and review of survey data on the organism–drug combination in question</td>
</tr>
<tr>
<td>Patients who respond to therapy despite being infected with an isolate later found to be resistant</td>
<td>Best approach not clear</td>
</tr>
<tr>
<td></td>
<td>Take into account severity of infection, patient immune status, consequences of recurrence of infection, etc.</td>
</tr>
<tr>
<td></td>
<td>Consider alternative therapy for infections with isolates that appear to be highly resistant to therapy selected</td>
</tr>
<tr>
<td>Mold infections</td>
<td>Susceptibility testing not recommended as a routine. Interpretive criteria have not been established</td>
</tr>
<tr>
<td>Selection of susceptibility testing method</td>
<td>Standardized methods</td>
</tr>
<tr>
<td></td>
<td>NCCLS broth-based methods</td>
</tr>
<tr>
<td></td>
<td>Yeasts; M27-A2</td>
</tr>
<tr>
<td></td>
<td>Molds; M38-A</td>
</tr>
<tr>
<td></td>
<td>Agar-based methods</td>
</tr>
<tr>
<td></td>
<td>Etest, numerous agents, yeast, and molds</td>
</tr>
<tr>
<td></td>
<td>NCCLS M44-P method for yeasts</td>
</tr>
</tbody>
</table>

Source: Adapted from Ref. 65a.
developed and published approved reference methods for broth dilution testing of yeasts (93) and filamentous fungi (94) and has proposed a standard method for disk diffusion testing of yeasts against fluconazole (62,95,96). Studies are now ongoing to further refine these methods and to examine the in vivo correlation with the in vitro data for molds (97).

Despite this progress, it remains to be seen how useful antifungal susceptibility testing will be in guiding therapeutic decision making. Guidelines for the use of laboratory studies, including antifungal testing have been developed (Table 11) (65a). Selective application of in vitro susceptibility testing, coupled with broader identification of fungi to the species level, should prove to be useful, especially in difficult to manage fungal infections (64,65a). Future efforts will be directed toward further validation of interpretative breakpoints for established antifungal agents and developing them for newly introduced systemically active agents. In addition, procedures must be optimized for testing non-*Candida* yeasts (e.g., *C. neoformans*, *Trichosporon*) and molds (90).

### VI. SUMMARY AND CONCLUSIONS

The infectious fungi now constitute one of the most important threats to the survival of immunocompromised hosts. There is little doubt that in addition to *C. albicans* and *A. fumigatus*, a vast array of fungi, previously considered to be non-pathogens, have now emerged as significant human pathogens. Recognition of these emerging fungal pathogens has resulted in a better understanding of their clinical presentation and response to the available therapeutic measures. Conventional laboratory-based methods for diagnosis of fungal infections remain useful but are often slow and lack sensitivity. Clearly, there is a need for improved diagnosis and management of these difficult infections. Newer broad-spectrum antifungal agents should prove useful but may require more sensitive methods for diagnosing the infections, as well as for estimating the extent of disease to significantly impact disease outcome. Broad application of both new and established antifungal agents may also select more resistant organisms from the vast pool of environmental opportunistic fungi. Such “emerging” fungal pathogens will pose yet another set of diagnostic and therapeutic challenges and will require that they are both visualized in tissue and identified in culture to truly define their pathogenesis and response to treatment.

### REFERENCES


I. INTRODUCTION

Fungal infections have become increasingly important in the last few decades as advances in modern medicine prolong the lives of severely debilitated patients. A wide variety of hosts with compromised host defenses are at risk for these infections. These include HIV-infected, cancer, transplant, surgical and Intensive Care Unit (ICU) patients, and also newborn infants. Neutropenia, use of broad spectrum antibiotics, indwelling catheters, immunosuppression and disruption of mucosal barriers due to chemotherapy and radiotherapy constitute some of the main predisposing factors for these opportunistic infections.

Formulating effective strategies to improve the outcome for patients with invasive fungal infections represents a formidable challenge. Making the diagnosis of invasive fungal infections early and accurately enough to allow institution of timely and effective antifungal therapy is critical, and better diagnostic tools are urgently needed to identify infected patients. However, the diagnosis of invasive fungal infections remains difficult; the lack of sensitive and specific noninvasive diagnostic tests constitutes a major impediment for therapeutic advance. The most important issue remains the need for tests or procedures that allow early detection of disease or, equally important, to provide reliable evidence for the absence of fungal infection. The diagnosis of invasive fungal infections is usually difficult to establish by clinical criteria. Moreover, noninvasive diagnostic techniques have been limited in most opportunistic fungal pathogens. Thus, conventional diagnostic tests (histology, microscopy, and culture)—reviewed in the previous chapters of this book—remain the cornerstone of proving the presence or absence of fungal disease; however, their sensitivity is low and isolation of fungi from clinical specimens take valuable time, ranging from days to weeks; therefore, their impact on clinical decisions to treat patients is often limited. Many times, microbiological cultures become positive at a late stage of infection, and delayed therapy is clearly associated with a poor
outcome. Development of methods for the detection of fungal antigens and antibodies, as well as molecular (PCR-based) methods represent promising noninvasive tools for the diagnosis of fungal infections. Because Candida and Aspergillus account for a significant number of these infections, this review focuses mainly on noninvasive diagnostic advances for invasive candidiasis and aspergillosis, and reviews the current status for nonculture based methods for other more common pathogenic and endemic mycoses.

II. SERODIAGNOSIS OF INVASIVE CANDIDIASIS

Candidiasis represents now the fourth most frequent nosocomial infection in hospitals in the US and worldwide (1–4). These infections are caused by yeast-like fungi of the genus Candida. These are normally commensal organisms found colonizing human mucous membranes. C. albicans remains the most frequent causative agent of candidiasis but other species have been increasingly associated with infections in an expanding population of immunocompromised patients (2,3).

Morbidity and mortality rates associated with invasive candidiasis remain unacceptably high (5), the main reasons being the difficulties encountered in the diagnosis and treatment of this type of infection. Development of nonculture based laboratory methods for invasive candidiasis faces the difficult challenge of differentiating normal commensalism/colonization from tissue invasion and from candidemia requiring antifungal treatment. Blood cultures for Candida species generally exhibit low sensitivity, with less than 50% of cultures from patients with disseminated disease being positive, even when lysis of centrifugation tubes is performed that reportedly increases sensitivity of the technique (6–8). To date, most of the nonculture based diagnostic techniques can be considered investigational, limited to research laboratories, with several commercial tests having ended in only limited success due to the intrinsic problems associated with the diagnosis of these infections.

A. Antibody Detection

Standard serological tests to detect anti-Candida antibodies usually have failed to discriminate between colonization and invasive candidiasis, since anti-Candida antibodies are ubiquitous in human sera, leading to poor specificity values (false positives) (9). Additionally, the tests may present a low sensitivity (false negatives) in severely immunosuppressed patients in whom the antibody response may be delayed, reduced, or altogether absent (9). Thus, despite more than 50 years of experience in this field, to date the development of useful tests based on antibody detections remains a formidable unconquered challenge.

Early studies on antibody detection for diagnosis suffered from the fact that they used mostly whole cells or crude antigenic preparations that were difficult to standardize. Additionally, most of these antigenic preparations contained cell wall mannann, and antimannann antibodies are almost ubiquitous in human sera (8,10). These early assays for antibody detection used techniques such as latex agglutination, complement fixation, immunodiffusion, counter-immunoelectrophoresis and indirect immunofluorescence. In general, the resulting tests lacked sensitivity and specificity and were found to be of limited diagnostic value (11,12).

More recent studies have attempted to improve on previous results by using purified antigens (or alternatively defined antigenic preparations) and more sensitive
techniques such as immunoblotting, radioimmunoassay, and most importantly enzyme-linked immuno sorbent assay (ELISA). Initially, there was a differentiation between antibodies against cell wall and cytoplasmic antigens; however, this differentiation is difficult because it has become evident that some “cytoplasmic” antigens can also be found as bona fide components of the cell wall (13).

Mannan is one of the main cell wall components of Candida. Most of the mannan in the wall structure is found in covalent association with proteins (mannoproteins) (10,13). As mentioned before, antimannan antibodies have been shown to be ubiquitous in human sera, presumably because the immune system can be stimulated as a result of colonization by C. albicans in the absence of disease (7–10). In any defined population, levels of antimannan antibodies are usually distributed about a mean; sera having the highest levels give positive precipitin tests when tested against cell wall mannan (14). When antimannan antibodies were measured in serially drawn sera from neutropenic patients, a frequency distribution plot showed that antibodies from patients with invasive candidiasis were elevated and tended to skew the normal distribution curve to the right. However, a clear bimodal distribution of these antibody values was not observed; thus, after establishing a cut-off value for anticell wall mannan antibodies, the best sensitivity value was about 65% (15). Sensitivities of 23–100% and specificities of 92–100% have been reported in ELISA assays for mannan detection (16). Most of these analyses considered mannan to be a single molecule, but failed to take into consideration its chemical and immunological complexity. More recently, several groups have reconsidered the diagnostic value of antimannan antibody detection. Several oligomannosidic epitopes were identified that react with antibodies in human sera (17–20). Based on these, the newer Platelia Candida antibody test (Bio-Rad, Redmond, WA) uses a standardized mannan preparation to coat ELISA plates to capture circulating antimannan antibodies in sera from patients, with reported specificity and sensitivity values of 94% and 53%, respectively (21). When performed simultaneously in combination with a mannan antigen detection test, the technique gave a specificity of 93% and a sensitivity of 80% (21,22). Similarly, van Deventer et al. (23) reported that a test measuring the antimannan antibodies was 64% sensitive and 89% specific in determining invasive candidiasis. In this report, antimannan antibody titers determined longitudinally in a group of immunocompromised patients with invasive candidiasis increased during the course of infection, as opposed to those who were only colonized.

Identification of antigens specific to the filamentous form of C. albicans could offer certain advantages, since filamentation is associated with invasive disease (24). By using an ELISA to detect antibodies against a defined cell wall extract from C. albicans germ tubes in sera previously depleted of antimannan antibodies, Navarro et al. (25) described a sensitivity of 89.2% and a specificity of 98.6% for the diagnosis of systemic candidiasis in a population of patients, including bone marrow transplant patients. Also, an indirect immunofluorescence test was developed that detected antibodies in patients’ sera directed against germ-tube specific antigens (26–28). This test was useful in the diagnosis of invasive candidiasis in different patient populations, and showed an overall sensitivity of 77–89% and a specificity of 91–100% (27,29,30). The test was also valuable for the therapeutic monitoring of patients with invasive disease.

MacDonald and Odds (31) compared the titers of antibodies to candidal secreted aspartyl proteinase (SAP) and to cytoplasmic antigens in the sera of healthy individuals, patients with candidiasis, and other hospitalized patients without candidiasis. Levels of anti-SAP antibodies in human sera were higher in patients with
candidiasis than in healthy individuals, while the antibody titer against candidal cytoplasmic antigens was similar in the two groups. Thus SAP could be a specific antigen for the serological diagnosis of candidiasis (31,32). Detection of anti-SAP antibodies for serodiagnosis has been attempted (33). Elevated levels of IgG antibodies to SAP were also observed in patients with invasive or disseminated candidiasis (34,35). However, it was reported that monitoring of anti-SAP antibodies alone was not useful for the diagnosis of invasive candidiasis (34).

Immunoblotting experiments with sera from patients suffering from systemic candidiasis showed the presence of a 47-kDa immunodominant antigen present in whole cell extracts of the fungus (36–38). This 47-kDa antigen was further identified as a heat-stable breakdown product of hsp90 with a cell wall location (39). Antibody to the 47-kDa antigen is present in serum samples from a high proportion of patients with chronic mucocutaneous candidiasis (CMC) and AIDS (36,40). Patients who recover from systemic candidiasis produce a major antibody response to the 47-kDa component, whereas fatal cases have little antibody or falling titers (36,38,41,42). In another report, Zoller et al. (43) used purified somatic antigens of *C. albicans*, including a 47-kDa component, in enzyme immunoassays for antibody detection in sera from patients with confirmed disseminated candidiasis. The assay had a sensitivity of 81.5% and a specificity of 97%. However, the identity of this 47-kDa antigen remains to be resolved, since it could actually be enolase (see below).

Despite the complex antigenic make-up and considerable heterogeneity of the antibody responses to candidal antigens in humans (9,13), several immunodominant antigens have been identified. Perhaps, the most prominent of these is the cytosolic glycolytic enzyme enolase (although it has to be noted that it has been demonstrated that enolase is also present in the cell wall of *C. albicans* (44)). Strockbine et al. (45) characterized the antigenic components in cytoplasmic extracts of *C. albicans* recognized by sera from patients with disseminated candidiasis. They found that these patients had circulating antibodies directed against a 48-kDa protein antigen, which was subsequently identified as enolase (46,47). Circulating antienolase antibodies may have potential value for the diagnosis of candidiasis (48,49). Thus, an ELISA using purified *C. albicans* enolase as target was devised to detect antibodies in sera from patients with proven candidiasis. Statistical analysis of the results obtained indicated that the assay was able to discriminate between invasive infection and simple colonization (48). However, the test suffered from low sensitivity. Using purified candidal enolase as antigen in immunoblotting experiments, another group detected antienolase IgG antibodies in serial samples drawn from 92.5% of the patients with systemic candidiasis examined with a specificity of 95% (49).

Greenfield and Jones (50,51) described a major *C. albicans* cytoplasmic antigen of a molecular mass of 54.3 kDa that was also present in *C. tropicalis* and *C. guilliermondii*. An ELISA test was developed to detect antibodies against this antigen in serum samples from patients with acute leukemia. Despite its excellent specificity (100%) unfortunately, the authors reported only a sensitivity of 21.4%, possibly indicating that patients with candidiasis often failed to produce antibodies against antigen.

**B. Antigen Detection**

To overcome problems (mainly specificity problems) with the detection of anti-*Candida* antibodies, many investigators have focused their effort in the detection of circulating *Candida* antigens (8,9,12,42). However, from experience to date, it is
clear that tests for determination of *Candida* antigens as markers for invasive disease need further fine-tuning to improve their sensitivity and specificity, so that they will be valuable in guiding clinical treatment decisions. Many times, the use of crude preparations of candidal antigens cannot be standardized enough to allow good test reproducibility among laboratories, which complicates development of such tests. Therefore, identification, characterization, and detection of defined fungal antigens may provide a suitable procedure for diagnosis of invasive-candidiasis (9,12).

Mannan is the major circulating antigen in patients with invasive candidiasis. Because antimannan antibodies are ubiquitous, mannan frequently circulates in the form of immunocomplexes, a dissociation of antigen–antibody complexes is required for optimal detection of circulating mannan (9,10,12,16). Sensitivities of 23–100% and specificities of 92–100% have been reported in ELISA assays for mannan detection (16). A commercial system to detect *Candida* mannan in serum, the Pastorex Candida test (Sanofi Diagnostics Pasteur, Mames-la-Coquette, France), appeared promising (11); however, Gutierrez et al. (52), who conducted a prospective clinical trial, found 0% sensitivity for this test. Lack of sensitivity is due to the rapid clearance of the antigen from patients’ sera and the test format (latex agglutination). Of note, the sensitivity of the Pastorex system was improved when serial assays using multiple consecutive serum samples were used (53). More recently, a double-sandwich ELISA using the same monoclonal antibody (EBCA1) used in the Pastorex *Candida* has been developed, which improved the detection limit up to 0.1 ng of mannan/mL and resulted in increased sensitivity. This test now constitutes the basis for the Platelia *Candida* antigen test (Bio Rad) with reported sensitivity and specificity values of 40% and 98%, respectively (21,22). As mentioned before, the utility of this assay is maximized when used in combination with the antibody detection test, but further evaluation and validation are needed to confirm its utility.

An extracellular proteinase activity from *C. albicans*, first reported by Staib (54), has been characterized as secreted aspartyl proteinases (SAPs). SAPs are important virulence factors in candidiasis (55); hence, the usefulness of SAP as a diagnostic antigen emanates from the idea that these enzymes are only produced during active tissue invasion during infection. Morrison et al. (56) purified this enzyme and removed contaminating mannan by column chromatography with sequential anion-exchange, gel permeation, and linear gradient anion-exchange steps. The polyclonal antibodies prepared against this purified SAP were used in a competitive binding enzyme immunoassay in an immunosuppressed rabbit model of disseminated candidiasis. This assay was able to detect SAP antigenuria within 24 h of IV challenge and could discriminate between gastrointestinal *C. albicans* colonization and disseminated candidiasis (57). Ruchel et al. (34) examined serum samples from patients with candidiasis for the presence of circulating SAP using a polyclonal anti-SAP antibody in an ELISA format. Proteinase antigen was detected in approximately 50% of suspected plus confirmed cases, indicating that detection of SAP has only limited diagnostic utility. However, a confounding factor could be the fact that SAP forms complexes in circulation.

*C. albicans* hsp90 circulates in body fluids of patients with disseminated candidiasis and its 47-kDa antigenic fragment was isolated from patients’ sera by affinity chromatography (37). An enzyme-linked immunodot assay using affinity-purified antibody against the 47-kDa moiety was capable of detecting circulating antigen in the serum of patients (36). With this assay, systemic candidiasis was detected in 77% of neutropenic patients, and in 87% of non-neutropenic patients. The sensitivity
and specificity of detection was improved over that of other commercially available products (36).

Antigenemia with the 48-kDa antigen (later found to be enolase) as detected by ELISA was observed in a murine model of disseminated candidiasis in the absence of fungemia and correlated with deep tissue infection (58). An assay was commercialized as a double sandwich assay using antienolase monoclonal antibody immobilized on nitrocellulose membrane for antigen capture, and subsequent detection with an anti-enolase polyclonal antibody (Directigen; Becton Dickinson) (58). To investigate the expression of this candidal cytoplasmic antigen in the serum of patients with cancer who are at high risk for deep invasive candidiasis, Walsh et al. (58) conducted a prospective clinical trial among patients from four medical oncology centers over a two-year period. They concluded that \textit{C. albicans} enolase antigenemia is a marker for deep tissue invasion even in the absence of fungemia. The serum enolase immunoassay complemented rather than replaced blood cultures for the diagnosis of such infections (7,58). The assay was very specific (96%), but the sensitivity was low (only 54% in patients with proven deep tissue invasion). Testing of multiple samples improved the sensitivity for antigen detection to 85% for patients with proven deep tissue infection and to 64% in proven cases of candidemia (58).

Gutierrez et al. (52) described similar values of specificity and sensitivity using the Directigen test. Unfortunately, this test is no longer commercially available. Mitsutake et al. (59) used an in-house developed dot-immunoblotting assay for the detection of enolase in serum samples from candidiasis and reported a sensitivity of 71.8% and a specificity of 100%.

Gentry et al. (60) developed a reverse passive latex agglutination assay for the detection of a structurally uncharacterized 56°C heat-labile antigen of \textit{C. albicans} (60). The test was commercialized as the Cand-Tec test (Ramco Laboratories, Houston, Texas). It used latex particles that were sensitized with polyclonal sera from rabbits immunized with heat-killed \textit{C. albicans} blastoconidia. The antigen seems to be a glycoprotein and may represent a neoantigen after processing by human cells. The test did not need the dissociation of immune complexes. Although early studies showed good sensitivity and specificity of this latex-agglutination tests (61), later studies were less favorable (62,63), particularly in patients with malignancies (64). Overall, although easy to perform, the test suffered from both poor sensitivity and specificity, and its usefulness for the reliable diagnosis of invasive candidiasis is limited.

### III. SERODIAGNOSIS OF INVASIVE ASPERGILLOSIS

Over the past two decades, \textit{Aspergillus fumigatus} has become the most prevalent airborne fungal pathogen, causing severe and usually fatal invasive infections in immunosuppressed patients (65–67). Invasive aspergillosis is now a major cause of death at leukemia treatment centers, and bone marrow and solid organ transplantation units (65–67). Other spp. of \textit{Aspergillus}, such as \textit{A. terreus}, \textit{A. flavus}, and \textit{A. niger} can also cause invasive aspergillosis (66). This severe opportunistic fungal infection is characterized by a high mortality rate in these at-risk patients (68) (the crude mortality rate of invasive aspergillosis approaches 100%). The diagnosis of these infections at an early stage of the disease remains a significant clinical problem, which is compounded by the fact that antifungal agents must be begun promptly in these highly immunosuppressed patients if therapy is likely to be successful (69,70).
However, the diagnosis of invasive aspergillosis is difficult; the lack of sensitive and specific noninvasive diagnostic tests remains a major obstacle. Conventional diagnostic tests (histology, microscopy, and culture) remain the cornerstone of proving the presence or absence of fungal disease; however, their sensitivity is low and, therefore, their impact on clinical decisions to treat patients limited. Cultures become positive at a late stage of infection and delayed institution of adequate antifungal therapy is clearly associated with a poor outcome. Performance of biopsy is often precluded by profound cytopenias or by the critical condition of the patient. More recently, the use of high-resolution computed tomography (CT), with the halo-sign as early indicator of fungal infection evolving into the air-crescent sign later in the course of infection, has become an important diagnostic tool (71,72). Unfortunately, although the halo sign is frequently seen in neutropenic patients, it is neither specific for aspergillosis nor is it commonly seen in solid-organ transplant recipients with aspergillosis (73,74). Consequently, in daily clinical practice, physicians combine clinical, radiological, and/or microbiological criteria to define the level of probability of invasive aspergillosis.

### A. Antibody Detection

Sera from most healthy individuals contain anti-Aspergillus antibodies due to continuous environmental exposure. In contrast to immunocompetent hosts, growth of A. fumigatus in the tissues of an immunocompromised host, who either lack a sufficient antibody response or who mount variable antibody response, is not correlated with an increase in anti-Aspergillus antibody titers. Indeed, presence of antibodies against Aspergillus in immunosuppressed individuals is more likely to represent circulating antibodies prior to the onset of immunosuppressive therapy, rather than antibodies formed during invasive infection (75). Increasing antibody titers at the end of immunosuppression are normally indicative of recovery from invasive aspergillosis, whereas declining antibody levels are normally associated with poor prognosis. These tests are further complicated by the use of uncharacterized antigenic preparations (75). Even if antigen-specific antibodies could be identified, because of the rapid progression of infection, a test is needed to detect very low levels of antibodies at a very early stage during infection. These facts and considerations highlight the difficulties of using antibody detection for the serodiagnosis of invasive aspergillosis in this patient population. In a rather comprehensive evaluation of eight Aspergillus antibody detection assays (three indirect hemagglutination tests, three enzyme immunoassays, and two complement fixation tests), sensitivity ranged from 14% to 36% and specificity from 72% to 99% (76). The authors concluded that commercially available antibody detection assays for the serodiagnosis of invasive aspergillosis are inadequate. It is clear that further evaluation is needed in order to clearly establish the diagnostic value of antibody detection in different types of patients with invasive aspergillosis.

### B. Antigen Detection

Because of the problems with the detection of antibodies for the serodiagnosis of invasive aspergillosis in immunocompromised patients, much attention has been paid to the detection of circulating antigens in biological fluids (serum, urine, and broncheoalveolar lavage fluid), obtained from patients (77). As in candidiasis, an essential step in the detection of antigen in body fluids is the dissociation of immune
complexes that result from the ubiquitous presence of *anti-Aspergillus* antibodies because of continuous environmental exposure in most individuals (75). Although several immunoreactive proteins have been detected that circulate in the blood of patients with invasive aspergillosis, this review focuses on the detection of galactomannan, which represents the most promising serologic tool for the diagnosis of these infections.

Galactomannan (GM), a component of the *Aspergillus* cell wall, was the first antigen detected in experimentally infected animals and in patients with invasive aspergillosis (78–80). Recently, Stynen et al. (81) have introduced a sandwich ELISA that is currently the most sensitive method developed for the diagnosis of invasive infection. Several studies performed in Europe have shown that this method shows promise for the early diagnosis of invasive aspergillosis, and the inter- and intra-laboratory reproducibility of the method is reasonably good (82–91). The FDA cleared the test—Platelia *Aspergillus* EIA (Bio Rad)—in May 2003 (92). This is a rapid (approximately 3 h) one-stage immunoenzymatic sandwich microplate assay method that employs the rat monoclonal antibody EB-A2, which recognizes the \((1\rightarrow5)\)-\(\beta\)-d-galactofuranoside side chain of the GM molecule. Since each GM molecule harbors several epitopes, the same monoclonal antibody can function as capture and detector antibody (75,81). This sandwich technique results in a significantly lower limit of detection of GM of 0.5–1.0 ng/mL of serum, whereas, the previous latex agglutination test using the same antibody—Pastorex *Aspergillus* latex agglutination test—had a 15 ng/mL threshold (81,93–95). Detection of circulating GM at a lower threshold should allow earlier diagnosis of invasive aspergillosis, which is of paramount importance in determining outcome. The galactomannan ELISA results are reported as a ratio between the optical density of the patient’s sample and that of a control with a low but detectable amount of galactomannan, and data are expressed as a serum galactomannan index (GMI). Most published studies used a cut-off GMI of less than 1.0 as a negative value, a value greater than 1.5 positive, and those between 1.0 and 1.5 were indeterminate, as recommended by the manufacturer. Those recommendations for a positive result required 2 or more samples to be tested positive. However, some studies have indicated that this cut-off may be lowered (90). Based on these studies, the approval by the Food and Drug Administration (FDA) is based on a cut-off of 0.5 (92). Importantly, positive results, as approved in the United States, are based on a single sample being tested positive more than once—rather than multiple samples testing positive. Data presented to the FDA showed a sensitivity of 80.7% in 31 patients with invasive aspergillosis and a specificity of 89.2% (92). The impact of this lowered threshold for positivity should increase sensitivity of the test, but its impact on specificity, particularly in patients at lower risk for invasive aspergillosis remain to be determined.

In addition to variable sensitivity, false-positive results have been a significant issue in interpreting the results of this test with false-positive results occurring in 1–8% of patients in most series (81,93,95–98). Importantly, false positive results have been reported more frequently in certain settings, including, children—in whom absorption of galactomannan from food seems higher (99), and also with some other fungi, like *Penicillium*, which has related antigens (96). More recently, false-positive results have been reported in patients receiving the semi-synthetic penicillin antibiotic piperillin-tazobactam, which is derived from a fermentation product of *Penicillium*, although the specific cause of the apparent false-positive results remains under investigation (100,101).
Importantly, in addition to the chosen cut-off, the gradual increase in the index in consecutive samples is a very strong indication of infection and should be considered when interpreting the results. The reported results with this test can be influenced by the extent of invasive aspergillosis at time of diagnosis, the prevalence of aspergillosis among the patients studied, the cut-off ratio used, and whether multiple consecutive positive tests were or not required for significance. In a patient population with a high incidence of aspergillosis the test had a sensitivity of almost 93% and specificity of 95% (91). However, in a study of both adult and pediatric patients with hematological malignancies who had a lower prevalence of aspergillosis, sensitivity was 28% and specificity was 99% (90).

Thus, performance and utility of this test seems to depend to a great extent on the patient population under study and the cut-offs used to consider positive results (102). Circulating galactomannan was detected in the serum from approximately 65% of patients before diagnosis was made by clinical examination and radiology, and at the same time as conventional diagnosis in 10% of patients. However, in approximately 25% of patients circulating antigen was detected after diagnosis was made. Also important is the fact that course of the antigen titer seems to correlate well with clinical outcome in patients; hence, this test could be important for monitoring therapeutic responses (102).

Studies of combining galactomannan with other diagnostic modalities such as CT scans of the chest suggest that this test can be a useful adjunct to establish a likely diagnosis of invasive aspergillosis (103,104). For example, in the study by Becker et al. (103), galactomannan was detected in both CT-directed BAL fluid and in serial serum samples. Although not approved for use in nonserum samples, this study showed that the sensitivity of serial serum samples was only 47%, which increased to 85% when combined with CT-directed BAL fluid galactomannan testing, with a specificity of 100% using that approach.

Galactomannan detection in CSF has also been used to diagnose Aspergillus meningitis and in a limited number of patients to follow the course of infection in those patients, although the use of the EIA for body fluids other than serum remains investigational (105).

IV. DETECTION OF FUNGAL METABOLITES AND OTHER NONANTIGENIC COMPONENTS

Detection of different nonantigenic components released by fungal cells during infections can also be employed in the diagnosis of invasive candidiasis. Among these, arabinotol (for the diagnosis of candidiasis) and glucan (for a nonspecific fungal diagnosis) are perhaps the most promising and have received most attention of late.

\(\alpha\)-arabinotol is a metabolite of many pathogenic Candida species and can be determined by gas chromatography or enzymatic analysis (7,106,107). However, C. krusei and C. glabrata, two species of increasing clinical importance, do not produce this metabolite (108). Most frequently, to correct for human-produced arabinotol and differences in kidney metabolism, the normalized \(\alpha\)-arabinotol/\(L\)-arabinotol or the \(\alpha\)-arabinotol/creatinine ratios are used. In a limited number of prospective clinical studies, elevated ratios in serum or urine from patients with invasive candidiasis were detected, which occurred before positive blood cultures. In addition, these ratios have been correlated with therapeutic response. Overall assay sensitivity seems to be in the proximity of only 50% (7,12). Further investigation of various
In well-designed studies is needed to establish the applicability of this method for the diagnosis of invasive candidiasis.

Glucan (β-1-3-glucan), which is another component of the cell wall of Candida, Aspergillus, and many other pathogenic fungi, can also be used diagnostically and possibly as surrogate marker of infection, even though it is not an immunogenic molecule. Human cells lack this polysaccharide, and thus it has been proposed as a good indicator of systemic fungal infection, if detectable in blood or other normally sterile body fluids. The Fungitec G test MK (Seikagaku/Tokyo, Japan; Glucatell, Associates of Cape Cod, Inc., Falmouth, MA), is a commercially available—and recently approved for diagnostic purposes in the United States—colorimetric assay that can indirectly determine the concentration of 1-3-β-glucan in serum samples (109). In this case, the detection system is based on the activation of a proteolytic coagulation cascade, whose components are purified from the horseshoe crab (110). The components of the assay include factor G, which triggers the β-1-3-glucan glucan-sensitive hemolymph-clotting pathway specifically, and a chromogenic Leu-Gly-Arg-pNA tripeptide, which is cleaved by the last component of this proteolytic cascade. The assay can measure picogram amounts of β-1-3-glucan and has been used to demonstrate the presence of this polysaccharide during a variety of systemic fungal infections, including candidiasis and aspergillosis, but not cryptococcosis nor infections caused by Zygomycetes which lack this component (59,109,111). The small quantities of β-1-3-glucan found in serum can be explained by the fact that β-1-3-glucan is an integral component of the cell wall skeleton and, in contrast to other cell wall carbohydrate components, is not normally released from the fungal cell. The high degree of specificity and sensitivity of this test suggest that it may be useful for the early and rapid diagnosis of deep-seated fungal infections. A sensitivity of 90% and a specificity of 100% were reported in a study of over 200 febrile episodes in patients who underwent treatment for hematological malignancies (111). Most experience to date has been obtained in Japan and results of further evaluation in other countries are awaited; however, standardization of this method may prove problematic.

V. PCR-BASED DIAGNOSIS

The detection of microbial DNA by PCR is without question one of the most powerful tools for the early diagnosis and identification of different types of microorganisms pathogenic to humans (112). Amplification of gene sequences unique to fungi may allow for early diagnosis of invasive fungal infections and subsequent treatment (113–115). Since fungal DNA sequences have been studied for the last few decades, and particularly now with the availability of data from genomic sequencing projects for different fungi, probes for both highly conserved regions as well as genus- and species-specific variable regions are available (113,116). This offers potential for sensitive panfungal markers for detection of invasive fungal infection, followed by identification at the species level of the causative agent. It is normally advisable to target a region of the fungal genome where repeated sequences are present, i.e., ribosomal or mitochondrial regions, to ensure good sensitivity of amplification (113,116). Important procedural considerations need to be taken for removing contaminating nonfungal DNA, breaking fungal cells for DNA extraction, and preventing destruction of fungal DNA. Irrespective of the technology used, most reports from different laboratories seem to indicate that the sensitivity of PCR-base
diagnosis is often better than other currently used diagnostic technologies (113,116). Additionally, PCR might be useful for monitoring the response to antifungal therapy. Contamination has been the main obstacle to the clinical application of PCR; it can occur by airborne spore inoculation during the extraction process, by product carry-over, and by the presence of nonviable fungal spores found in reusable equipment, even after autoclaving (116,117). One additional caveat is that these are all in-house protocols developed by different groups of investigators; they use different samples, e.g. serum vs. plasma vs. whole-blood, different protocols for sample preparation, and different genes. Hence, comparisons and standardization between different assays are virtually impossible (113,114).

Early protocols for the detection of fungal DNA in human specimens focused on the DNA detection of single species or genus. Different studies in animals demonstrated that PCR with blood is more sensitive than culture for detecting candidemia (118). A higher sensitivity was also found for clinical samples obtained from patients with candidemia and those with histologically confirmed invasive candidiasis (119). Different types of clinical samples were analyzed including blood, serum, and blood culture bottles (114). An important observation was that Candida PCR of blood was negative in most patients and animals with gastrointestinal colonization with Candida species (118,120,121). During the past decade, PCR assays for the diagnosis of candidiasis have been reported using a variety of genes, such as ERG11, hsp90, secreted aspartyl proteinase, chitin synthase, actin, tubulin, mitochondrial DNA, a number of rRNA gene fragments derived from internal transcribed regions (ITS), and 5S, 18S and 28S rRNA [reviewed in Ref. (113)].

PCR has also been successfully used for early detection of Aspergillus DNA in peripheral blood (122,123). Moreover, PCR monitoring of high-risk patients allowed early diagnosis of invasive aspergillosis with good sensitivity and specificity, both in BMT recipients and in allogeneic stem cell transplant recipients indicating that in patients with a negative PCR result the probability of an invasive fungal infection was extremely low (122,123). A potential problem complicating the use of diagnostic PCR to detect Aspergillus DNA in respiratory specimens is the potential false positives because of contamination or colonization. Despite this fact, a number of reports have successfully used PCR-based methods for the diagnosis of invasive aspergillosis in different types of patients and clinical samples (124–136). Overall, results from these tests yielded similar levels of sensitivity and specificity (most often superior to other diagnostic tests), but comparative trials are still lacking (114,115).

Because of the changing epidemiology of fungal infections and the increasing recognition of the pathogenic potential of a number of fungal species in these patients, the ability to detect a wide range of medically important fungi is important. Different PCR-based protocols have been developed to such objective. The method developed by Hopfer et al. (137) targeted a multicopy rDNA highly conserved throughout the fungal kingdom, obtaining positive PCR results for all genera and species of fungi tested (137). By performing restriction fragment length polymorphism (RFLP) analysis of the resulting amplicons, they could subsequently differentiate between different groups of medically important fungi. Similarly, a test was developed that could detect a rDNA fragment present in all pathogenic fungi except Mucor spp. (138). Sandhu et al. first sequenced the 28S rDNA from 50 medically important fungi and designed universal primers and species-specific probes (139). Their results indicated a high level of specificity. Van Burik et al. (140) developed a novel panfungal PCR assay for the detection of fungal infections in blood from patients. The panfungal primers, which targeted the small-subunit rRNA gene were
optimized separately for *Candida albicans* and *Aspergillus fumigatus*. Another method uses a combination of seven digoxigenin labeled probes following amplification of a 482–503-bp fragment of 18S rRNA genes and could detect DNA from seven *Candida* spp and six *Aspergillus* spp (122,123) with excellent sensitivity.

The methods mentioned above use conventional PCR for the diagnosis of invasive fungal infections. The majority of these procedures include time consuming in-house DNA extraction protocols and require the use of gel-electrophoresis or other slow detection steps. The use of standardized DNA extraction protocols and real-time PCR may address many of the limitations of conventional PCR (141). Recently, a quantitative real-time PCR assay using the LightCycler instrument (Roche Molecular Diagnostics) has been reported to show great potential for the rapid diagnosis of candidiasis and aspergillosis (142). The LightCycler technology combines the fast in vitro amplification of DNA with immediate fluorescence detection of the amplicon. This allows the real-time quantification of the amount of DNA. A proven method for the highly specific detection of the PCR products uses the fluorescence resonance energy transfer (FRET) system with sequence-specific hybridization probes. A recent report by Pryce et al. (143) describes the use of a new real-time PCR assay with FRET and melting curve analysis to detect *C. albicans* and *A. fumigatus* DNA in whole blood and its preliminary evaluation in a number of high-risk patients. In this report, the real-time assay demonstrated an analytical sensitivity of 10 fg of purified fungal DNA, was highly reproducible, and detected *C. albicans* and *A. fumigatus* DNA in two patients with proven and in one patient with possible invasive fungal infection. Similarly, other investigators have used the TaqMan assay (Applied Biosystems) for the automated detection of fungal DNA in clinical isolates and in experimentally infected animal tissues (130,144–146). Loeffler et al. (130) compared the results of quantitative culture, PCR-ELISA, and a quantitative LightCycler assay of blood and organ specimens of experimentally infected mice and rabbits. The PCR assay was almost 20-fold more sensitive than culture from both blood and organ cultures. None of the 68 blood cultures from mice and rabbits were positive for *Aspergillus fumigatus*, whereas, PCR detected Aspergillus DNA in 17 out of 68 blood samples. Quantitative PCR analysis of blood samples showed a fungus load of 10–100 cfu/mL of blood.

Other groups of investigators are implementing similar technologies, mostly for *Candida* and *Aspergillus* infections, but other pathogenic fungi can readily be accommodated in these assays (147–153). It is anticipated that the use of quantitative real-time PCR could be readily introduced into the routine clinical microbiology laboratory and may result in drastically reduced turnaround times for results.

The potential clinical utility of PCR for the early diagnosis of invasive aspergillosis was evaluated by Hebart et al. (127) who evaluated 84 patients undergoing allogeneic stem cell transplantation. Of 1193 blood samples analyzed, 169 (14.2%) were positive by PCR. In 7 patients with newly diagnosed invasive aspergillosis, PCR positivity preceded the first clinical signs by a median of 2 days (range, 1–23 days) and preceded clinical diagnosis of IA by a median of 9 days (range, 2–34 days). The PCR assay revealed a sensitivity of 100% and a specificity of 65%, and none of the PCR-negative patients developed invasive aspergillosis during the study period, suggesting that prospective PCR screening may allow for early identification of patients at high risk for subsequent onset of invasive aspergillosis. Those same investigators showed that PCR-based techniques was sensitive in detecting early invasive aspergillosis in patients with febrile neutropenia (123).
VI. NONCULTURE BASED DIAGNOSIS OF PATHOGENIC AND ENDEMIC MYCOSES

The occurrence of true pathogenic, and endemic mycoses, including cryptococcosis, histoplasmosis, blastomycosis, and coccidioidomycosis in immunosuppressed hematological patients are less common than infections caused by Candida, Aspergillus, and opportunistic moulds (154,155). Nevertheless, the diagnosis of these infections through classical culture-based methods is often difficult. This is because their sensitivity, like with the opportunistic pathogens described above, is low and the time required for a positive culture result of these is often slow as the growth of these organisms may delay diagnosis and treatment significantly. Consequently, interest has been high in developing nonculture-based methods for these infections as well.

A. Cryptococcosis

The most useful nonculture-based method in systemic mycoses is the one developed for detecting the capsular polysaccharide antigen of Cryptococcus. While direct visualization of the organism in cerebrospinal fluid or other body fluids is sufficient to establish a presumptive diagnosis of cryptococcosis (156), cultures of the organisms may take several days to detect and identify the organism. Detection of cryptococcal antigen by latex agglutination based on latex particles coated with antibody raised against cryptococcal capsule has been widely used to diagnose this infection (112). False-positive results can occur with some other fungi—particularly Trichosporon species which shares a common antigenic component (157). More recently, the PREMIER Cryptococcal antigen assay (Meridian Diagnostics, Inc.) is an EIA that has become widely used for the diagnosis of cryptococcosis, because of its lack of reactivity with rheumatoid factor, fewer false positives, excellent sensitivity, and being easy to run on a large number of samples (158). The sensitivity of the assay in serum is greater than that in cerebrospinal fluid, even in patients with meningitis, although the sensitivity of the newer assays approaches is more than 95% and false-negative results can occur particularly in capsule-deficient strains (159). Higher titers clearly predict poorer outcomes, although the value of serial measurement is limited, with only serial CSF values correlating with response (160). Development of antibody to Cryptococcus is common following infection and may indicate a more favorable long-term prognosis, although it has limited value as a clinical tool and is seldom measured (159,161).

B. Histoplasmosis

While isolation of Histoplasma capsulatum from tissues remains the standard for establishing a diagnosis of histoplasmosis, cultures for the organism may take as long as 2-4 weeks to grow and be identified. Thus, nonculture-based methods, including both antibody and antigen detection, may significantly reduce the time required to diagnose this endemic organism. Antibody detection may give a clue to the diagnosis of this infection, even in immunosuppressed hosts. Antibodies to the H antigen of histoplasmin develop during active histoplasmosis, while those to M antigen are indicative of prior infection and is the first to rise with seroconversion (162). Nevertheless, limitations of serological detection of histoplasmosis include lack of humoral response in those patients with more severe immunosuppression, and because of the lack of sensitivity or specificity of that response (162).
useful, particularly in immunosuppressed hosts, is the detection of histoplasma polysaccharide, which is detectable in disseminated infection and correlates with response to therapy (163–165). This assay system developed by Wheat et al. (165), originally a radioimmunoassay (RIA) is now performed as an EIA, and detects antigen in urine as well as serum, with higher sensitivity in the urine. In addition, antigen can be detected in the CSF as a means to establish the presence of histoplasmosis in the central nervous system (166).

C. Blastomycosis

The diagnosis of blastomycosis is usually made either by isolating the organism in culture or by identifying the typical broad-based budding yeast in pathological material or lesion scrapings. Serological approaches can occasionally be helpful in establishing the diagnosis as antibodies to *Blastomyces dermatitidis* are produced in response to the infection. Early tests detected antibodies to *Blastomyces* A antigen, a yeast antigen (167), but recent efforts by Klein et al. (168) have focused on WI-1 antigen. Antibodies to this 120-kDa cell surface protein are detected earlier than A antigen and decline by 6 months after illness in patients who respond to therapy. A major problem complicating interpretation of serological tests for blastomycosis is cross-reactivity with other mycoses, including histoplasmosis, coccidioidomycosis, paracoccidioidomycosis, and even nonfungal infections (167,169).

D. Coccidioidomycosis

The diagnosis of coccidioidomycosis can be established by demonstration of the characteristic spherule in infected tissues, which is relatively insensitive, or by isolating the organism in tissues—that can be associated with risk of infectivity to laboratory personnel for unsuspected infections and requires the use of biological safety hoods. Thus, serological techniques for this mycosis are important in establishing a diagnosis. Serologic studies using crude antigens prepared from filtrates, or lysates of mycelial, or spherule-endospore phases are useful in establishing a diagnosis of coccidioidomycosis and in determining prognosis (170). Currently used qualitative tests include EIA, immunodiffusion (ID) and tube precipitin (TP) for the detection of IgM (IDTP) and EIA, ID and CF for the detection of IgG (IDCF) (171). IgM antibody may be detected within the first few weeks while IgG is detected after a few weeks of infection and usually disappears in several months if the infection resolves. A positive IDCF is highly suggestive of infection, and titers of 1:16 or greater typically indicate disseminated, extrapulmonary disease. Serum IDCF titers can be negative with single-site extrapulmonary infection. A positive CSF IDCF is useful in the diagnosis of the disease because cultures of CSF are uncommonly positive and serum IgG levels may not be elevated. Serial serum titers can be used to assess efficacy of therapy: rising titers are a poor prognostic sign, while falling titers are associated with a favorable clinical response (172).

VII. SUMMARY

The diagnosis of invasive fungal infection remains a significant challenge in the management of hematological patients. Since traditional culture based diagnosis is often not sensitive and invasive procedures are reluctantly undertaken in these
compromised hosts, nonculture-based methods offer substantial opportunities to establish an early diagnosis of these often lethal infections. Much attention has been focused on nonculture-based methods not only to establish an early diagnosis of systemic candidiasis or invasive aspergillosis because of the frequency of these infections in immunosuppressed hosts, but are also available for true pathogenic fungi and endemic mycoses. Nonculture-based methods for diagnosing serious Candida infection remain limited, with significant challenges of distinguishing invasive infection from colonization. Recent advances in aspergillosis have improved early diagnosis, including both detection of the antigen galactomannan and PCR-based techniques, but significant limitations to these methods remain, which significantly reduce their clinical utility. Ongoing research efforts hope to capitalize on data derived from the sequencing of the Aspergillus genome, which is aimed at identifying new targets and new methods for diagnosing this often lethal infection.

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I. OVERVIEW

Imaging is increasingly important in the management of the immunocompromised patient with suspected invasive fungal infection, largely because early-targeted treatment is the key to improved outcome. Modern cross-sectional imaging provides a powerful means of detecting occult disease and of narrowing the differential diagnosis when invasive fungal diseases might otherwise go undetected. For patients whose condition permits, imaging also serves as a guide for tissue sampling and as an essential tool for longitudinal assessment of treatment response.

Although etiologic diagnosis usually depends on culture, direct visualization of a specific fungus in clinical specimens, or on certain serologic tests, initial imaging findings are of great utility in identifying further diagnostic and treatment strategies when an etiologic diagnosis is not made. The probability that a particular imaging finding will be associated with a specific infection depends mostly on the prior probability of that infection, i.e., on the entire clinical context in which imaging abnormalities is identified. The prior probability of infection depends on the underlying condition, the type and severity of immunosuppression, the past medical history, coexisting conditions, the duration of infection, the pathogenesis of the infectious process, and clinical tests and findings (1). The unique contribution of imaging to the differential diagnosis depends mainly on the type, extent, and distribution of lesions detected with cross-sectional imaging, i.e., CT, MRI, and US.

A few clinical circumstances may be associated with very distinctive imaging findings that can provide highly suggestive diagnoses of invasive fungal infection. In the HSCT recipient, and in the patient with a hematological condition associated with neutropenia, the CT “halo sign” or the “air crescent sign” are highly suggestive of invasive pulmonary aspergillosis. Similarly, in the AIDS patient with a low CD4 count, diffuse “miliary” nodules are highly likely to indicate a disseminated endemic fungal infection.
II. CLINICAL–ETIOLOGIC CONSTELLATIONS

The lung is an important portal of entry for fungal infection, so chest imaging is of central importance in the early detection of disease. Initial imaging findings of invasive fungal infection are in general, nonspecific, but when taken in clinical context, they may greatly narrow the differential diagnostic possibilities. From an imaging point of view, the vast majority of invasive mycoses affecting the immune-deficient patient fall into one of four clinical–etiological constellations that have varied but prototypical imaging findings.

The first constellation is caused by ubiquitous opportunistic fungi that rarely cause invasive infection in the immune-competent host, but often cause life-threatening primary fungal pneumonia in the immune-deficient patient, particularly in the HSCT recipient, and in the patient with a hematologic condition associated with severe neutropenia. The angio-invasive fungus *Aspergillus* is by far the most common example, and the angio-invasive *Mucorales* fungus is a rare example. The most prototypical initial CT finding in this constellation is the pulmonary macronodule (≥1 cm diameter); less commonly, consolidation and the consolidative infarct. With prompt CT imaging, these findings are often discoverable prior to systemic fungal dissemination.

The second constellation is caused by ubiquitous opportunistic fungi that rarely cause primary invasive pneumonia even in the immune-deficient patient. These infections are usually widely disseminated at the time of first detection. A variety of portals of entry are utilized. These fungi rarely cause primary pneumonia, but they can cause secondary pneumonias from hematogenous dissemination to the lungs from an extra-pulmonary site of infection, and from super-infection of lung, damaged by prior infection from other microbes or by noninfectious processes (2,3). *Candida* sp., are by far the most common example, and *Sporothrix schenckii*, the soil fungus for which the lung is an uncommon portal of entry is a very rare example. CT imaging of extra-pulmonary candidiasis tends to produce characteristic disseminated 5–15 mm diameter nodules. Secondary pneumonias associated with candidiasis are highly variable and unpredictable in appearance, often demonstrating diffuse or multifocal infection that is often multimicrobial.

The third constellation is caused by ubiquitous pathogenic fungi that use the lung as the primary portal of entry, but uncommonly cause clinically obvious or progressive infection in the immune-competent patient, but commonly cause life-threatening disseminated fungal infection in the immune-deficient patient, especially in the host with severe T-cell immune-deficiency. *Cryptococcus neoformans* is the main example. The most common imaging findings in this constellation include focal or disseminated lesions in the brain and meninges. Lung lesions include focal nodules or focal consolidations, or diffuse miliary nodules. The vast majority of patients first discovered with pulmonary cryptococcosis already have disseminated CNS infection.

The fourth constellation is caused by geographically endemic fungi that produce only trivial or subclinical pneumonia in immune-competent hosts, but cause disseminated infection and progressive pulmonary infection in patients with marked T-cell immune-deficiency. In the United States, endemic fungi include, *Histoplasma capsulatum*, *Coccidioides immitis*, and *Blastomyces dermatitidis*. On initial CT studies, chest findings often consist of residue of previously dormant primary infection, or widespread lesions such as miliary or larger-sized nodules consistent with systemic dissemination to the lungs. Abnormalities are also commonly found in solid viscera and lymph nodes.
Identifying one of these constellations can be useful in narrowing the differential diagnosis, planning further etiologic diagnostic testing, and deciding on presumptive treatment. Identifying a constellation, however, can be complicated by variations from the prototypical imaging findings described above, and by synchronous or metachronous fungal infections, nonfungal infections, or noninfectious complications (4).

Seriously immunocompromised patients who are at risk of invasive fungal infection are also at increased risk of a wide range of other potentially serious infectious and noninfectious conditions. These include bacterial and viral pneumonias, aspiration, septic or bland pulmonary emboli and infarcts, pulmonary hemorrhage, drug toxicities, lymphoproliferative disorders, vasculitis, and localized and disseminated malignancy.

This chapter includes discussions on the use of imaging techniques pertinent to the diagnosis of suspected fungal infection in the immunocompromised patient, and imaging findings of infections caused by fungi in the four clinical–etiologic constellations described above.

III. IMAGING TECHNIQUES

The chest, sinuses, CNS, abdominal organs, and bones are the main affected anatomic regions that require imaging assessment for invasive fungal infection. Efficient use of imaging can expedite and simplify patient management, and can help avoid errors. The chest is the central focus for imaging suspected invasive fungal infection because the lung is the most common portal of entry.

A. Chest Imaging

1. Computed Tomography

Many studies have confirmed that CT is both much more sensitive (5,6) and more specific (7) than radiography in the detection and diagnosis of pulmonary lesions. Modern CT provides high-resolution tomographic anatomic detail that has become the gold standard for lung imaging, and reduced respiratory artifacts in the tachypneic patient with pneumonia. There is growing availability of state-of-the-art scanners that provide improved speed, image quality, and radiation dose reduction. As a result, there is justifiably growing reliance on CT for both screening detection as well as for differential diagnosis. CT is the most dependable means of detecting chest lesions associated with clinically occult invasive fungal infection, and the most accurate means of determining the location, extent, distribution, and type of lung lesions; the imaging parameters most necessary in narrowing the differential diagnosis. CT also serves as the modality of choice for guiding percutaneous lung biopsy, a procedure that in the immunocompromised patient can be expected to identify etiologic organisms about 80% of the time (8). Because many patients are not suitable candidates for expeditious biopsy, specific etiologic diagnosis is often not known. Thus, early treatment is often based on the most likely diagnosis based on clinical background, imaging, and other tests (9,10). Today, few important clinical management decisions in the immunocompromised patient with suspected fungal infection are made without reference to chest CT studies.

2. Radiography

Despite the now dominant position of CT in chest imaging, radiography continues to be a practical day-to-day management tool in the evaluation of chest infection.
Standard, state-of-the-art, high-quality, erect, full inspiration, posteroanterior digital chest radiographs obtained in a modern radiology department provide a reasonably dependable gross survey of chest anatomy. Unfortunately, even these high-quality studies frequently produce false-negative results in the immunosuppressed patient with fungal infection. Low-quality, anteroposterior, supine, shallow inspiration bedside chest radiography that has even lower sensitivity and specificity than high-quality studies cannot be relied upon to exclude chest disease; or when positive, be relied upon to accurately portray the type and extent of lung abnormality.

Thus, in a patient at high risk of fungal infection, a CT study is almost always needed to confirm the type, severity, extent, and distribution of disease when radiography is positive, and is often necessary to exclude lung disease in the symptomatic patient even when radiography is negative.

B. Extrapulmonary Imaging

Magnetic resonance imaging (MRI) and CT are the main modalities used to image the sinuses, bones and joints, and CNS. Contrast-enhanced CT is the main modality used for imaging the abdominopelvic compartment in patients with suspected invasive fungal infection. As compared with either ultrasound or MRI, CT provides the best means of global assessment of the entire abdominopelvic compartment. Sonography has the advantage of being suitable for bedside studies when necessary, and not requiring iodinated intravenous contrast. Like sonography, MRI, is best suited for focused problem-solving examinations of specific organs or regions, rather than for overall assessment of the abdominopelvic compartment. Whole body scintigraphic screening for infection with F-18 fluorodeoxyglucose positron emission tomography (PET) is rapidly replacing Gallium-67-labeled radiopharmaceuticals, but it has not been much used to detect invasive fungal infection because there are cross-over positive results with malignant tumors (11), and other noninfectious inflammations, e.g., drug-induced pulmonary toxicity (12).

IV. IMAGING IN SPECIFIC INVASIVE MYCOSES

A. Aspergillosis

Aspergillus is the main example of the clinical–etiologic constellation caused by ubiquitous opportunistic fungi that rarely cause invasive infection in the immune-competent host, but often cause life-threatening primary fungal pneumonia in the immune-deficient patient. The HSCT recipient and the patient with a hematologic condition associated with severe neutropenia are at particularly high risk of infection. Two peak periods of especially high infection risk for the HSCT recipient occur during the first 30 days following engraftment when neutropenia may be severe, and later after the first 90 days when chronic graft versus host disease (GVHD) may require prolonged high levels of immunosuppression. Thus, slightly more than half of Aspergillus infections in HSCT recipients occur after hospital discharge (13). Sporadic invasive aspergillosis also occurs in patients with other underlying causes of immune-suppression but at a lower incidence than in the above high risk groups, such as in solid organ transplant recipients, high-dose corticosteroid recipients, and in patients with AIDS.

The lung is the main portal of entry, and the most common primary site of invasive infection (~90%) (14). Aspergillus is the most common cause of primary
opportunistic invasive pulmonary fungal pneumonia in the immune-deficient patient (14).

Angio-invasion is the dominant pathogenetic method of disease progression, and responsible for the most important imaging findings. Immune mechanisms that normally prevent the tiny inhaled ubiquitous *Aspergillus* spores, which are regularly inhaled into the peripheral airspaces, from germinating into invasive hyphae allow the development of a core of infection. Vascular invasion by *Aspergillus* hyphae produces a tangle of small infected necrotic vessels that become incorporated into the core of infection (15). The resulting vascular thrombosis and infarction are responsible for a perimeter of hemorrhage and infection surrounding the core of infection (16). This pathogenetic sequence is responsible for the development of the pulmonary nodule and the perimeter of ground-glass opacity due to infection and hemorrhage, i.e., the “halo sign” (17). Occasionally, hyphal elements invade adjacent large vessels to cause thrombosis, pseudoaneurysm, and the risk of rupture and exsanguination. In a small fraction of patients (~10%), *aspergillosis* infection progresses via airway invasion, i.e., via the radial airway route resulting in centrilobular infection, which includes the bronchi, bronchioli, and peripheral airspaces (18).

1. **Similarities to Mucormycosis**

Mucormycosis is a much rarer cause of this constellation. Invasive aspergillosis and mucormycosis have indistinguishable clinical presentations, pathogenetic mechanisms, pathology, and imaging findings (19–21). For this reason, the vast majority of the discussion of imaging findings in invasive aspergillosis can be extrapolated to mucormycosis. Angio-invasive mucormycosis differs angio-invasive aspergillosis primarily in its rarity (22), even in the at-risk groups described above, and in its resistance to standard anti-*Aspergillus* therapy with amphotericin B. Mucormycosis is frequently treated with surgical resection. Thus, lesions attributed to *Aspergillus* without confirmatory mycology should always be regarded as potential signs of mucormycosis, especially when standard treatment with standard anti-Aspergillus drugs is not effective.

2. **Pulmonary Aspergillosis**

Initial CT imaging findings in invasive aspergillosis are dominated by the nodule and associated “halo sign.” Cavities and the “air crescent sign” are primarily features of late invasive aspergillosis. Consolidations and consolidative infarcts are much less common than nodules as initial CT features. Subcentimeter lung nodules, singly or in clusters, and the bronchiolitis-bronchopneumonia pattern are uncommonly detected on initial CT studies. Ancillary pleural or pericardial effusions, and hilar or mediastinal adenopathy are each found in a very small fraction of patients (~10%).

   a. **Macronodule.** The main finding in invasive aspergillosis is a pulmonary nodule ≥1 cm in maximum diameter, i.e., the macronodule. It is defined as a localized space-occupying, ovoid soft tissue opacity that displaces rather than conforms to the shape of the pre-existing aerated lung. The soft tissue nodule completely obscures the background bronchovascularature. The macronodule is the imaging analog of the angio-invasive core of infection in the peripheral airspaces (16), and the most common initial CT finding in patients with mycologically proven invasive aspergillosis. More than 90% of patients with mycologically proven invasive aspergillosis have at least one pulmonary nodule (10). The macronodule is such a common
feature on initial CT that its absence argues against the likelihood of invasive aspergillosis (10,16).

The “roughly nodular infarct” is a subcategory of the macronodule that has a characteristic hump-shape and subpleural location; sometimes referred to as a “Hampton’s hump” (23).

Macronodules can be caused by any invasive fungal infection, as well as by many other infectious processes including nocardiosis, tuberculosis, and lung abscess. Common noninfectious causes include bland pulmonary infarcts, lung cancer, lung metastases, lymphoproliferative disorders, and vasculitis.

b. Halo Sign. The halo sign is a modifier of the macronodule. It is defined as a perimeter of CT ground-glass lung opacity that surrounds a pulmonary nodule (10,15–17,24) (Fig. 1). The ground glass perimeter should be substantial enough to permit clear visualization of background vasculature through it (10). The nodule with a halo sign is differentiated from the nodule with unsharp margination because of irregularity of outline or partial volume effect caused by thick CT sections or respiratory motion.

On initial CT study of patients with mycologically proven invasive aspergillosis, about one-third of patients have one or more macronodules with a halo sign (14).

In the patient with a compatible illness who is at particularly high risk of invasive aspergillosis, i.e., the HSCT recipient, and the patient with a hematologic condition associated with neutropenia, the halo sign is considered a specific indicator of the fungal infection (14–17,25,26).

The halo sign is also an important imaging sign because it tends to identify patients with invasive aspergillosis who are most likely to respond to aggressive specific antiAspergillus therapy (10,25).

There is relatively little experience with MRI in early invasive aspergillosis. Nodular and segmental non-nodular lung lesions have demonstrated target-like

**Figure 1** Halo Sign.
T1-weighted central hyperintensities, and rim-enhancement after intravenous gadolinium injection (27). Hyperintensity on T1-weighted imaging correlates with subacute hemorrhage permeated by Aspergillus organisms (27). A comparative study found that the CT halo sign was much more specific than the analogous MRI halo sign in identifying patients with invasive aspergillosis (28).

The differential diagnosis of the halo sign is broad. The halo sign is not unique only to infection by angio-invasive Aspergillus. Other causes include the rare angio-invasive Mucorales sp., and other even rarer normally saprophytic angio-invasive fungi, such as Trichosporon sp., (29), Penicillium sp., (30) and Fusarium sp., (31). The pathogenetic mechanism responsible for the halo sign can also be found in angio-invasive bacterial infections, most notably those caused by Pseudomonas aeruginosa (32). The CT halo sign has also been reported in lung infections due to endemic fungi, e.g., Coccidioides immitis, as well as due to infections caused by Nocardia sp., Mycobacterium tuberculosis, cytomegalovirus, and herpes simplex virus. Noninfectious conditions reported to cause the halo sign include bronchoalveolar cell carcinoma, lymphoproliferative disorders, metastatic angiosarcoma, Kaposi sarcoma, Wegener granulomatosis, eosinophilic lung disease, and organizing pneumonia (24,33,34).

c. Air Crescent Signs and Cavities. The air crescent sign on CT is defined as a CT nodule containing a semilunar pocket of gas surmounting a partially detached sequestrum of devitalized lung (16,35) (Fig. 2).

In the patient with a compatible illness who is at particularly high risk of invasive aspergillosis, i.e., the HSCT recipient, and the patient with a hematologic condition associated with neutropenia, the air crescent sign is considered a specific indicator of the fungal infection (14–17,25,26). The air crescent sign is attributed to a crescentic cap of air between a devitalized sequestrum pulmonary aspergillosis that has separated from the remaining viable lung.

![Figure 2 Air Crescent Sign.](image)
Over time, necrosis tends to develop in nodular foci of *Aspergillus* infection often leading to cavitation. Sometimes an air crescent sign will develop. The air crescent sign is detected in only 5–10% of patients on initial CT studies (10). In a week to 10 days following an initial chest CT, macronodules with halo signs will become progressively less frequent (28,36), while macronodules with cavitation and air crescent signs will become more frequent.

The air-crescent sign tends to develop after recovery from neutropenia. Like the halo sign, the air crescent sign is an indicator of likely invasive aspergillosis when it is detected in a patient at high risk of the infection (16,37,38).

Thick-walled and thin-walled cavities without air crescents can be found in about 10% of initial CT studies of patients with invasive aspergillosis, but these findings do not seem to have the same diagnostic predictive value as the air crescent sign, from which they should be differentiated (39).

In limited experience with invasive aspergillosis, MRI studies have demonstrated necrotic target lesions with a rim with T-2 weighted hyperintensity, and gadolinium rim enhancement (28).

The differential diagnosis of the air crescent sign is broad, but like the halo sign, invasive aspergillosis is the most common cause when it is found in the HSCT recipient or the patient with hematologic malignancy with neutropenia who has a compatible illness. The sign is also found in many conditions other than invasive aspergillosis, e.g., nocardiosis, tuberculosis, bacterial lung abscess, cavitary hemotoma, and cavitary lung cancer (40,41). The sequestrum of the air crescent sign needs to be differentiated from the often free-floating fungus ball of saprophytic aspergillosis. The sign also needs to be differentiated from thin and thick-walled cavities that lack air crescents.

d. Consolidation and Consolidative Infarct. Consolidation is defined as a non-space-occupying lung opacification of the peripheral airspaces, such that the background of underlying bronchovascular structures is totally obscured, except where bronchi contain residual gas, i.e., air bronchograms (42). Consolidations usually maintain the general shape of the pre-existing aerated lung anatomy. The consolidative infarct is a subcategory of consolidation that is wedge-shaped and pleural-based (16,17). In angio-invasive aspergillosis, the consolidative infarct is attributed to a segment- or larger-sized lung infarct (16).

On initial CT studies, about one-third of patients with invasive aspergillosis demonstrate one or more consolidations, or consolidative infarcts (10).

The differential diagnosis of localized lung consolidation includes any invasive fungal infection, as well as a wide range of pneumonias due to bacteria, *Legionella*, and viruses. Common noninfectious causes include aspiration, infarction, hemorrhage, partial atelectasis, lymphoproliferative disorders, and radiation injury.

e. Centrilobular, Tree-in-Bud, and Peri-bronchial Opacities. Centrilobular opacities are discrete subcentimeter nodular opacities located in the center of secondary lobules, and are often found in conjunction with opacified segments of small branching bronchi and bronchioli, i.e., tree-in-bud opacities, and/or patches of peribronchial consolidation. They form what is called the bronchiolitis–bronchopneumonia pattern.

These findings, identified either separately or in combination, are taken as general indicators of bronchiolitis or bronchopneumonia that result from spread of infection along the airways (18). In the specific clinical context of the patient at high risk of opportunistic or endemic fungal infection, such findings may be because of airway invasive progression of fungal infection, such as because of *Aspergillus* or *Cryptococcus*. 
These findings have been noted in about 10% of patients with invasive pulmonary aspergillosis, and attributed to airway invasion (10, 18, 43, 44).

A chronic form of airway invasive aspergillosis, i.e., chronic tracheobronchial aspergillosis infection spreads along the airways, resulting in bronchial wall thickening and nodularity, bronchostenosis, and bronchiectasis that can lead to peribronchial consolidation and atelectasis, especially in HSCT and lung transplant recipients, and in AIDS patients (18, 45).

The differential diagnosis of the bronchiolitis–bronchopneumonia pattern is broad, and not specific for invasive aspergillosis. It may be found in a wide variety of other conditions, including atypical mycobacterial infection, tuberculosis, and bronchopneumonia of any cause (18).

Direct intrathoracic invasion into extra-pulmonary tissues such as the pleura and pericardium (~10%), and hila and mediastinum (~1%) is very low on initial CT studies (10). Blood-borne dissemination into the lungs may result in disseminated lung nodules, or diffuse consolidation.

f. Treatment Response. Follow-up imaging of initial pulmonary findings shows that the halo and air crescent signs vary inversely with each other over time, i.e., the frequency of the halo sign falls, and the frequency of the air crescent sign rises. In one longitudinal CT study, 72% of patients had halo signs early in the course of disease, but only 22% of patients had halo signs after the first 10 days (28). In another study, the frequency of the halo sign rapidly decreased to about one fifth of its initial frequency 7–10 days following an initial CT scan (36). Some data suggest that there may even be a significant fall off in the frequency of the halo sign during the first three days following the initial CT scan (36).

Over the first seven days following discovery, a three to four-fold increase in the volume of CT lung opacities has been observed in patients who do not seem to show other signs of unsatisfactory response to treatment (36). This phenomenon may be because of increased host responsiveness to infection and not because of treatment failure.

3. Extrapulmonary Aspergillosis

Cerebral aspergillosis occurs by direct extension from sino-nasal aspergillosis, or from systemic hematogenous dissemination (Fig. 3). Sino-nasal aspergillosis may demonstrate soft tissue masses or abscesses in the nasal cavities or paranasal sinuses. Extension to the brain or orbit may be seen through the cribiform plate, sometimes associated with bone destruction or cavernous sinus thrombosis (46). Initial brain lesions are similar in appearance to those in the lung; usually well-defined macronodular hypodensities or large vessel infarcts surrounded by edema on CT. On MRI, cerebral lesions are T2W hyperintensities surrounded by edema that enhance after intravenous gadolinium (47). In later stages, central necrosis and rim enhancement are the primary findings (48).

Visceral dissemination is associated with solid nodular masses, abscesses or infarcts in the liver, spleen, and kidneys.

B. Candidiasis

Candida is the main example of the clinical–etiological constellation caused by ubiquitous opportunistic fungi that rarely result in primary invasive pneumonia, but commonly cause solid organ and/or lung dissemination that is often identifiable
on an initial imaging study. Candidiasis is commonly associated with synchronous infection by other pathogenic and opportunistic microbes. Those at high risk are usually severely immunosuppressed patients, especially those with leukemia and those who are neutropenic.

1. **Pulmonary Candidiasis**

*Candida* pneumonia may be due to hematogenous dissemination or to super-infection of lung that has been damaged by prior or contemporaneous infection, by other microbes or by some other noninfectious processes.

In pulmonary candidiasis, imaging findings defy meaningful categorization because the fungus is often found in lung tissue without convincing evidence of significant lung damage (49). It is usually found in severely immunocompromised patients who have disseminated candidiasis that often coexists with invasive infection because of other opportunistic and pathogenic microbes (50).

The pulmonary imaging findings that have been attributed to invasive Candidiasis are often bilateral and diffuse as a result of systemic dissemination. Focal findings may also occur in areas of damaged lung that has become infected. On CT, a wide variety of both focal or diffuse lesions have been identified, including nodules (50), lesions of the bronchiolitis-bronchopneumonia pattern (51), and diffuse miliary nodules (1–3mm discrete nodules too numerous to count). Focal nodules detected in pulmonary candidiasis do not seem to have the same clinical relevance as early nodular lesions found in invasive aspergillosis, where early detection and treatment may have an impact on outcome (52). The lack of correlation between the detection of localized and diffuse imaging findings on improved outcome holds whether candidiasis is considered “primary,” i.e., due to airway aspiration, or “secondary,” i.e., due to hematogenous dissemination. Contemporaneous initial abdominal CT studies may also demonstrate disseminated nodular lesions in the liver, spleen, or kidneys.

The differential diagnosis of diffuse lung opacification in the immunosuppressed patient is broad and mainly dependent on the particular type of lung lesions that make up the diffuse opacification. The detailed differential diagnosis based on the type and distribution of particular lung lesions is beyond the scope of this chapter.
In general, in the immune-deficient patient, diffused lung opacification may be found in candidiasis, PCP, viral pneumonia, toxoplasmosis, and disseminated endemic or opportunistic mycoses. It can also be found in pulmonary edema, Kaposi sarcoma, ARDS, capillary leak syndromes, pulmonary hemorrhage, alveolar proteinosis, transfusion reactions, drug toxicity, idiopathic interstitial pneumonias, and metastatic cancer.

2. Extra-pulmonary Candidiasis

The portal of entry is variable, e.g., because of transcutaneous infusion lines or aspiration. The infection is usually disseminated in multiple organs at the time of the initial CT study. CT studies may demonstrate pulmonary lesions alone or in combination with extrapulmonary infection.

   a. Hepatosplenic Candidiasis. Chronic hepatosplenic candidiasis is a condition often of patients recovering from prolonged neutropenia, usually after remission induction chemotherapy for acute leukemia (53). The recovered neutrophil function in chronic candidiasis allows for focal granuloma formation (54).

   In chronic hepatosplenic candidiasis, studies of the abdomen with CT, MRI or US characteristically demonstrate multiple, 5–15 mm, well-defined, solid nodules in the liver, spleen, and/or kidneys. In acute hepatosplenic candidiasis before neutrophil function has recovered, these same nodules consist of liquefied or partially liquefied abscesses, rather than solid focal lesions.

   Patients suspected of chronic disseminated candidiasis are best studied with contrast-enhanced CT. Upper abdominal US may also be useful because lesions that are not visible on CT may be apparent with US (55). The converse is also true. On CT, multiple, well-defined, low-attenuation nodules of 5–15 mm are detected in the liver, spleen, and/or kidneys (56) (Fig. 4). Disseminated granulomatous infection can be found most often in the liver, spleen, or kidneys (56) when chest radiographic studies are usually normal.

![Figure 4](image-url)  
Hepatosplenic candidiasis.
In US, the nodules are well defined and hypoechoic, often with a “wheels within wheels” appearance consisting of a hypoechoic outer rim, hyperechoic inner rim, and hypoechoic infectious-necrotic core. Sometimes the hypoechoic nodules have hyperechoic cores called “Bull’s eye” lesions (55,57). In MRI, deep visceral candidiasis appears as well-defined, T-2 hyperintense nodules (58).

Similar appearing, multiple subacute or chronic macroscopic hepatosplenic nodules (hypoechoic on US and hypoattenuating on CT) can be found in immunosuppressed patients with other disseminated fungal infections, such as disseminated aspergillosis and fusariosis, miliary tuberculosis and disseminated mycobacterial infection, and multiple bacterial abscesses.

b. Treatment Response. Even after satisfactory clinical response to treatment, hepatosplenic nodules tend to persist for long periods (55,57,59).

C. Cryptococcosis

*Cryptococcus neoformans* is the main example of the clinical–etiological constellation caused by ubiquitous pathogenic fungi, which use the lung as the primary portal of entry that uncommonly cause clinically obvious or progressive infection in immunocompetent patients, but also commonly cause life-threatening disseminated fungal infection in immune-deficient patients. The main high-risk groups have severe T-cell immune-deficiency, especially those with AIDS and very low CD4 counts. The pathogenesis sequence includes inhalation of fungi, pneumonia, and prompt blood-borne dissemination in the immune-deficient patient. The pulmonary infection is the result of either a new primary pulmonary infection, or an activated dormant focus of infection.

1. Pulmonary Cryptococcosis

In the immunocompetent patient, cryptococcosis can produce a wide variety of initial pulmonary imaging findings, most often of the focal type. In dormant infection, there may be no imaging residue, or a silent subpleural soft tissue nodule or mass. Other positive chest findings may include hilar lymphadenopathy and pleural effusion.

In the immune-deficient patient, cryptococcosis is the most common cause of fungal pneumonia in the severely immune-deficient AIDS patient, usually associated with concomitant CNS infection (86%) that may be clinically silent (60). Conversely, about one-fourth of AIDS patients who present with CNS cryptococcosis have clinically silent pulmonary lesions (Fig. 5). Other common sites of disseminated mycosis include skin, bone, or genitourinary tract.

In the immune-deficient patient, a subpleural pulmonary nodule is a characteristic localized finding (61), and may be observed to progress into a peribronchial, segmental, or Iobar opacity or into a fulminant, widespread ARDS-like picture (61). Hematogenous dissemination to the lung often takes on a pattern of miliary nodules (1–3 mm diameter discrete nodules too numerous to count) identical to the appearance of hematogenous spread of endemic mycoses (61).

The differential diagnosis of diffuse miliary nodules includes other hematogenously disseminated mycoses, such as endemic mycoses and other nonfungal infectious etiologies. The other infections that are likely to cause diffuse miliary nodules in the immunocompromised patient include disseminated tuberculosis, disseminated nontuberculous mycobacterial infection, and viral pneumonia. Other
causes of diffuse miliary nodules include noninfectious granulomatous disease, such as sarcoidosis and pneumoconiosis, and hematogenous metastases to the lung. See Sec.IV.B.1 for a discussion of the more general differential diagnosis of diffuse lung opacification in the immunosuppressed patient.

2. *Extra-pulmonary Cryptococcosis*

   a. **Cerebral Cryptococcosis.** Cerebral cryptococcosis is the most common invasive fungal infection of the CNS. On CT and MRI, it presents in approximately equal frequencies as (a) one or more nonenhancing solid lesions, hypodense pseudocysts, or space-occupying lesions in the basal ganglia, as (b) meningitis/meningoencephalitis with gyral enhancement, or as (c) normal studies (62). Commonly, there are signs of hydrocephalus (62). Imaging can identify the most useful site for biopsy when necessary (63). MRI studies show meningeal enhancement. Other signs include a solid parenchymal mass without hemorrhage (granuloma), atrophy, cerebral edema, or hydrocephalus. Basal ganglia lesions are hypointense on T1-weighted images, hyperintense on T2-weighted images, and usually hyperintense on T1-weighted images after intervention gadolinium.

   b. **Abdominal Cryptococcosis.** Abdominal cryptococcosis can affect the solid abdominal viscera such as the liver, and may be seen as multiple, small, and low attenuation nodules similar to other those found with disseminated infections by opportunistic or other endemic fungi.
In the AIDS patient with a very low CD4 count and diffuse nodular CT lung opacities, diagnostic considerations for small miliary nodules should include disseminated endemic fungal infection, disseminated mycobacterial infection, *pneumocystis carinii* pneumonia, viral pneumonia, pulmonary edema, diffuse lung damage (ARDS), treatment-induced lung reactions, bronchiolitis obliterans-organized pneumonia, lymphoproliferative disorders, Kaposi sarcoma, hematogenous metastases, and allergic pneumopathy. Up to one-third of pulmonary nodules have been diagnosed with halo signs (64), or cavitation (61). The differential diagnosis of a cavitary pulmonary nodule in the AIDS patient must include PCP (61). Hilar and/or mediastinal adenopathy, and pleural effusions can also be found (60,61).

In the AIDS patient with neurologic findings and a very low CD4 count at-risk, the differential diagnosis must include toxoplasmosis, a cause of parenchymal lesions with ring enhancement, solid enhancement, and nonenhancing focal edema.

D. **Endemic Mycoses**

*Histoplasma capsulatum*, *Coccidioides immitis*, and *Blastomyces dermatididis* are the main causes of a clinical-etiologic constellation resulting from geographically endemic fungi encountered in the United States. Infection in immune-competent hosts usually produces trivial or subclinical pneumonia that spontaneously resolves, but in immunosuppressed patients it can cause disseminated life-threatening infection. Dissemination has usually already occurred at the time of initial detection. The main risk factor is severe impairment in T-cell immunity, such as in the AIDS patient with very low CD4 count. (61,65).

The lung is the main portal of entry of endemic mycoses. Infection in immunodeficient patients usually arises from one of two pathogenetic sequences. In the first, deficiency of T-cell immunity results in failure to maintain inactivity of a dormant focus of a previous primary infection. In the second, deficiency of T-cell immunity results in failure to prevent new primary pulmonary infection, in an endemic area. In each case, there is rapid hematologic dissemination to extra-pulmonary sites and the lungs. Nonpulmonary sites of initial infection include the paranasal sinuses and skin. Sites of extra-pulmonary dissemination that have significant imaging implications include the brain and meninges, and the solid abdominal viscera, gastrointestinal tract, and bones.

Initial chest radiographs are negative as often as half the time even when the disease is disseminated. Positive initial chest findings are varied (66), but tend to occur in three types of presentations. In one presentation, initial studies show focal or multifocal lung lesions such as nodules or consolidations because of a reactivated dormant focus, or a progressive primary infection.

In a second presentation, there are diffuse lung lesions because of hematogenous dissemination from a progressive primary pulmonary infection or from secondary sites of extra-pulmonary infection. The diffuse lung lesions may be composed of discrete miliary nodules (1–3 mm diameter), larger nodules, or consolidation. In radiography, the discrete miliary lesions seen on CT may appear as vague, nonspecific reticulonodular opacities. When the miliary nodules of hematogenous dissemination are present, larger nodules or consolidation of the focus of previously dormant primary lung infection, or the main new site of progressive primary infection may be visible.

In the third presentation, the initial imaging finding is due to extra-pulmonary dissemination to the CNS, intra-abdominal organs, or lymph nodes.
For a discussion on the differential diagnosis of the macronodule and consolidation in the immunocompromised patient, see Sec. IV.A.2. For a discussion on the differential diagnosis of diffuse lung opacification in general, see Sec. IV.B.1. For a discussion on the differential diagnosis of diffuse miliary nodules, see Sec. IV.C.1.

The same underlying conditions and immunodeficiency that make these patients susceptible to endemic mycoses also put them at increased risk of infection caused by other organisms such as other fungi, bacteria, viruses, and protozoa, including especially mycobacteria, Nocardia asteroides, and Legionella sp., varicella-zoster virus, herpes simplex virus, cytomegalovirus, Epstein-Barr virus, P. carinii, and T. gondii. Each of these alternative infections, and a variety of noninfectious conditions must be kept in mind when arriving at a differential diagnosis of imaging findings (67,44). Hilar, mediastinal, and intra-abdominal lymphadenopathy are common features of endemic mycoses, and need to be differentiated from similar findings in disseminated tuberculosis, atypical mycobacteria, metastatic tumor, and lymphoma.

1. Histoplasmosis

a. Pulmonary Histoplasmosis. Histoplasma capsulatum causes endemic infection in central United States. Like other endemic fungi, it generally produces a mild, self-limited infection of the lungs, and only rarely causes progressive or disseminated infection (<0.05 %) (68). The immune-competent patient with histoplasmosis is capable of reacting to infection with well-developed ruberculoid granulomas that usually become dormant and calcify.

In the immunocompromised patient, histoplasmosis is uncommon even in endemic areas (3). When it occurs, infection is often disseminated on first discovery. Dissemination is the result of activation of a dormant focus, or progressive primary infection. Impaired T-cell immunity limits the ability to produce well-developed granulomas.

In the lung, a diffuse radiographic abnormality reflecting hematogenous dissemination is identified in about half of the patients (69). The diffuse lung lesions consist of miliary nodules, macronodules, vague reticulonodular opacities or consolidations (Fig. 6). See Sec. On IV.A.2 and IV.C.1 for differential diagnosis. Almost half the time, chest radiographs are interpreted as normal even in disseminated disease (69,70). Focal radiographic lung opacities are identified in only about 10% of patients (69). Enlarged hilar and mediastinal lymph nodes, calcified granulomas, and cavitation are uncommonly identified (<5% each) (69). Increased focal opacity and/or cavitation within the diffuse lung disease may indicate a site of an activated dormant focus. Even when imaging does identify findings consistent with disseminated histoplasmosis, concurrent tuberculosis must be considered, especially in the AIDS patient with a low CD4 count.

b. Extra-pulmonary Histoplasmosis. Extra-pulmonary findings include hepatosplenomegaly, lymphadenopathy, and solid organ enlargement. The specific diagnosis is based on H. capsulatum in a tissue specimen or grown in culture or the presence of Histoplasma antigen in blood or urine. At the onset of infection, histoplasmosis is often widely disseminated, and can be rapidly diagnosed with antigen levels in urine or serum, or by blood cultures or pathologic specimens. Disseminated extra-pulmonary histoplasmosis is an important diagnostic consideration in the immunocompromised patient from an endemic area who develops a febrile illness associated with pneumonic consolidation, paratracheal mediastinal and intra-abdominal lymphadenopathy, superior vena cava syndrome, and in the patient with
miliary lesions in the lung, spleen, or adrenal glands. These lesions may or may not be calcified.

2. **Coccidioidomycosis**

   a. **Pulmonary Coccidioidomycosis.** The lung is the primary portal of entry of *Coccidioides immitis*, a cause of endemic mycosis in southwestern United States. In the immunocompetent person, an intact T-cell immunity helps confine coccidioidomycosis to the lungs and intrathoracic lymph nodes during which the disease is usually self-limited, and flu-like. Only a small fraction of symptomatic immunocompetent patients exhibits imaging evidence of pneumonia (about 5%). When they do, the findings are most often segmental or lobar airspace opacities, sometimes associated with hilar adenopathy and/or pleural effusion. Any residue of these infections are usually one or more soft tissue nodules or cavities that gradually resolve, or evolve into thin-walled cysts in a matter of several months (71).

   In the immunocompromised patient with defective T-cell immunity, a dormant focus of coccidioidomycosis may activate, or in an endemic region a new primary pulmonary infection may become progressive and/or promptly disseminate to involve the skin, bones, joints, kidneys, and meninges. Dissemination carries a high risk of mortality (about 70%). CNS involvement is usually fatal.

   Blood-borne pulmonary dissemination results in diffuse micro nodular (miliary) abnormality (72) or diffuse reticulonodular opacities, sometimes with hilar lymphadenopathy and pleural effusions (73). In about one-fourth of patients with disseminated disease, localized lung disease will be found. CT and radiographic images often demonstrate diffuse miliary or reticulonodular opacities in patients with hematogenous dissemination to the lungs (74). Hilar and mediastinal adenopa-
thy are frequent findings in disseminated coccidioidomycosis. Pericardial involvement may lead to pericardial effusion, cardiac tamponade, or constrictive pericarditis.

See the following sections for discussions on differential diagnosis: nodules and consolidations in Sec.IV.A.2., diffuse lung opacification in Sec.IV.B.1, diffuse miliary nodules in.

b. Extra-pulmonary Coccidioidomycosis. In a CT and/or an MRI, dissemination to the brain may show meningitis, i.e., marked thickening of basal meninges and intense contrast enhancement. Other CNS findings include communicating hydrocephalus in the cervical subarachnoid space and cisterns (basilar, sylvian, and inter-hemispheric) (46,75). Focal parenchymal MRI abnormalities may suggest ischemia or infarction, brain abscess, or granulomas (76). Extra-pulmonary dissemination can also produce bone abscesses, synovitis, abscesses, or granulomas in the liver and other infra-abdominal organs in CT or MRI.

3. Blastomycosis

a. Pulmonary Blastomycosis. *Blastomyces dermatitidis*, a soil fungus coendemic with *H. capsulatum* in south-central and mid-western United States. It is an uncommon cause of invasive mycoses in severely immunocompromised HIV patients. The two main portals of entry are the lung and the skin. Etiologic diagnoses depend solely on culture or direct visualization of the fungus in clinical specimens because there are no reliable serological tests. Cell-mediated immunity appears to be less important for protection against blastomycosis than it is for protection against other endemic mycoses.

In immunocompetent patients with acute blastomycosis, imaging findings usually consist of airspace opacification most of which also have air bronchograms (86%) (77). Mass lesions usually indicate chronic disease (78). CT studies demonstrate airspace disease, and mass-like opacities in more than half of patients (78,79). Other presentations, such as nodules, cavities, and interstitial lung findings are uncommon (~10%). Miliary lesions and diffuse alveolar damage are rare in the immunocompetent patient (less than 10% of patients) (78).

In the immunosuppressed patient, imaging findings consist of consolidations and mass lesions that are often extensive and progressive (80), and may be complicated by the superimposition of disseminated miliary lung lesions or an ARDS-like pattern (about a quarter of patients (81) (Fig. 7). Cavitation and pleural abnormalities occur in a minority of patients.

Microbial confirmation and/or histopathology is required to document an etiologic diagnosis. See the discussions on differential diagnosis: nodules and consolidations in the sections on Pulmonary Aspergillosis, diffuse lung opacification in Pulmonary Candidiasis, diffuse miliary nodules in Pulmonary Cryptococcosis.

b. Extra-pulmonary Blastomycosis. Dissemination to liver, spleen, bone marrow, pancreas, brain, meninges, and endocrine glands are commonly seen in AIDS patients. CNS involvement typically demonstrates evidence of meningitis or cerebral abscess. The highest rate of CNS dissemination and the most severe disease are found in patients with advanced HIV disease.

E. Other Rare Invasive Mycoses

*Scedosporium apiospermum* is an asexual anamorph of the fungus *Pseudallescheria boydii*, an emerging cause of disseminated infection in immunocompromised patients.
that may rarely simulate the imaging findings of invasive aspergillosis, but is resistant to amphotericin B (82).

*Sporotrichosis* is a dimorphic soil fungus that usually gains access to the body through the skin, a rare cause of a chronic re-infection tuberculosis-like pattern with apical cavities in immunocompetent patients after heavy soil exposure (83). In the immunocompromised patient with a deficiency of cell-mediated immunity, the fungus rarely causes disseminated lesions in the lungs, brain, and bones similar to those found in disseminated endemic fungal infections (84).

Rare angio-invasive fungi include three normally saprophytic fungi, i.e., *Trichosporon* sp., (29) *Penicillium* sp., (30), and *Fusarium* sp., (31). These fungi rarely cause angio-invasive infection with imaging features similar to invasive aspergillosis and mucormycosis. They may also cause disseminated disease.

**V. SUMMARY**

This chapter provides an overview of the role of imaging in the management of invasive fungal infection in the immunocompromised patient.

It stresses the importance of cross-sectional imaging in the detection and differential diagnosis of infection, and the importance of prior probability in interpreting imaging findings.

It identifies a few coherent prototypical patterns out of a wide variety of imaging findings in the patient with a compatible illness. Four such clinical–etiological imaging constellations include:

![Pulmonary Blastomycosis](image_url)
1. One or more pulmonary nodules with halo signs or air crescent signs due to lung infection by a ubiquitous angio-invasive opportunistic fungus typified by *Aspergillus* in an HSCT recipient or in a patient with a hematologic condition with neutropenia.

2. Multiple chronic 5–15 mm nodules in the liver and/or spleen due to a hematogenously disseminated infection by a ubiquitous opportunistic fungus typified by *Candida* in an HSCT recipient or in a patient with a hematologic condition recovering from neutropenia.

3. One or more enhancing cerebral gyri, cerebral nodules, and/or diffuse miliary or focal lung opacities due to a disseminated ubiquitous fungus typified by *Cryptococcus* in a patient with severe T-cell immunodeficiency.

4. Diffuse miliary lung nodules and/or intra-abdominal abnormalities due to a hematogenously disseminated geographically endemic fungus in the United States typified by *Histoplasma capsulatum*, *Coccidioides immitis*, and *Blastomyces dermatitidis* in a patient with marked T-cell immunodeficiency.

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16
Polyenes

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I. INTRODUCTION

The polyenes are a family of several hundred naturally derived macrolide antibiotics. Those most well studied to date possess an internal cyclic ester, with 4–7 conjugated double bonds. They exhibit a broad spectrum of antifungal activity and have a common mechanism of action (1). The polyenes bind to sterols in all eukaryotic cells, but some have greater affinity for ergosterol found in fungal cell membranes, as opposed to cholesterol found in mammalian cell membranes. This difference in affinity between fungal and mammalian sterols has allowed for human use, a select few of the polyenes that have been studied. Amphotericin B, nystatin, and pimaricin are the most commonly used polyenes in clinical practice. This chapter will focus exclusively on amphotericin B and its lipid formulations.

Amphotericin B is a lipophilic, rod-like macrolide, first isolated by Gold and colleagues (2) from an aerobic actinomycete (Streptomyces nodusus), found in the Orinoco Valley of Venezuela in 1955. Because of its broad spectrum of antifungal activity, the increasing importance of invasive mycosis in clinical medicine, and the lack of effective alternative therapy, amphotericin B soon became the treatment of choice for invasive fungal infections, particularly in the immunocompromised host (3,4). Like other polyenes studied, amphotericin B binds to sterols, primarily ergosterol, in the fungal cell membrane. The binding of amphotericin B to the sterols of susceptible fungi allows for the development of pores, which increase cytoplasmic membrane permeability leading to loss of intracellular potassium and other small intracellular molecules and eventual fungal cell death (Fig. 1). Antifungal activity does not require metabolism of amphotericin B and is rapid in onset. Oxidative damage to fungal cells caused by amphotericin B has also been suggested as an additional mechanism of antifungal action (1,5).

Amphotericin B has a broad range of activity against most pathogenic fungi in vitro. Standards for susceptibility testing of amphotericin B against isolates of yeasts and molds have been developed by the national committee for clinical laboratory
standards (6,7) (NCCLS). Although susceptibility testing does not always clearly separate sensitive from resistant organisms, a number of investigators have reported a relative correlation between outcome and results they obtained from in vitro testing of amphotericin B, against clinical isolates utilizing modifications of the NCCLS methodology (8,9). Emergence of previously less common fungi as causes of life-threatening invasive infections (10,15), however, make it important to mention fungi, which have poor susceptibility to amphotericin B. These include the moulds *Pseudallescheria boydii* (*Scedosporium apiospermum*) (12), *Aspergillus terreus* (13), *Fusarium* spp. (14), and the yeasts *Trichosporon* spp. (15,16), and *Blastoshizomyces capitatus* (17).

II. AMPHOTERICIN B DEOXYCHOLATE

Amphotericin B is a lipophilic molecule that is poorly absorbed from the gastrointestinal tract and is insoluble in water at physiologic pH. In order to allow for intravenous use, amphotericin B is dispersed as a colloid formation in sodium deoxycholate with sodium phosphate buffer. Amphotericin B will aggregate in electrolyte solutions and must be reconstituted in 5% dextrose solutions. The use of inline filters with pore size of 0.22 μm or less may remove significant amounts of drug from solution and is not recommended (18). All of the lipid formulations of amphotericin B also require reconstitution in 5% dextrose water and inline filters are not recommended.

Amphotericin B deoxycholate is usually infused over 2–4 hr with doses of 0.5–1.0 mg/kg/day. A maximum of approximately 1.5 mg/kg/day of conventional amphotericin B deoxycholate has been utilized for more serious infections; however, toxicity at this dosage usually limits length of therapy. The drug is best administered through a central venous catheter due to associated phlebitis. Although the pharmacokinetics of amphotericin B are not well understood, concentrations of the drug in biological fluids have been measured by a variety of methods including high pressure liquid chromatography (19), immunoassay (20), and bioassay (21). Amphotericin B separates from deoxycholate in serum with greater than 95% binding to serum proteins. The drug rapidly leaves the circulation, distributing throughout tissues presumably by combining with cholesterol containing membranes. Amphotericin B
then re-enters the circulation slowly with only a small percentage being excreted in the urine or bile acid (22). Blood levels of amphotericin B are not affected by hepatic or renal dysfunction and the drug is not cleared by hemodialysis. The drug concentrates in the reticulo-endothelial system with higher concentrations in the liver and spleen. Amphotericin B penetrates poorly into brain, meninges, pancreas, muscle, and bone. Peak blood levels of amphotericin B after infusion of standard doses amphotericin B deoxycholate are approximately 0.5–2.0 μg/mL. The initial half-life is 24 hr with a β phase of approximately 15 days. Amphotericin B can be detected in blood even 7 weeks after end of treatment, suggesting continued release from tissues (21).

Unfortunately, a number of acute and chronic toxicities have made treatment with amphotericin B deoxycholate difficult for patients to tolerate. Infusion-related reactions occurred such as fever, rigors, and chills occur in as approximately 50% of patients (23,24), but were usually treated symptomatically without discontinuation of the drug in the setting of life-threatening infection. When severe, however, rigors can be accompanied by bronchospasm with wheezing and hypoxia. These less common infusion-related reactions to amphotericin B deoxycholate, which also include hypertension, hypotension, and hypoxemia, may adversely affect antifungal drug therapy. Although infusion-related toxicities frequently diminish after several days, a variety of medications including acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs), diphenhydramine, and hydrocortisone have been utilized as premedications in an attempt to ameliorate the discomfort of therapy with amphotericin B deoxycholate (25). Merperidine at doses of 25–50 mg has been utilized successfully to blunt serious rigors, but may lead to seizures after accumulation of the neurotoxic metabolite normeperidine in patients with serum creatinine >2 mg/dL. Few controlled studies have been done to show benefit of premedication and the use of these drugs is not without risk. Use of NSAIDs in the thrombocytopenic patient is not recommended, and long-term use of steroids may worsen the immune suppression that lead to development of invasive mycosis in the compromised host initially.

Reduction in dosage or discontinuation of therapy with amphotericin B deoxycholate is more commonly because of the adverse effects of this polyene on renal function. Amphotericin B deoxycholate treatment causes constriction of the afferent renal arterioles leading to reduced blood flow to glomeruli and renal tubules (26). Continued use of amphotericin B deoxycholate frequently leads to azotemia, renal tubular acidosis, and impaired urinary concentrating ability with subsequent electrolyte imbalance. Factors that have been identified for increased risk of nephrotoxicity with amphotericin B deoxycholate treatment include underlying chronic renal disease, concurrent use of other nephrotoxic agents (e.g., aminoglycosides and cyclosporine), duration of therapy, and a mean daily dose of ≥35 mg/day (27,28). Amphotericin B deoxycholate-related nephrotoxicity has been shown to increase the duration of hospital stay and cost (27,29,30). Moreover, although nephrotoxicity associated with amphotericin B, deoxycholate has long been considered reversible after drug discontinuation; treatment leading to the need for hemodialysis has been shown to increase the risk of death (27,28).

A number of strategies have been utilized in an attempt to reduce the risk of nephrotoxicity related to treatment with amphotericin B deoxycholate. These include reduced dosage, alternate day therapy, the use of mannitol, saline loading, low-dose dopamine, and prolonging the infusion time (31–35). The use of low-dose dopamine has been common in many centers; however, a study by Camp et al shows no benefit in a group of high risk leukemia and BMT patients. In addition, there were signifi-
cantly more adverse events including cardiac arrhythmias in the dopamine treated patients (33). Reducing the dose of drug or giving the same dose on alternate days risks reduced efficacy, Mannitol has not been found to be particularly effective (31), but saline loading has been found to be beneficial (32). Eriksson et al. (34) found that they could reduce the risk of nephrotoxicity associated with amphotericin B deoxycholate by prolonging the infusion time to 24 hr. This reduced risk extended to slightly higher daily doses of the amphotericin B deoxycholate up to 2 mg/kg day in a follow-up study by Imhof et al. (35). The daily doses given in the second study did not equal the daily doses routinely achieved when amphotericin B is given as lipid formulation. Moreover, because of its incompatibility with electrolyte containing solutions, dedicating one intravenous line to amphotericin B deoxycholate over 24 hr each day may become problematic in complicated patients requiring multiple drug infusions (11).

Along with reduced glomerular filtration rate with resulting increase in serum creatinine, electrolyte losses are an important result of amphotericin B deoxycholate-related nephrotoxicity. Magnesium loss is a commonly overlooked occurrence and needs to be considered when amphotericin B-related hypokalemia appears to be refractory to replacement therapy.
Other toxicities that may occur with amphotericin B deoxycholate treatment include fatigue, anorexia, nausea, vomiting, and weight loss. Anemia due to hemolysis and/or renal insufficiency with suppression of erythropoiesis may also be seen. Leukoencephalopathy has been reported in patients receiving systemic chemotherapy, intrathecal chemotherapy, or whole brain radiation (36). Wright et al. (37) reported the development of severe pulmonary reactions leading to acute respiratory distress syndrome when granulocyte transfusions were given to a group of patients who simultaneously received treatment with amphotericin B deoxycholate. Other investigators have not been able to document the same pulmonary reaction when white cell transfusions and amphotericin B are given together, and therefore, the interaction between the two has been questioned (38-40). In the study by Wright et al. (37), all patients receiving white cell transfusions had concurrent Gram-negative sepsis. It has been speculated that if the relationship between white cell transfusions and the use of amphotericin B exists, the presence of Gram-negative endotoxin may be required as a cofactor, leading to excess trafficking of the infused white cells to the microvasculature in the lungs and subsequent respiratory distress. Regardless, when given together in the same patient, it has been suggested that the antifungal and white cells be given at least 4-12 hr apart.

Even in the setting of maximally tolerated doses of drug, treatment with amphotericin B deoxycholate frequently fails to control invasive fungal infections in the immunocompromised host. Mortality rates for invasive aspergillosis remain high for neutropenic patients with the majority of these severely immunocompromised patients dying of infection despite therapy (41-43).

III. LIPID FORMULATIONS OF AMPHOTERICIN B

Incorporation of the highly lipophilic drug amphotericin B into liposomes to improve its therapeutic index, was recognized almost two decades ago. New et al. (44) reported reduced amphotericin B-related toxicity associated with the use of liposomal-associated drug in an animal model of leishmaniasis. This was followed by reports from Graybill et al. (45) and Taylor et al. (46) that liposomal-associated amphotericin B could significantly reduce the toxicities associated with free drug, without compromising efficacy in animal models of cryptococcosis and histoplasmosis. Lopez-Berestein and Juliano developed a lipid formulation of this polyene by combining a mixture of the two phospholipids dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidyglycerol (DMPG) in a 7:3 molar ratio containing 5-10% mole ratio of amphotericin B and showed a significant improvement in therapeutic index, as compared to conventional drug in a neutropenic mouse model of disseminated candidiasis (47). They subsequently reported the preliminary compassionate use experience with this lipid formulation in patients treated at the MD Anderson Cancer Center (Houston, Texas). They suggested that life-threatening invasive fungal infections could be controlled even in patients who had failed to respond to treatment with conventional doses of amphotericin B deoxycholate. Moreover, the liposomal product they had created had significantly less nephrotoxicity than conventional amphotericin B even at higher doses (48,49). Similar findings were observed at the Institute Jules Bordet (Brussels, Belgium), in a group of cancer patients treated there with a small unilamellar liposomal formulation of amphotericin B (50). Although these initial products were not further developed, the use of lipid-based biotechnology by the pharmaceutical industry has led to the development
<table>
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<th>Compound</th>
<th>Brand name</th>
<th>Lipid configuration</th>
<th>Size (n,m)</th>
<th>Lipids</th>
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<th>Mean Vdss in mL/[min kg] (dosage)</th>
<th>Mean CL in mL/[min kg] (dosage)</th>
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<td>80</td>
<td>Increased</td>
<td>7.3 (1.0 mg/kg) 17.2 (2.5 mg/kg) 57.6 (5.0 mg/kg)</td>
<td>0.58 (1.0 mg/kg) 0.69 (2.5 mg/kg) 0.22 (5.0 mg/kg)</td>
<td>0.27 (1.0 mg/kg) 0.33 (2.5 mg/kg) 0.17 (5.0 mg/kg)</td>
<td>69 (1.0 mg/kg) 206 (2.5 mg/kg) 713 (5.0 mg/kg)</td>
<td>Increased</td>
<td>(51–53)</td>
</tr>
<tr>
<td>ABCD Amphocil</td>
<td>Disklike</td>
<td>Cholesteryl sulfate</td>
<td>120–140</td>
<td>Decreased</td>
<td>0.84 (0.5 mg/kg)</td>
<td>5.7 (0.5 mg/kg) 7.2 (1.0 mg/kg) 7.9 (1.5 mg/kg)</td>
<td>0.42 (0.5 mg/kg) 0.36 (1.0 mg/kg) 0.47 (1.5 mg/kg)</td>
<td>21 (0.5 mg/kg)</td>
<td>(49,52)</td>
<td></td>
</tr>
<tr>
<td>ABLC ABELCET</td>
<td>Ribbonlike</td>
<td>Dimyristoyl phosphatidylcholine, dimyristoyl phosphatidylglycerol</td>
<td>1,600–11,000</td>
<td>Decreased</td>
<td>0.27 (0.5 mg/kg) 1.1 (2.5 mg/kg)</td>
<td>3.9 (0.5 mg/kg) Not determined 1.3 (0.5 mg/kg)</td>
<td>Similar Decreased 2.8 (0.5 mg/kg) 8.9 (2.5 mg/kg)</td>
<td>Similar Decreased</td>
<td>(52,53,55,56)</td>
<td></td>
</tr>
</tbody>
</table>

Note. “Decreased,” “increased,” and “similar” are in reference to values for amphotericin B.
ABCD = amphotericin B colloidal dispersion; ABLC amphotericin B lipid complex; AUC = area under the curve; C pharmacokinetics in children; Cmax = maximum drug concentration; Cl = clearance; L-AMB liposomal amphotericin B; NA = not applicable; VdSS = volume of distribution.

*Model dependent.
of three commercially available lipid formulations of amphotericin B (amphotericin B lipid complex (ABLC), amphotericin B colloidal dispersion (ABCD), and liposomal amphotericin- [L-AMB]) that have been available for clinical use worldwide for the last several years (Table 1) (51–56). The lipid formulations of amphotericin B have the same antifungal spectrum as, but are significantly less nephrotoxic than, amphotericin B deoxycholate at doses up to 4–6 mg/kg day (24,57–63). With increased availability and experience in the use of the lipid formulations of amphotericin B, along with the newer broad spectrum azoles and the echinocandins, amphotericin B deoxycholate’s role as the “gold standard” has recently come in to question (64).

A. Amphotericin B Lipid Complex

ABLC, manufactured by Enzon Pharmaceuticals, Bridgewater, New Jersey, is similar to the original formulation of Berestein and Juliano in that it contains amphotericin B complexed with the two phospholipids DMPC and DMPG in a 7:3 molar ratio. ABLC, however, is a ribbon-like structure measuring 1600–11,000 nm in diameter and is not made up of liposomes. Studying a mixture of phospholipids and amphotericin B similar to the Berestein/Juliano product, Janoff et al. (65) at The Liposome Company discovered that it contained a variety of liposomal and nonliposomal structures of lipid bilayers. It appeared that the nonliposomal structures in the formulation that they called “ribbons” were responsible for the reduction in toxicity. A varying concentration of amphotericin B was mixed with the combination of phospholipids DMPC and DMPG in a fixed molar concentration of 7:3 Freeze-etch electron microscopy of formulations made with 0,5,25, and 50 mol% of amphotericin B showed significant differences in the structures formed (Fig. 2) (66,67). Amphotericin B in a 5 mole%
concentration resulted in a product similar to the original Berestein/Juliano mixture with both liposomes and ribbon-like structures. With increased concentration of amphotericin B, liposomes disappeared, leaving tightly packed ribbon-like structures. At concentrations $>50 \text{ mol}\%$ amphotericin B, the formulation appeared as ribbon structures; however, the lipids and total amphotericin B were no longer closely complexed, with the appearance of free amphotericin. ABLC was the name given to the formulation of ribbon-like structures created by complexing DMPC and DMPG in a 7:3 molar ratio with a 1:1 molar concentration of amphotericin B.

Hemolytic activity at low concentrations (<3 mole% of amphotericin B) was similar to conventional amphotericin B. As the concentration of amphotericin B was increased and the ribbon structures formed, there was a marked reduction in toxicity. As the ribbon structures formed, the amount of free amphotericin in solution decreased, presumably accounting for the reduced toxicity.

ABLC has been compared to amphotericin B in a number of animal models including mice, rats, rabbits, and dog. Amphotericin B concentrations in the liver, spleen, and lungs of mice and rats appear to be much higher after a single dose of ABLC than after a single dose of amphotericin B deoxycholate. Amphotericin B levels in the kidney of mice after injection of 1 mg/kg of ABLC appeared similar to levels achieved with conventional amphotericin B. However, plasma levels of amphotericin were significantly lower after dosing with ABLC as compared to amphotericin B deoxycholate. Moreover, when increasing the dose of the lipid complex, the levels of drug in the liver, spleen, and lung tissue rise dramatically with little change in the level of amphotericin found in the kidney and with essentially no rise in plasma levels (68–70). The LD$_{50}$ after a single i.v. dose for ABLC was found to be 40 mg/kg whereas for conventional amphotericin B it was 3 mg/kg. Multiple dose studies of ABLC continued to show reduced toxicity even at 10 times the standard dose of conventional amphotericin B in mice (69) and four times the standard dose in rabbits (71). The efficacy of ABLC was found to be comparable to conventional amphotericin B in a number of animal models of fungal infection. Moreover, in a number of cases, the lipid complex was found to be effective when conventional amphotericin B was found ineffective in controlling the fungal infection (68,69,72,73). As an example, in a model of experimental murine disseminated candidiasis, Mitsutake et al. (71) found that ABLC was as effective as conventional amphotericin B at doses of 0.5 and 1.0 mg/kg but more efficacious at 10 mg/kg. The ability to deliver much higher doses of amphotericin B in the form of lipid complex without reaching the maximum tolerated dose may account for the improved therapeutic index (68).

Bhamra et al. (74) reported results of pharmacokinetic studies comparing the behavior of ABLC relative to amphotericin B deoxycholate in plasma in vitro and in circulation in rats. Rat blood or plasma spiked with ABLC was assayed for amphotericin B released from the complex after centrifugation. At 0–15 min approximately 90% of amphotericin B remained complexed in the phospholipid formulation. The amphotericin B released from the complex was found to be associated with plasma lipoprotein and nonlipoprotein proteins. The area under the curve (AUC$_{0-24\text{hr}}$) for total amphotericin B in whole blood of rats given a single i.v. dose of 1 mg ABLC per kg of body weight was four-fold lower than that in rats given 1 mg of amphotericin B deoxycholate per kg. Complexed amphotericin B was rapidly removed from the circulation and was distributed to the tissues. Further study looked at rats treated intravenously with 10 mg/kg/day of ABLC compared to 0.5 mg/kg/day of amphotericin B deoxycholate for 15 days. Blood samples taken
at 15 and 180 min after the last dose of drug, showed total levels of amphotericin B in rats administered ABLC to be 3–5 times greater than those given amphotericin B deoxycholate. The concentration of uncomplexed, protein bound amphotericin B in plasma of ABLC treated rats was only 1–2 times that of those treated with amphotericin B deoxycholate, despite a 20-fold difference in dose given. The rapid uptake of amphotericin B by tissues in the form of the lipid complex and the very low levels of circulating protein-bound amphotericin B in plasma was suggested to possibly account in part for the improved therapeutic index of this lipid formulation.

Pharmacokinetics of ABLC in humans resembles those in animals. Circulating blood levels of amphotericin B were much lower in male volunteers after single-dose infusion of ABLC than after conventional amphotericin B deoxycholate (55). The lipid complex is believed to be rapidly taken up by the reticulo-endothelial system and concentrated in the liver, spleen, lungs, and other tissues of the body. Tissue levels of amphotericin B were measured at autopsy of a heart transplant patient after 3 days of treatment with ABLC. Relatively high concentrations of amphotericin were detected in the spleen (290 g/g), liver (196 g/g), and lungs (222 g/g). Lower concentrations were detected in kidney (6.9 g/g), lymph nodes (7.6 g/g), brain (1.6 g/g), and heart (4.9 g/g) (75).

The safety, tolerance, and pharmacokinetics of ABLC were studied in a cohort of pediatric patients enrolled in a phase I/II trial (54). Six children received ABLC at 2.5 mg/kg/day for 6 weeks for a total dose of 105 mg/kg for the treatment of hepatosplenic candidiasis. Mean baseline serum creatinine of 0.85 ± 0.12 mg/dL was stable at the end of therapy at 0.85 ± 0.18 mg/dL and at 1-month follow-up at 0.72 ± 0.12 mg/dL. There was no increase in transaminases. Mean plasma concentrations over the dosing interval and AUC_{0–24 hr} increased between doses 1 and 7 but were similar between doses 7 and 42, suggesting that a steady state was achieved by day 7 of therapy. After the final dose of ABLC therapy (42nd), mean AUC_{0–24 hr} was 11.9 ± 2.6 g/mL/hr, mean plasma concentration over the dosing interval was 0.50 ± 0.11 g/mL, maximum concentration of drug was 1.69 ± 0.75 g/mL, and clearance was 3.64 ± 0.78 (mL/kg/min). Response of hepatic and splenic lesions was monitored by serial computed tomographic and magnetic resonance imaging scans. Five patients evaluated for response to ABLC showed complete or partial resolutions of physical findings and radiographic lesions of infection. During the treatment course, there was no evidence of progression of infection, breakthrough fungemia, or recurrence of hepatosplenic candidiasis post-therapy. Hepatic lesions continued to resolve even after completion of treatment with ABLC. This study suggests that ABLC administered in multiple doses was safe and effective in the treatment of children with hepatosplenic candidiasis.

ABLC has been given in substantially larger doses than conventional amphotericin B. When given over several months, these large doses of amphotericin B appear to be relatively less toxic than those of conventional amphotericin B. Six patients with invasive fungal infection were reported by Kline et al. (76) to have received large cumulative doses (23.3–73.6 g) of ABLC over 21–121 weeks. These patients were reported to tolerate the therapy well. Although the mean serum creatinine level for this group of patients rose from 1 mg/dL (range, 0.4–1.9 mg/dL) at the start of ABLC to 1.5 mg/dL (range, 1.0–2.0 mg/dL) at the end of therapy, none had dose-limiting toxicity necessitating discontinuation of treatment.

ABLC has been compared to conventional amphotericin B in a number of small phase II and III clinical trials of patients with coccidiodomycosis and cryptococcosis. In a study by Sharkey et al. (77), ABLC was studied in a sequential dose
escalation for the treatment of cryptococcosis in HIV-infected patients. Fifty-five patients were randomly assigned to 6 weeks of therapy with ABLC (1.2–5.0 mg/kg/day, with ascending doses for three sequential cohorts) or conventional amphotericin B (0.7–1.2 mg/kg/day). Forty-six patients received 12 or more doses. Transfusion requirements, mean decreases in blood hemoglobin, and mean increases in serum creatinine were significantly greater in patients treated with conventional amphotericin B when compared to ABLC. The total number of adverse events, infusion-related events, and occurrences of hypomagnesemia and hypokalemia were similar in the two groups of patients. Among patients treated with ABLC at a dose of 5 mg/kg (daily for 2 weeks and then three times per week for 4 weeks), symptoms and signs of infection resolved in 18 patients (86%). This study suggests that ABLC is effective in the treatment of HIV-related cryptococcal meningitis and is associated with less hematologic and renal toxicity when compared to conventional amphotericin B.

ABLC was compared to amphotericin B deoxycholate in a randomized, controlled trial of the treatment of invasive candidasis (57). Two hundred and thirty-one patients from 27 centers were randomized (2:1) to receive ABLC 5 mg/kg/day vs. amphotericin B 0.6–1.0 mg/kg/day for hematogenous and invasive candidiasis. One hundred and fifty-three patients were assigned to treatment with ABLC and 78 to amphotericin B. Response rates (68% for ABLC vs. 68% for amphotericin) were not significantly different (P > 0.5). Nephrotoxicity; however, was less significant with ABLC. In patients treated with ABLC, serum creatinine doubled by the end of therapy in 28%, when compared to 47% of patients treated with conventional amphotericin B (P = 0.007).

There is extensive experience with the use of ABLC in emergency-use protocols for patients with invasive fungal infections, who were felt to be refractory or intolerant to treatment with amphotericin B deoxycholate. Walsh et al. (78) reviewed the safety and antifungal efficacy of ABLC in 556 cases of invasive fungal infection treated on single patient emergency use protocols for patients refractory or intolerant of conventional antifungal therapy. In order to be eligible to receive therapy with ABLC, patients had to have met one of the following criteria: (1) patients must have failed treatment with previous systemic antifungal therapy, including amphotericin B at a cumulative dose of at least 500 mg; (2) developed nephrotoxicity defined as a serum creatinine of 2.5 mg/dL in adult or 1.5 mg/dL in children while undergoing antifungal therapy with amphotericin B or other drugs; (3) had severe acute toxicity secondary to amphotericin B or (4) had pretreatment renal insufficiency defined as a serum creatinine of 2.5 mg/dL or creatinine clearance rate of <25 mL/min precluding treatment with amphotericin B. In this open-labeled study, serum creatinine levels decreased significantly from baseline (P < 0.02) during the course of ABLC treatment. Moreover, in the 162 patients who began therapy with a baseline serum creatinine 2.5 mg/dL, the mean serum creatinine value decreased significantly from the first to the sixth week of treatment (P < 0.0003). There was either complete or partial response to treatment with ABLC in 57% (167/291) of cases of mycologically confirmed invasive fungal infection, which were evaluable for therapeutic response. This included 55/130 (42%) cases of aspergillosis, 28/42 (67%) cases of disseminated candidiasis, 17/24 (71%) cases of zygomycosis, and 9/11 (82%) cases of fusariosis.

Wingard (79) reported the results of efficacy and toxicity from a subgroup of 95 bone marrow transplant recipients with presumed or documented invasive fungal infection treated with ABLC on this open-label emergency use clinical trial. Seventy one (75%) had undergone allogeneic bone marrow transplantation and 24 (25%)
had received an autologous transplant. The most common underlying diagnosis before transplant was leukemia (59 patients). Forty-one patients were neutropenic (<500/mm³) at baseline. Fifty-nine patients undergoing bone marrow transplantation were felt to be clinically evaluable for response. Thirty-one patients (53%) responded to treatment: 23 patients (39%) were designated as cured, and eight patients (14%) were designated as improved. All 95 patients were evaluable for nephrotoxicity. Overall, only two patients discontinued therapy due to renal toxicity. Moreover, for 30 patients who began ABLC treatment with a serum creatinine of >221 mol/L, significant improvement in renal function was observed at weeks 1–3 (P < 0.01) and 6 (P < 0.001). Trends in serum creatinine during ABLC therapy between autologous and allogeneic transplant recipients were similar.

In a preliminary report of the treatment of invasive aspergillosis (80), 151 patients treated with ABLC on the emergency-use protocols were compared to medical records of 122 control patients treated with conventional amphotericin B deoxycholate. Complete and partial responses were seen in 43% of patients treated with ABLC vs. 23% of patients treated with amphotericin B deoxycholate. Moreover, in patients with a baseline serum creatinine of 2.5 mg/dL at the beginning of treatment with ABLC, significant decreases in serum creatinine were observed at 2 and 5 weeks (P < 0.004) despite continued therapy at 5 mg/kg/day. Although historical control trials are difficult to interpret, these data suggest that ABLC is effective in the treatment of invasive aspergillosis, and less nephrotoxic than treatment with amphotericin B deoxycholate. These include infections due to *Fusarium* species and *Zygomycetes* (81), in which conventional amphotericin B rarely controls disease and the value of ABLC for individual patients was particularly apparent.

Sundar and Murray (82) reported the use of ABLC in 21 Indian patients with visceral leishmaniasis, who did not respond to or relapsed after 28–60 days of pentavalent antimony therapy. Five infusions (3 mg/kg each), given every second day over 9 days (total dose, 15 mg/kg), resulted in a curative response in all patients treated with this regimen. In four other patients who had not responded to antimony, an apparent cure was also induced by ABLC, given 3 mg/kg/day for 5 consecutive days (total dose, 15 mg/kg). Fever and chills developed routinely during the initial 2-hr infusions; however, these reactions were tolerated and diminished with successive infusions. Six months after treatment, all 25 patients were healthy, had parasite-free bone marrow aspirates, and were considered cured.

Based upon current data, ABLC is considered to be active against a variety of invasive mycoses in immunocompromised hosts even in the setting of prior failure of amphotericin B deoxycholate. ABLC is also less nephrotoxic than conventional therapy, which may account for its improved therapeutic index. This drug is currently approved for clinical use in the United States, as well as numerous countries worldwide, for patients with invasive fungal infections who are refractory or intolerant to treatment with amphotericin B deoxycholate. Additional clinical study of ABLC is in progress to further define its role in the treatment of life-threatening fungal infections.

### B. Amphotericin B Colloidal Dispersion

Amphotericin B colloidal dispersion (InterMune, Brisbane, California) is a combination of amphotericin B and cholesteryl sulfate in a 1:1 ratio. This formulation of amphotericin B forms disk-like structures approximately 115 nm in diameter when combined with the cholesteryl sulfate (83). The discs are made up of aggregates of
tetramers of amphotericin B and cholesteryl sulfate coalesced into spiral arms. Each
tetramer consists of two molecules of amphotericin B with two molecules of chole-
steryl sulfate with a hydrophobic core and hydrophilic regions exposed to water
(Fig. 3). Hanson and Stevens (84) documented in vitro activity of ABCD against
41 isolates of 15 pathogenic species of fungus. Mean inhibitory concentrations and
mean fungicidal concentrations of ABCD appeared similar to those of conventional
amphotericin B.

Plasma levels of amphotericin B after a single intravenous bolus injection in the
rat are significantly less at 1 hr when ABCD is compared with amphotericin B deox-
ycholate. The half-life of the lipid formulation, however, is much longer, and its
volume of distribution is much greater. Tissue concentrations of amphotericin B
in the liver are 2–3 times higher after injection of ABCD vs. amphotericin B deox-
ycholate. At the same time, amphotericin B concentrations in the kidney are signifi-
cantly reduced even after 5 mg/kg of the colloidal dispersion (85). Similar findings of
lower plasma concentrations, increased liver deposition, and decreased kidney con-
centration of amphotericin B have been reported in the dog model (86). Dosages of
as much as 5 mg/kg/day could be given to dogs before ABCD produced adverse
effects similar to those of 0.6 mg/kg/day of amphotericin B deoxycholate.

The efficacy of ABCD compared with amphotericin B deoxycholate has been
studied in animal models of coccidioidomycosis, cryptococcosis, and aspergillosis
(73,87,88). Despite lower doses of amphotericin B deoxycholate (1–3 mg/kg)
required to clear organs of fungus in a murine model of coccidioidomycosis com-
pared with ABCD (5.0 mg/kg), conventional amphotericin B was found to be 5–8
times more toxic resulting in an improved therapeutic index for the lipid formulation
(73).

In a murine model of cryptococcosis, ABCD was found to have equal efficacy,
compared to amphotericin B on a milligram per kilogram basis. ABCD was also
found to be less toxic, again resulting in an improvement in therapeutic index for

Figure 4  The putative structure of amphotericin B colloidal dispersion (ABCD). Source:
From Ref. 137.

Cholesteryl Sulfate  Amphotericin B
the lipid formulation when compared to amphotericin B deoxycholate. ABCD treatment of persistently granulocytopenic rabbits in a model of pulmonary aspergillosis resulted in improved survival when comparing 5 mg/kg/day of ABCD and 1 mg/kg/day of amphotericin B deoxycholate (88). The improved therapeutic index may be because of an enhanced rate of tissue clearance, decreased pulmonary injury, and reduced nephrotoxicity in the rabbits treated with 5 mg/kg/day of ABCD. These findings were further confirmed in a study of the evolution of pulmonary infarcts in experimental pulmonary aspergillosis. Through ultrafast computed tomographic scanning and an image-analysis algorithm, a dose-dependent clearance of pulmonary infiltrates in rabbits treated with ABCD was found (89). In an immunocompromised rabbit model of experimental disseminated aspergillosis, Patterson et al. (90) again demonstrated a dose-dependent response of ABCD. Animal models of both disseminated and pulmonary invasive aspergillosis suggested that ABCD was less effective than amphotericin B deoxycholate in tissue clearance of fungus at equal doses of 1 mg/kg (88–90).

Bowden et al. (91) reported results of a phase I sequential dose-escalation study of ABCD in 75 bone marrow transplant recipients with invasive fungal infections (primarily Aspergillus or Candida species). This study was designed to evaluate the toxicity profile, maximum tolerated dose, and clinical response to ABCD. Dosages were escalated from 0.5–8.0 mg/kg/day in 0.5 mg/kg per patient increments with an upper limit of 6 weeks of treatment duration. No infusion-related toxicity was observed in 32% of the patients; 52% had grade 2 toxicity, and 5% had grade 3 toxicity. Significant renal toxicity was not observed at any dose level. Maximum tolerated dose was considered to be 7.5 mg/kg, based upon rigors, chills, and hypotension in three of five patients at 8.0 mg/kg. The overall complete or partial response rate across dose levels and infection types was 52%, 53% of patients with fungemia had complete responses, and 52% of patients with fungal pneumonia had complete or partial responses. ABCD was considered to be safe at doses up to 7.5 mg/kg, with tolerable infusion-related toxicity and documented antifungal activity in this patient population.

The safety of ABCD in five open-label phase I and II clinical trials was recently reviewed by Herbrecht (92). In a total of 572 selected patients treated ABCD for invasive fungal infections, ABCD was administered to 442 patients after therapy with amphotericin B. In 192 patients, conventional amphotericin B had been withdrawn because of toxicity. One hundred and forty patients had pre-existing nephrotoxicity. No alterations in serum creatinine were seen with dosages of ABCD as high as 6 mg/kg/day even in those patients with pre-existing renal failure. ABCD therapy also resulted in no significant changes in liver function from baseline as measured by serum levels of aspartate aminotransferase, alkaline phosphatase, and total bilirubin in comparison. Apart from thrombocytopenia, there was no significant alteration in hematologic parameters. Adverse events attributable to ABCD requiring discontinuation of therapy occurred in 70 patients (12.2%). The most toxicity resulting in discontinuation of ABCD in this group of patients was infusion-related adverse events, occurring in 5.4% of patients.

White et al. (93), retrospectively, compared the records of 82 patients with proven or probable aspergillosis who were treated in clinical trials with ABCD with 261 patients with aspergillosis who were treated with amphotericin B, at six cancer or transplantation centers, between January 1990 and June 1994. Although the groups were balanced in terms of underlying disease, ABCD recipients were younger and more likely to have pre-existing renal insufficiency than amphotericin B recipients.
Amphotericin B recipients were more likely to be neutropenic at baseline than ABCD recipients (42.5% vs. 15.9%). Patients in the ABCD treated group had higher response rates (48.8%) and survival rates (50%) than those patients treated only with conventional amphotericin B deoxycholate (23.4% and 28.4%, respectively) \((P < 0.001\) for both comparisons). ABCD recipients were less likely to develop renal impairment when compared to amphotericin B deoxycholate recipients (8.2% vs. 43.1%, respectively; \(P < 0.001\)). ABCD was considered to be less nephrotoxic and similarly efficacious as amphotericin B deoxycholate in this study of the treatment of invasive aspergillosis. A prospective double-blind, randomized, and controlled multicenter trial comparing conventional amphotericin B and ABCD (at a dose of 6 mg/kg/day) in patients with invasive aspergillosis was recently reported by Bowden et al. (59). Although no significant difference in overall efficacy was noted, ABCD was significantly less nephrotoxic than amphotericin B deoxycholate. Another phase III trial compared ABCD and conventional amphotericin B for empiric treatment of the persistently febrile neutropenic patient (58). Although this study again showed that ABCD was less nephrotoxic, this lipid formulation had increased infusion related toxicity when compared to amphotericin B deoxycholate.

C. Liposomal Amphotericin B, L-AMB (AmBisome™)

L-AMB (Gilead Sciences, Inc., San Dimas, CA) is the third lipid formulation of amphotericin B to be approved by the Food and Drug Administration for clinical use in the United States. It has been used for the treatment of proven or suspected invasive fungal infections in numerous countries worldwide. This formulation of amphotericin B differs from ABLC and ABCD in that the lipids involved form small unilamellar lipid vesicles (true “liposomes”) that are uniform and spherical in size, averaging 60–70 nm. The lipid bilayer is made up of hydrogenated soy phosphatidylcholine and distearoyl phosphatidylglycerol, stabilized by cholesterol, and combined with lipophilic amphotericin B in a 2:0.8:1:0.4 molar ratio (94) (Fig. 4). Antifungal activity of L-AMB in vitro was found to be comparable to that of amphotericin B when a number of clinical isolates from a large cancer center were tested (95).

A number of animal models have been used to study the pharmacokinetics, toxicity, and efficacy of L-AMB (96–104). Pharmacokinetic evaluation of liposomal amphotericin B in mice, rats, and rabbits revealed similar peak plasma levels. Similar to the other lipid formulations of amphotericin B, L-AMB is preferentially concentrated in the liver and spleen of animals. The rate of uptake by the reticulo-endothelial system; however, appears to be much slower than that of ABLC or ABCD. It is hypothesized that the larger lipid complexes and dispersions may be more readily phagocytosed by the macrophages of the reticulo-endothelial system when compared with the smaller unilamellar vesicles. The negative charge on the L-AMB particle also may delay uptake by the reticulo-endothelial system. These mechanisms may account for the much higher peak plasma levels and prolonged circulation time of the liposomal form of amphotericin B as opposed to its larger counterparts.

L-AMB has been found to be less nephrotoxic than conventional amphotericin B in mice, rats, and rabbits. There does however, appear to be a slight rise in liver transaminases with repeated infusions of liposomal amphotericin B at high dosages in rodents (101). The LD\(_{50}\) after a single injection of liposomal amphotericin B was \(>175\) mg/kg in mice and 50 mg/kg in rats. This LD\(_{50}\) was 30–60 times greater than that of a single injection of conventional amphotericin B (96). Boswell et al. (101)
recently reported on the pharmacokinetics and toxicity profile of L-AMB in a rat model. Single and multiple dose pharmacokinetics were evaluated for doses of 1, 3, 9, and 20 mg/kg/day. Mean plasma amphotericin B concentrations reached 500 and 380 g/mL (males and females respectively) following 30 days of L-AMB at 20 mg/kg. The overall apparent half-life was 11.2 ± 4.5 hr (males) or 8.7 ± 2.2 hr (females). The overall clearance was 9.4 ± 5.5 mL/hr/kg (males) and 10.2 ± 4.1 mL/hr/kg (females). L-AMB appeared to have a saturable disposition. This resulted in a nondose proportional AUC for amphotericin B and a lower clearance at higher doses. Histopathological evaluation revealed transitional cell hyperplasia of the epithelium of the urinary tract that was dose dependent. Moderate hepatocellular necrosis was seen at the highest dose (20 mg/kg/day). However, the toxicities seen in this animal model were considered to be considerably less than that expected with amphotericin B at much lower doses.

Murine models of disseminated candidiasis, cryptococcosis, and blastomycosis have been used to study the efficacy of L-AMB. Gondal et al. (102) showed survival benefit in mice infected with *Candida albicans*, if treated early with all doses of liposomal amphotericin B studied except for 1 mg/kg. Delay in treatment until 3 days after inoculation, required 5 mg/kg of the drug to achieve optimal benefit. The maximum dose of conventional amphotericin B given to mice by single injection was 2 mg/kg, with 1.5 mg/kg/day the maximum tolerated dose with multiple injections. Although Phals and Schaffner (104) found L-AMB to be significantly less toxic in their mouse model of candidiasis, these investigators suggested that liposomal amphotericin B was 4–8 times less active in clearing the infection. L-AMB was given in divided daily doses in this study, and may have altered the pharmacodynamics of the compound.

A study by Francis et al. (103) reported the results of a comparison of amphotericin B deoxycholate with the small unilamellar liposomal form of amphotericin B in a neutropenic rabbit model of pulmonary aspergillosis. Rabbits were evaluated for survival, lung tissue infection, and hemorrhagic pulmonary lesions by the use of ultrafast CT scans. Treatment with the liposomal form of amphotericin was studied at 1, 5, and 10 mg/kg, compared with 1 mg/kg of amphotericin B deoxycholate. Although all doses of L-AMB showed survival benefit compared with amphotericin B, the rate of reduction of pulmonary injury was greatest above 5 mg/kg/day. Although the 10 mg/kg/day dosage was capable of irradiating tissue infection, it was found to be more nephrotoxic. Based on these findings, 5 mg/kg/day was proposed as the optimal dosage between safety and efficacy in this model of pulmonary aspergillosis.

The pharmacokinetics, safety, and tolerance of L-AMB were studied in 24 persistently febrile neutropenic patients receiving this liposomal formulation of amphotericin B as empiric antifungal therapy in a sequential dose-escalation study of 1.0, 2.5, and 5.0 mg/kg (105). Serial measurements of serum creatinine, potassium, and magnesium were not significantly changed from baseline, and there was no net increase in hepatic transaminases during the duration of therapy. There were, however, increases in serum bilirubin and alkaline phosphatase levels in patients from all dosage groups. L-AMB followed a nonlinear dosage relationship that was consistent with reticuloendothelial uptake and redistribution. This study demonstrates that L-AMB was safe and well tolerated when administered as empirical antifungal therapy in febrile neutropenic patients receiving cytotoxic chemotherapy. This study is not designed to assess efficacy; however, no breakthrough fungal infections developed during the course of empiric antifungal treatment with L-AMB.
Pharmacokinetics of L-AMB in 10 patients treated with dosages of 2.8–3.0 mg/kg/day were compared with the pharmacokinetics observed in six patients treated with amphotericin B deoxycholate at a dosage of 1.0 mg/kg/day by Heinemann et al. (106). When administered approximately three-fold greater doses of amphotericin B as L-AMB formulation, patients were found to have a median maximal concentration of drug 8.4-fold higher (14.4) than that in patients treated with amphotericin B deoxycholate (1.7 g/mL). The median AUCs in the L-AMB treated patients also exceeded the AUCs in patients treated with amphotericin B deoxycholate by nine-fold. This was partly explained by a 5.7-fold lower volume of distribution (0.42 L/kg) in L-AMB treated patients ($P = 0.001$). Elimination of amphotericin from the serum was biphasic for both liposomal and deoxycholate amphotericin B. Compared to amphotericin B deoxycholate; however, the plasma half-life of L-AMB was twice as short ($P = 0.003$). L-AMB was less nephrotoxic than conventional amphotericin B, despite being given at higher dosages in this study.

European investigators have had extensive experience with the use of L-AMB in immunocompromised patients with proven or suspected invasive fungal infections. Much of this experience has been published as the results of a number of phase II clinical trials (106–110). Similar to the initial clinical experience with ABLC and ABCD in the United States, patients in Europe were eligible to receive therapy with liposomal amphotericin B if they failed to respond to or could not tolerate treatment with amphotericin B deoxycholate. Additionally, they could also receive the lipid formulation if they had significant underlying renal insufficiency before antifungal therapy was started. The most common fungal pathogen isolated was Candida species and Aspergillus species. Patients with underlying malignancies, AIDs, and bone marrow and solid organ transplant recipients were the most commonly treated. Even in patients with documented invasive fungal infections who had previously failed treatment with amphotericin B deoxycholate, clinical responses were noted. Neutropenic patients also appeared to respond to therapy, although recovery from neutropenia, remission from underlying malignancy, and continued therapy with the liposomal form of amphotericin B appeared to be necessary for resolution of the fungal infection. Pediatric experience with the use of L-AMB in immunocompromised children has also been published (111–115), suggesting efficacy and safety in this patient population.

Prentice et al. (60) conducted a randomized phase III clinical trial in Europe comparing the empirical use of conventional amphotericin B deoxycholate vs. L-AMB for patients with persistent fever and neutropenia unresponsive to antibacterial therapy. 134 adults and 204 children were randomized in two separate, but parallel prospective, multi-institutional trials. Patients were eligible for study if they were neutropenic ($< 500 / \text{mm}^3$) and had fever of unknown origin ($> 38^\circ \text{centigrade}$) for more than 96 hr not responding to antibacterial antibiotics. They were randomized to receive 1 mg/kg/day amphotericin B deoxycholate, 1 mg/kg/day liposomal amphotericin B, or 3 mg/kg/day of liposomal amphotericin B. In patients not receiving concurrent nephrotoxic agents, no one treated with 1 mg/kg/day was observed to have a doubling of serum creatinine from baseline. 3% of patients treated with 3 mg/kg/day were found to double their serum creatinine from baseline as opposed to 23% of patients treated with amphotericin B deoxycholate. Analysis of breakthrough fungal infections and time to resolution of fever revealed no overall difference between the study arms. The authors concluded that L-AMB was significantly less toxic than conventional amphotericin B deoxycholate.
Walsh et al. (24) reported the results of a multicenter trial comparing empirical antifungal therapy with L-AMB vs. amphotericin B deoxycholate in a similar patient population in North America. Unlike the European trial, this study was double-blind and was designed to have improved statistical power to assess antifungal efficacy. A total of 687 patients were randomized to receive empirical antifungal therapy with either conventional amphotericin B (CAB) or liposomal amphotericin B (L-AMB). The two arms of the study were assessed for both antifungal efficacy as well as safety. Overall survival rates (93% for L-AMB vs. 90% for CAB) and fever resolution during neutropenia (58% for L-AMB vs. 58% for CAB) were similar in both arms of the study. However, there was a significant reduction in the development of breakthrough fungal infections in patients treated with L-AMB as compared to amphotericin B deoxycholate (5% L-AMB vs. 9% CAB) \((P = 0.021)\). Treatment with L-AMB was associated with significantly less infusion related fever, including increases in temperature of \(1^\circ\) Centigrade \((P \leq 0.01)\), chills and rigor \((P \leq 0.01)\), and cardiorespiratory events such as dyspnea, hypotension, hypertension, tachycardia and hypoxia \((P = 0.01)\) when compared with conventional amphotericin B. All patients in the North American study were assessed for nephrotoxicity regardless of concurrent treatment with other nephrotoxic agents. Only 19% of patients treated with L-AMB vs. 34% of patients treated with CAB developed a significant rise in serum creatinine from baseline defined as, in children a rise \(>2\) times baseline, and in adults a rise \(>2\) times baseline, along with a rise \(\geq 1.2\) mg/dL. This double-blind, randomized controlled trial confirmed the superior safety profile of liposomal amphotericin B previously seen in European trials. Moreover, the larger number of patients included in the North American study allowed improved assessment of efficacy. Although there was no overall difference in outcome, there was a statistically significant decrease in the number of breakthrough fungal infections in patients treated with L-AMB.

There are only a few published reports of prophylactic studies of lipid formulations of amphotericin B to date. Tollemar et al. (116) has reported the results of a double-blind, randomized trial comparing L-AMB at 1 mg/kg/day with placebo for antifungal prophylaxis in a group of patients undergoing bone marrow transplantation in Sweden. 84 allogeneic and 15 autologous bone marrow transplant recipients were entered on this phase III study. There was no significant difference in the incidence of documented invasive fungal infection in patients receiving prophylactic L-AMB as compared to patients receiving placebo (3% vs. 8%, respectively). All documented fungal infections were in allogeneic transplant recipients. There was no survival advantage seen in the group of bone marrow transplant patients treated prophylactically with liposomal amphotericin B even in the subset of allogeneic transplant recipients. The authors suggest that the low incidence of invasive fungal infection and the small number of bone marrow transplant patients entered in the study made it difficult to show benefit from antifungal prophylaxis with L-AMB. Further study of antifungal prophylaxis with lipid formulations of amphotericin B in patients at higher risk for invasive fungal infection, such as recipients of unrelated donor marrow or those who develop graft vs. host disease post-transplant, are in progress.

Tollemar et al. (117) also reported the results of a trial of liposomal amphotericin B in liver transplant recipients. A reduction in invasive fungal infections was found, but the benefit was mostly because of a decrease in Candida infections, a result that one surmises could be achieved with fluconazole, at less expense and toxicity. Liver transplant patients with certain risk features are at high risk for invasive fungal infections. The utility of lipid amphotericin B formulations was evaluated in
an historical control study recently (118). In this very high-risk subset of patients, there were suggestions of benefit, especially seen in patients receiving hemodialysis. Lung transplant recipients are particularly susceptible for invasive aspergillosis. These appear to invade tissue via the airway, especially at the anastomosis site. Perfect and colleagues have conducted several trials of delivery of amphotericin directly to the site of infection by aerosolization of amphotericin B or amphotericin B in lipid complex. Early results appears promising and the lipid formulation is better tolerated and appears more easily delivered to the lower respiratory tract (119).

Patients with undergoing induction therapy for acute myelogenous leukemia are especially susceptible for *Candida* and *Aspergillus* infections. In a randomized trial of liposomal amphotericin B, given at a dose of 3 mg/kg three times weekly compared with the combination of fluconazole plus itraconazole, both groups had very low rates of invasive fungal infections and the rates of infection were not different (120). However, there was significantly more nephrotoxicity and elevated levels of bilirubin with liposomal amphotericin B. Leukemic patients who have had invasive *aspergillus* infections, which have been treated but require additional antineoplastic therapy, are at very high risk for recurrence. “Secondary” prophylaxis with amphotericin B has been shown to be associated with a reduced rate of reactivation; similarly, several case series show that lipid amphotericin B has a similar protective benefit (121).

Cryptococcal meningitis is one of the AIDS-defining illnesses in patients infected with HIV. Initial therapy with intravenous amphotericin B deoxycholate followed by oral fluconazole has been considered standard therapy for this fungal infection for the last several years. A phase III randomized trial of L-AMB at a dose of 4 mg/kg/day or conventional amphotericin B deoxycholate at 0.7 mg/kg/day for 3 weeks followed by oral fluconazole 400 mg/day for 7 weeks in 28 evaluable patients with AIDS-related cryptococcal meningitis was conducted by Leenders et al. (61) in the Netherlands. Patients treated with L-AMB and amphotericin B deoxycholate were found to have similar rates of clinical response; however, treatment with liposomal amphotericin B showed a more rapid conversion of CSF fungal culture to negative ($P < 0.05$; median time between 7 and 14 days for L-AMB vs. >21 days for amphotericin B deoxycholate by Kaplan–Meier estimate).

Similar to ABLC, liposomal amphotericin B has been studied for the treatment of cutaneous and visceral leishmaniasis in both experimental and clinical settings (122,123).

### D. Lipid Emulsion Mixtures of Amphotericin B Deoxycholate

The development of each of the lipid formulations of amphotericin B previously described has taken as much as a decade before they were approved for routine clinical use. The processes involved in their preparation and quality control are complex and expensive. For these reasons, a number of investigators have studied the use of an admixture of commercially available lipid emulsion in a 20% with conventional amphotericin B. Kirsh et al. (124) reported reduced amphotericin B toxicity without loss of antifungal activity in a murine model of murine candidiasis when amphotericin B was mixed with lipid emulsion; Chavanet et al. (125) recently reported improved efficacy and reduced toxicity in a neutropenic rabbit model of candidiasis. Randall et al. (126), however, found no difference in the degree of nephrotoxicity between
amphotericin B deoxycholate prepared in 5% dextrose vs. 20% fat emulsion in a canine model of adult male Beagles.

Limited pharmacokinetic studies suggest that the combination of conventional amphotericin B with lipid emulsion has a similar profile to ABLC or ABCD, with lower peak concentrations and AUC values in serum than conventional amphotericin B, corresponding to faster deposition of the lipid emulsion amphotericin B mixture in tissues (106,127). There have been several reports describing the clinical use of this mixture for HIV-infected patients with candidiasis or cryptocococcol meningitis, as well as in patients with fever and neutropenia (125,128–132). Although Lopez et al. (133) found that amphotericin B at concentrations of 1 and 2 mg/mL were stable in 20% fat emulsion for four days at 20–25°C exposed to fluorescent light, studies by Trissel (134) as well as Ranchere et al. (135) have found this mixture to be quite unstable. Even when amphotericin B was first diluted in 5% dextrose, the amphotericin B/intralipid combination was found to be unstable with the development of a yellow precipitate. Despite the suggestion by Shadkhan et al. (136) that the mixture can be stabilized by extended agitation, methods of drug preparation have not been standardized. The admixture of this amphotericin B with fat emulsion in the pharmacy has not been approved by the U.S. Food and Drug Administration, and exposure of patients to parenteral lipid formulations may carry the risk of infections, coagulopathy, and hepatic disease. Further study of standardized preparations of this mixture are required to define the safety and efficacy of this combination before it can be recommended for routine clinical use.

E. Comparisons of the Lipid Formulations of Amphotericin B

Although there is general agreement that all of the lipid formulations are less nephrotoxic than amphotericin B deoxycholate, a number of questions still remain unanswered. Although there are clear differences in the structure and pharmacokinetics of the lipid formulations of amphotericin B (137,138), what clinical significance does this have, if any (139,140)? Two small controlled trials showed liposomal amphotericin B to be less nephrotoxic than ABLC, however, severe nephrotoxicity leading to the need for hemodialysis was rare with both products (141,142). Although infusion-related reactions are least common with liposomal amphotericin B (24,58,141), reactions such as chest, back, or flank pain have been seen in up to 5% of patients treated with this formulation (143). Does dosage make a difference? Although neutropenic animal models of candidiasis have not shown superior efficacy, models of disseminated aspergillosis have suggested an advantage to the higher daily doses of amphotericin B that can be achieved by administering the drug in a lipid formulation. Unfortunately, despite dose-excalation studies showing safety at doses as high as 15 mg/kg/day (144), clinical trials have not shown superior efficacy with higher doses of lipid formulation (59,145). Studies of empirical therapy have also used doses of 1–3 mg/kg/day. Although there appeared to be no difference in efficacy between 3 and 5 mg/kg/day in one study (141), 1 mg/kg/day appeared less effective in terms of defervescence as compared to 3 mg/kg/day in another study. Current recommendations at this point suggest that higher dosages in the range of 4–6 mg/kg/day be utilized in the setting of documented infections, particularly with invasive aspergillosis and other filamentous fungal infections. Lower doses in the range of 1–3 mg/kg/day may be reasonable for empirical therapy (146).
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The Systemically Acting Azoles

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I. INTRODUCTION

Ketoconazole, the first oral systemically actingazole, was introduced in 1981 and at the time it represented an advance in antifungal therapy. Prior to this, choices of systemic antifungal therapy were limited to intravenous (IV) amphotericin B or miconazole, oral flucytosine. All these agents were associated with significant toxicity, and flucytosine had a narrow spectrum of activity. The use of ketoconazole was limited over time as a result of the subsequent introductions of fluconazole in 1990, and itraconazole in 1992. These azoles were designed to be more specific for fungal cells, and to be associated with less human toxicity. Consequently ketoconazole is now relegated to second-line status for the treatment of many systemic mycoses. Voriconazole, the most recent addition to this class, possesses a broad spectrum of activity and thus, it represents yet another advance in this growing class of antimycotics. The safety and efficacy of fluconazole, itraconazole, and voriconazole, make them significant additions to the antifungal arsenal. These azoles represent relatively safe and effective alternatives to other systemically acting antifungal agents. This chapter will review the pharmacology of the azole class of antifungal agents.

A. Chemistry

The systemic azoles are synthetic compounds and are divided into the imidazole and triazole classes based on their chemical structure (Fig. 1). Ketoconazole, the remaining systemically acting imidazole, contains two nitrogen atoms on the five-membered azole ring, whereas the triazoles, fluconazole, itraconazole, and voriconazole, contain three nitrogens in the five-membered azole ring. The chemical structure and properties of ketoconazole and itraconazole are somewhat similar. Ketoconazole is a lipophilic, weak dibasic compound with low-water solubility at pH greater than 3 (1). Itraconazole is a weak base that is extremely lipophilic, essentially water-insoluble (<5 µg/mL) and ionized at only low pH (2). Both ketoconazole and itraconazole undergo optimal dissolution at pH less than 3 (1,2). The poor aqueous solubility made the development of IV formulations of these compounds challenging, thus ketoconazole is available only as a solid oral dosage form.
Fluconazole is also a weak base, but it is only slightly lipophilic, and thus more polar than either ketoconazole or itraconazole. As a result, it circumvents much of the hepatic metabolism required by the other azoles, achieves high-serum concentrations, and exhibits low-protein binding (3). In addition, since fluconazole is water soluble, it is readily formulated as an IV dosage form.

Voriconazole is a derivative of fluconazole (Fig. 1). Chemically, voriconazole differs from fluconazole in that a fluropyrimidine group replaced one triazole moiety and a methyl group was added to the propanol backbone of the molecule (4). These alterations enhance the potency and expand the spectrum of activity to include filamentous fungi, especially *Aspergillus* sp. (4).

**II. MECHANISM OF ACTION**

**A. Effects on 14α-Demethylase**

The sterol ergosterol is a key component of the fungal cell membrane that is not present in mammalian cells. Therefore, this sterol is an ideal target for antifungal activity. Ergosterol is critical to the integrity of the fungal cell membrane and functions in regulating membrane fluidity and asymmetry (5). Sterols that are incorporated into the cell membrane must lack C-4 methyl groups in order for membrane integrity to be maintained (5). This is accomplished in the sterol biosynthesis pathway by cytochrome P450 (CYP) – dependent 14α-demethylase. Inhibition of the enzyme 14α-demethylase leads to depletion of the essential cell membrane sterol, ergosterol, and accumulation of sterol precursors, including 14α-methylated sterols (lanosterol, 14-dimethyl lanosterol, and 24-methylenedihydrolanosterol) (Fig. 2). The net result of this inhibition is the formation of a plasma membrane with altered structure and function (5).

The systemic azoles are generally believed to exert a fungistatic effect by inhibiting this step via binding of theazole ring to the heme iron of the enzyme, which catalyzes the CYP-dependent 14α-demethylation of lanosterol (7). However, ketoconazole may have a secondary mechanism of action. Ketoconazole also inhibits several membrane-bound enzymes and may interact directly with membrane lipid
biosynthesis (5). Likewise, in addition to inhibiting 14α-demethylation of lanosterol, voriconazole may also affect chitin synthesis (8). However, studies show that the triazoles act primarily on the enzyme 14α-demethylase (5,9).

The 14α-demethylase inhibitory activity of the triazoles may vary with genus. For example, in addition to inhibiting 14α-demethylase in Cryptococcus neoformans, itraconazole and fluconazole inhibit the reduction of the 3-ketosteroid obtusifolione to obtusifoliol, presumably by inhibiting 3-ketoreductase (Fig. 2) (5). The resultant accumulation of the 3-ketosteroid obtusifolione and other methylated sterol precursors increases the fragility of the membrane. In Histoplasma capsulatum var. capsulatum, investigators have hypothesized that 3-ketoreductase may be more sensitive to inhibition by itraconazole than by fluconazole (10). This may explain why itraconazole is more active than fluconazole against this pathogen.

The 14α-demethylase inhibitory activity of the triazoles also varies with species, in fluconazole susceptible Candida albicans voriconazole completely blocks ergosterol and obtusifoliol synthesis, whereas fluconazole only partially inhibits ergosterol and completely blocks obtusifoliol synthesis (9). Similarly, in Candida krusei, both voriconazole and fluconazole completely blocked obtusifoliol synthesis, but voriconazole inhibited ergosterol synthesis more than fluconazole (9). Variability in the 14α-demethylase inhibitory potencies among the azoles likely explains the differences in their activity.

B. Pharmacokinetics

The triazoles differ subtly in chemical properties, which form the basis of the pharmacokinetic differences between the agents and the propensity of this class to interact with other medications. These properties can limit the use of these agents,
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particularly ketoconazole or itraconazole. The pharmacokinetic properties of the commonly used systemic acting azoles are provided in Table 1.

1. Ketoconazole

For systemic use ketoconazole is available only as 200 mg tablets. Due to its chemistry and low water solubility, ketoconazole undergoes optimal dissolution at pH less than 3. Food does not consistently alter the absorption and systemic availability of ketoconazole (11). The absorption of ketoconazole is rapid and somewhat complete. The relative bioavailability of the ketoconazole tablet is approximately 80% (11). Ketoconazole is highly lipophilic, therefore in the serum it is highly protein bound (99%) and widely distributed in the body (11). As a result of high-protein binding, the unbound concentrations of ketoconazole in body fluids [i.e., urine, cerebral spinal fluid (CSF), etc.] are low (11). Ketoconazole is hepatically metabolized to inactive metabolites by CYP3A4, and exhibits non-linear (i.e., dose-dependent) elimination. Less than 5% of an administered dose of ketoconazole is eliminated unchanged in the urine (11). Ketoconazole also interacts extensively with the heme moiety of CYP3A resulting in non-competitive inhibition of oxidative metabolism of many CYP3A substrates (12). To a lesser extent ketoconazole also inhibits other CYP enzymes involved in drug metabolism (13). Ketoconazole also interacts somewhat with the transport P-glycoprotein (P-gp) (14).

P-gp is a transmembrane efflux pump/transport protein that is expressed in a variety of tissues, where it interacts with a diverse array of substrates, including itraconazole and ketoconazole, but not fluconazole (14). Although it has not been characterized, the interaction between voriconazole and P-gp is likely negligible. The P-gp is extensively expressed in the GI tract, liver, cells of the blood–brain barrier, and in the renal epithelia of the proximal tubule (15). In these tissues P-gp acts to limit systemic exposure of its substrate. In the GI tract P-gp reduces drug absorption, whereas in the liver and proximal tubules of the kidney it enhances drug elimination. In the blood–brain barrier, P-gp limits distribution to central nervous system (CNS) tissues (12). This protein is the basis of several azole drug–drug interactions.

2. Itraconazole

Itraconazole is available as 100 mg capsules containing itraconazole-coated pellets, and solubilized in a 40% hydroxpropyl-β-cyclodextrin (HP-βCD) 10 mg/mL solution for oral and IV use. The absorption of itraconazole from the capsule form is slow and incomplete, consequently the drug is subjected to significant metabolism in the intestine and liver before reaching the systemic circulation. As a result, absorption from the capsule is variable and optimal under acidic gastric conditions or in the fed state (16). In contrast, HP-βCD significantly enhances the solubility of itraconazole. Therefore, the oral solution form requires no dissolution so its absorption is not influenced by gastric pH and is rapid. As a result, itraconazole is delivered to the intestinal epithelium in high concentrations, which may cause transient saturation of intestinal CYP3A4 (i.e., a component of first pass metabolism) (17,18). With less first pass metabolism, higher and more consistent serum concentrations can be achieved. The absorption of itraconazole from the oral solution is optimal in the absence of food (17). Consequently, with an empty stomach peak plasma concentrations of both itraconazole and its primary metabolite, hydroxyitraconazole, are higher and are achieved earlier, compared to non-fasting conditions (17,18). Absorption of the oral solution on an empty stomach is more rapid and less variable.
than the absorption of the capsule under fed conditions. This results in a pharmacokinetic profile with less inter- and intrapatient variability (19). However, even in the presence of food, higher serum concentrations are achieved with the oral solution than with the capsule. The absolute bioavailability of the oral solution is 55% and approximately 30% higher than that of the capsule formulation, nonetheless the two formulations are considered bioequivalent in many pharmacokinetic parameters (16,19).

The HP-βCD is a carrier molecule modified from natural breakdown products of starch, and when given orally it is poorly absorbed from the intestine, stimulates gastrointestinal secretion and propulsion, and causes diarrhea. While, this compound improves the pharmacokinetics of the oral dosage form of itraconazole, it hinders the IV form of itraconazole. Following IV administration, the amount of itraconazole that is cleared by the kidney is negligible, but HP-βCD is primarily eliminated by the kidneys (80-90%) (20,21). With severe renal impairment (creatinine clearance (CrCl) ≤ 19 mL/min) renal elimination of this compound decreases sixfold (22). Currently the use of IV itraconazole is contraindicated in patients with CrCl ≤ 30 mL/min due to concern that the renal accumulation of HP-βCD may cause histological damage to the kidney (22). These changes have been observed in animals, but there is no human data describing this toxicity.

Itraconazole is highly lipophilic, therefore in the serum it is highly bound (99.8%) to albumin and widely distributed in the body (2). As a result of high protein binding, the unbound concentrations of itraconazole in body fluids (i.e., CSF, saliva, and urine) are very low (2). Itraconazole has high affinity for tissues (i.e., vaginal mucosa, horny layer of nails, etc.), and concentrations in these tissues can persist well after the serum concentrations are undetectable (2). Like ketoconazole, itraconazole also exhibits non-linear elimination. However, itraconazole is extensively metabolized to many metabolites by CYP3A4. The principle metabolite, hydroxyitraconazole is formed primarily during gut wall metabolism and is bioactive (16). The complete metabolic pathway of itraconazole is not fully understood and it may interact with additional CYP enzymes. Itraconazole primarily inhibits the CYP3A4 enzyme (13,23). In addition, it is a substrate and inhibitor of P-gp, and its interaction with this protein is the basis for several notable drug interactions involving itraconazole (i.e., vinca alkaloids, digoxin, etc.) (13,23).

3. Fluconazole

Fluconazole is available as 50, 100, 150, and 200 mg tablets as well as a 2 mg/mL solution for IV administration, and in a powder form for reconstitution as a 10 or 40 mg/mL oral suspension. The oral formulations of fluconazole are rapidly and nearly completely absorbed, with a bioavailability in excess of 93% (24). Consequently, serum concentrations after oral dosing are similar to those obtained with IV dosing. Therefore, the IV formulation should be used only in patients who cannot take oral medications, or in whom oral absorption cannot be assured. The administration of fluconazole in through enteral feeding tubes has been studied and most data seem to indicate that the systemic availability of fluconazole is relatively unaffected by this mode of administration. However, one large study demonstrated that serum concentrations obtained with standard doses administered via an enteral feeding tube may not be adequate to treat Candida glabrata infections (25). Absorption of fluconazole is not dependent on acidic gastric conditions or the presence of food (24). Fluconazole binds minimally to plasma proteins (11%), and
circulates mostly as free drug, thus it distributes extensively into a variety of body fluids including the CSF, urine, as well as hepatic, renal, and CNS tissues (24).

The chemical properties of fluconazole also allow it to circumvent much of the intestinal and hepatic metabolism required by ketoconazole or itraconazole for elimination. Fluconazole exhibits linear pharmacokinetics, that is, increases in dosage produce proportional changes in serum concentration and systemic exposure. Fluconazole is unique among systemic azoles, in that approximately 91% of an orally administered dose is excreted in the urine, mostly (80%) as unchanged drug, two inactive metabolites account for the remaining 11% (26). Although fluconazole undergoes minimal CYP-mediated metabolism, like the other azoles, in vitro it inhibits CYP3A4, albeit much more weakly than other agents in this class (27). However, in vitro fluconazole also inhibits several other CYP enzymes (27). When evaluating in vitro studies of the CYP inhibitory potential it is important to note fluconazole binds non-competitively to CYP, and in vivo it circulates largely as free drug. Therefore, determination of the ability of fluconazole to inhibit CYP in vitro may not accurately predict its potential to inhibit CYP in vivo.

4. Voriconazole

Voriconazole is available in both IV and oral formulations. The IV formulation is formulated as a powder for reconstitution containing 200 mg voriconazole solubilized with sulfobutyl ether β-cyclodextrin. When reconstituted the final solution contains 10 mg/mL. The oral tablets contain either 50 or 200 mg of voriconazole. Like fluconazole, dissolution of voriconazole is not affected by increases in gastric pH. Voriconazole absorption is rapid and nearly complete following oral dosing, with a relative bioavailability of approximately 90% (28). Peak serum concentrations are achieved within 2 hr of oral dosing. Voriconazole is moderately bound to plasma proteins, and is widely distributed throughout the body. In case reports, CSF concentrations achieved with standard dosing have been approximately 30–60% of plasma concentrations (28,29). Voriconazole concentrations in brain tissue concentrations are higher than those in the CSF (30).

In adults, increases in voriconazole dosage produce disproportional changes in drug levels (i.e., non-linear pharmacokinetics). In contrast, in children, increases in voriconazole dosage produce proportional changes in drug levels (i.e., linear pharmacokinetics). Moreover, higher doses are required in children (28). These age related differences are probably due to the saturation of CYP enzymes in adults or age related differences in CYP expression.

Voriconazole undergoes extensive hepatic metabolism by CYP enzymes. However, the metabolism of voriconazole is more complex than other azoles and involves several different CYP enzymes, namely, CYP2C9, CYP2C19, and CYP3A4 (28,30). Voriconazole is primarily metabolized by CYP2C19, which exhibits genetic polymorphism. To date there have been eight variant alleles identified with this polymorphism. All variants are associated with reduced enzyme activity that manifests as a poor metabolizing phenotype (PM). The PM phenotype is an inherited autosomal recessive trait, and is present in 3–5% of Caucasians, 12–23% of Asian populations, and 38–79% of Polynesians and Micronesians (31). Populations that exhibit the homozygous PM phenotype will have approximately a four fold increase in drug exposure compared to those who exhibit the homozygous efficient metabolizer (EM) phenotype. Populations that are heterozygous for the EM phenotype will have
nearly a two fold increase in drug exposure compared to the homozygous EM phenotype (28).

Voriconazole is also metabolized by CYP2C9, which also exhibits polymorphisms. To date six variant alleles have been identified with this polymorphism, and two are associated with reduced enzyme activity. Therefore, clearance of a substrate, such as voriconazole will be reduced when these variants are present. The variant alleles are expressed among Caucasians, and less frequently among African-Americans. They are not expressed in Asian populations (31,32). Lastly, CYP3A4 is also involved in voriconazole metabolism, however, to date significant polymorphisms have not been identified with this enzyme. Genotyping is not clinically indicated, but nonetheless clinicians should be aware of the complexities of voriconazole metabolism. In addition to being a substrate of these three CYP enzymes, voriconazole inhibits them as well. Therefore, it has the potential to interact with a wide array of other medicines. As a consequence of its complex CYP-mediated metabolism, less than 5% of voriconazole is eliminated by the kidneys unchanged (28). The affinity of voriconazole for P-gp has not been fully evaluated. However, since it is a derivative of fluconazole, and has activity in CNS infections, it is likely not a P-gp substrate.

C. Adverse Effects

In general the azoles are associated with few serious adverse effects and are considered a relatively safe class of drugs. Table 2 summarizes common and serious adverse

<table>
<thead>
<tr>
<th>Organ System</th>
<th>KTZ</th>
<th>FCZ</th>
<th>ITZ</th>
<th>VCZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI tract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Vomiting</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Dermatological</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itching</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rash</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>Stevens–Johnson</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hepatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased transaminases</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Endocrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal insufficiency</td>
<td>++</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Mineralocorticoid excess</td>
<td>++</td>
<td>_</td>
<td>++</td>
<td>_</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Visual disturbances</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>++</td>
</tr>
</tbody>
</table>

Abbreviations: KTZ, ketoconazole; FCZ, fluconazole; ITZ, itraconazole; VCZ, voriconazole; (--), uncommon; (+), common; (++), most common within class.
effects of the azoles. The advent of the triazoles greatly improved the safety of this class. As a class, dose related GI symptoms are the most common side effects, particularly with the oral itraconazole solution (11). These effects are typically seen at the upper ends of the recommended dosage ranges of these compounds, and rarely are the symptoms severe enough to warrant discontinuation of therapy. Transient increases in serum aminotransferase concentrations are also commonly observed with all agents in this class. In general, patients who experience azole-associated increases in serum aminotransferases are asymptomatic, but these increases can, on rare occasion, evolve into fatal drug-induced hepatitis (11). Consequently, baseline liver function tests should be performed prior to initiating azole therapy, and the patient should be monitored periodically for evidence of drug-induced hepatitis (11). The azoles can also produce allergic skin rashes that are generally mild, and subside with discontinuation of the drug. The azoles produce teratogenic effects in mice and therefore their use should be avoided in pregnancy (category C).

1. Ketoconazole

The biosynthesis pathways of fungal and mammalian sterols have several CYP-mediated steps in common. For example the azoles can block synthesis mammalian sterols, specifically cholesterol, at the 14α-demethylation step. However, the triazoles have reduced affinity for the mammalian enzymes, and thus supra-pharmacologic doses would be required to produce the degree of inhibition observed in fungal cells (5). However, ketoconazole is non-specific in its inhibition of sterol biosynthesis, which is the primary difference between the imidazole, and the triazoles. Consequently, inhibition of human sterol biosynthesis is most commonly observed with use of ketoconazole, than with the triazoles.

Ketoconazole reversibly inhibits the synthesis of several mammalian sterols including testosterone, estradiol, and cortisol, especially when given in daily doses in excess of 400 mg (33). Consequently, a variety of endocrine disturbances can be observed in patients receiving ketoconazole. Within 6 months of starting therapy up to 20% of men can experience a dose-dependent inhibition of testosterone that causes symptoms such as gynecomastia, or diminished libido (33). In addition, oligospermia, or impotence can also occur. Women also can experience endocrine abnormalities associated with ketoconazole therapy. Menstrual irregularities can occur in up to 16%, and reversible alopecia can be seen in 8% (33). These endocrine abnormalities subside with drug discontinuation. In rare cases ketoconazole can cause adrenal insufficiency as a consequence of its ability to inhibit steroidogenesis (11). Limiting the dose to no more than 400 mg/day lowers the risk of endocrine or GI adverse effects associated with ketoconazole (34).

2. Itraconazole

Itraconazole in dosages of 400 mg/day or less generally produces little toxicity. Reports of adverse effects increase with prolonged courses of at least 400 mg/day. Nonetheless this toxicity rarely necessitates discontinuation of the drug (34). The primary adverse effects associated with itraconazole administration are GI symptoms, particularly nausea, abdominal pain, and diarrhea (34). These symptoms are generally described as mild by patients receiving the capsule or IV formulations of itraconazole. However, the symptoms are most frequently reported with the use of the oral solution and are generally more severe (35). In clinical trials of the oral solution in patients with AIDS, the frequency of GI adverse events were severe
enough to necessitate withdrawal of therapy in 8–10%, which is a higher discontinuation rate than seen other populations (35). The propensity of the oral solution to cause diarrhea is likely due to the cyclodextrin, HP-βCD that helps to solubilize itraconazole. When given orally, HP-βCD is converted its basic glucose molecules by cyclodextrin transglycolase, which is produced by intestinal microflora (35). The glucose breakdown products are then absorbed and metabolized in the liver (22). The diarrhea associated with the oral solution is generally attributed to an osmotic effect of the HP-βCD in the GI tract (35).

Adverse effects with the IV formulation are generally mild and rare. The most common reactions reported with this formulation are phlebitis and other injection site reactions (35). As mentioned previously, use of the IV solution in patients with significant renal impairment is contraindicated. The concern stems from the fact that when administered IV, HP-βCD is primarily eliminated rapidly by glomerular filtration, and animal data suggesting high concentrations of cyclodextrins can damage renal tissue (20,21,36). Whether these animal data are applicable to HP-βCD, is unknown. Cyclodextrins are classified as “α,” “β,” or “γ” by the number of α-1, 4-linked glucose units in their molecular structure (36). β-Cyclodextrin has limited aqueous solubility due to intramolecular hydrogen bonding (36). The low aqueous solubility of β-cyclodextrin carries toxicological consequences. In mice and rats the parenteral administration of β-cyclodextrin leads to increases in blood urea nitrogen (BUN), a reduction in the rate of weight gain, decreases in liver mass (36). In addition, kidney mass is increased and the activity of numerous enzymes in the kidney are diminished (36). β-Cyclodextrin undergoes tubular reabsorption and concentrates in vacuoles, where, it precipitates due to its low aqueous solubility, (36). Histologically the resultant damage manifests as nephrosis (36). Clinicians should understand that HP-βCD, the cyclodextrin used in the IV and oral itraconazole solutions, is a hydroxylated β-cyclodextrin. The non-selective hydroxylation of β-cyclodextrin increases its water solubility and therefore may decrease its potential to cause renal toxicity (36). Rare life-threatening reactions (liver failure and CHF) can also occur with itraconazole therapy. Clinicians should keep this in mind when using itraconazole to treat non–life-threatening infections of the skin and nailbeds. Lastly, in contrast to ketoconazole, when given in standard doses, itraconazole has little or no inhibitory effect on human steroidogenesis. However, occasionally with doses in excess of 400 mg/day or with protracted courses, patients may experience a mineralocorticoid excess syndrome manifested by hypokalemia, hypertension, and edema (36).

3. Fluconazole
Fluconazole is considered the safest azole, and doses 4–5 times in excess of the recommended daily dose have been well tolerated. Similar to ketoconazole and itraconazole GI symptoms are the most commonly reported adverse effects, but with fluconazole they occur in less than 2% of patients and are frequently considered mild. As previously mentioned, transient elevations in serum transaminase levels occur, but progression to severe drug-induced hepatitis is exceedingly rare. Like itraconazole, fluconazole does not interfere with human steroidogenesis. Nonetheless, reversible alopecia has been reported in up to 20% of patients receiving at least 400 mg/day for more than 2 months (37). This adverse effect resolves within 6 months of stopping therapy or reducing the dose by 50% (37).
4. Voriconazole

In general like other azoles voriconazole is well tolerated. However, voriconazole is commonly associated with a unique adverse effect. Approximately 30% of patients experience reversible visual disturbances (30). Although noxious, these disturbances rarely lead to discontinuation of therapy. The reported visual disturbances include changes in color discrimination, blurred vision, increased sensitivity to light, and the appearance of bright spots. These disturbances are acute and diminish shortly into a course of therapy, and apparently there is no lasting damage to the retina (30). The underlying mechanism for this adverse effect is unknown.

Other common adverse effects include skin rash and elevations in serum transaminase levels. Like the other azoles, the elevations in serum transaminase levels are generally benign, however, life-threatening hepatitis associated with voriconazole use has been described (38). The risk of hepatitis associated with voriconazole use, appears to correlate with elevated serum drug concentrations (38). More rigorous monitoring of liver function tests is recommended for patients who will be treated with voriconazole (30).

III. IN VITRO ACTIVITY

A. Susceptibility Testing

1. Susceptibility Testing and Interpretive Breakpoints

For many years clinically relevant antifungal susceptibility testing was non-existent. Consequently antifungal therapy was largely empiric and not guided by susceptibility data. Clinically relevant antifungal susceptibility testing has developed over the past two decades. Currently the National Committee for Clinical Laboratory Standards (NCCLS) has published an approved reference method for yeasts (M27-A). This method is fairly established for interpreting susceptibility data for azole antifungal agents. The NCCLS has also published a proposed reference method for molds (M38-P). However, the development of this method is still in its infancy. A comprehensive discussion of the specific methods used to test the susceptibility of fungal pathogens against the azoles is beyond the scope of this chapter. The reader is referred to two excellent comprehensive reviews that were recently published on the subject (5,39). Breakpoints exist for Candida sp. tested against

<table>
<thead>
<tr>
<th>Agent</th>
<th>Susceptible (S) (µg/mL)</th>
<th>Susceptible-Dose Dependent (S-DD) (µg/mL)</th>
<th>Resistant (R) (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoconazole</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>≤8</td>
<td>16–32</td>
<td>≥64</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>≤0.125</td>
<td>0.25–0.5 a</td>
<td>≥1</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>unk</td>
<td>unk</td>
<td>unk</td>
</tr>
</tbody>
</table>

*aFor isolates classified S-DD, plasma concentrations should be maintained in excess of 0.5 µg/mL.
Abbreviations: n/a, No specific breakpoints proposed, data suggest MIC > 0.125 µg/mL associated with diminished response (5). unk, Not yet determined.
the azoles (Table 3). There were no specific breakpoints proposed for ketoconazole against yeasts or molds. However, aggregate data suggest that *Candida* sp. with an MIC of >125 μg/mL as determined by the reference M27-A method will have reduced susceptibility (39). Breakpoints for fluconazole were established based on data from mucosal and invasive disease. The breakpoints contain a unique category known as “susceptibility is dose-dependent” (S-DD). This breakpoint considers the pharmacokinetics of fluconazole and emphasizes the importance of optimizing fluconazole blood and tissue concentrations for isolates with elevated MICs (39).

For itraconazole breakpoints against *Candida* sp. exist only for mucosal infections (39). Like fluconazole, there is an S-DD breakpoint that suggests the susceptibility depends upon drug delivery to the infection site (39). Depending on the formulation, itraconazole absorption is unpredictable and somewhat erratic. Therefore, lack of drug delivery to the infection site may be the true cause of the apparent reduced susceptibility. The correlation between itraconazole serum concentrations or MICs and outcome are unclear (39).

The NCCLS has yet to establish breakpoints for voriconazole against clinically relevant fungal pathogens, and the relationship between clinical outcome and in vitro susceptibility has yet to be determined (28). Interpretive breakpoints for *C. neoformans* and other molds against the azoles have yet to be established.

**B. In Vitro Spectrum of Activity**

1. **Ketoconazole**

Based upon in vitro observations, ketoconazole is considered to be fungistatic. Ketoconazole is very active in vitro against many yeasts including *C. neoformans* and *C. albicans*. Ketoconazole is active, but has less potency than other agents against non-albicans *Candida* sp., such as *C. glabrata*, *C. krusei*, and *C. parapsilosis*. In addition, ketoconazole also is active against common dimorphic or endemic fungi such as *Coccidioides immitis*, *H. capsulatum*, *B. dermatitidis*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii* (11). The activity of ketoconazole against *Aspergillus* sp. is variable, and it is not active against *Aspergillus fumigatus*. Despite a fairly broad spectrum of activity, clinically ketoconazole has been relegated to a second-line drug by the more potent and safer triazoles.

2. **Fluconazole**

The in vitro activity of fluconazole is generally considered to be fungistatic (40). In addition, its activity is essentially limited to yeasts. Fluconazole is highly active against *C. neoformans* and *C. albicans*. Moreover, fluconazole is also active against certain non-albicans *Candida* sp., such as *C. parapsilosis*, *C. tropicalis*, and *C. lusitaniae* (41,42). However, the activity of fluconazole against other albicans *Candida* sp. is poor. *C. krusei* is inherently resistant, and *C. glabrata* is often resistant to fluconazole. Fluconazole has activity against *C. immitis*, but it has poor activity against other dimorphic fungi such as *H. capsulatum* and *B. dermatitidis*. Fluconazole is devoid of activity against *Aspergillus* sp., *Fusarium* sp., and the agents of Zygomyces.
3. Itraconazole

The activity of itraconazole is species- and strain-dependent. In vitro itraconazole exerts fungicidal activity against filamentous fungi, and some strains of *C. neoformans* (40). However, against yeasts itraconazole is generally fungistatic. Itraconazole has a very broad spectrum of in vitro activity and is very active against dermatophytes and many yeasts including *C. neoformans*, *C. albicans*. Moreover, itraconazole is also active in vitro against certain *non-albicans Candida* sp., such as *C. parapsilosis*, *C. tropicalis*, and *C. lusitaniae* (42). In addition, itraconazole has varying in vitro activity against other *non-albicans Candida* sp., such as *C. glabrata*, *C. krusei* (42). Itraconazole has excellent in vitro activity against common dimorphic or endemic fungi including *B. dermatitidis*, *C. immitis*, *H. capsulatum*, *H. duboisii*, and *P. brasiliensis* (11,43). Itraconazole also possesses good activity against *S. schenckii* (11). Prior to the development of voriconazole, itraconazole was the only azole with clinically significant activity against *Aspergillus* sp. Like other early azoles, itraconazole is devoid of activity against *Fusarium* sp., and the agents of *Zygomycetes*. In addition, itraconazole is unique in that hydroxyitraconazole, its primary metabolite in humans, is bioactive (16).

There are few data assessing the activity of hydroxyitraconazole against fungal pathogens. The antifungal activity of hydroxyitraconazole was tested in a study of quantitative differences between biologic and chemical analytical methods used to measure itraconazole concentrations in body fluids. Accordingly, the antifungal activity of hydroxyitraconazole was found to be nearly identical to that of itraconazole. Hydroxyitraconazole was twice as active against *C. pseudotropicalis*, which was the isolate used in the bioassay (44). Because the bioassay measures total activity and does not distinguish the contribution of hydroxyitraconazole from itraconazole, these data explain why, compared to chemical analytical methods, bioassays overestimate itraconazole concentrations in body fluids (44). Of more interest, the activity of hydroxyitraconazole *A. fumigatus*, *A. terreus*, *C. neoformans*, and *C. immitis* was the same as that of itraconazole (44). In drug interaction studies, the two compounds were additive against *A. fumigatus*, and slight synergy was observed against *C. pseudotropicalis* and *C. neoformans* (44). Interestingly, more pronounced synergy were observed against *C. immitis* (44). Whether this additive or synergistic activity occurs in vivo or how much hydroxyitraconazole contributes to the clinical activity of itraconazole is unknown. However, in the body, it circulates at 1–2 times the concentration of the parent compound after IV or oral administration, respectively (16).

4. Voriconazole

Similar to itraconazole, voriconazole exerts fungicidal activity against select opportunistic fungi (40). In addition, fungicidal activity against certain *non-albicans Candida* isolates and *C. neoformans* has been observed (40). However, against most yeasts, voriconazole exerts fungistatic activity (40). Voriconazole is the most potent azole available, and it possesses as very broad spectrum of activity against dermatophytes, yeasts, and molds. Voriconazole is active against all *Candida* sp., including those with acquired (i.e., *C. albicans*) or inherent (i.e., *C. glabrata* and *C. krusei*) resistance to fluconazole (30). Voriconazole is very active against other yeasts including *C. neoformans*, and *Trichosporon beigeli*, which is typically resistant to the other azoles and polyenes (30).

Voriconazole is also highly active against filamentous fungi such as *Aspergillus* sp., and its activity against *A. fumigatus* and *A. flavus* is comparable to that of
itraconazole (28). Most notably it possesses activity against resistant *Aspergillus* sp., including *A. terreus*, which is often resistant to amphotericin B, and a clinical isolate of *A. fumigatus* resistant to itraconazole (30,45). Voriconazole is very active against the dimorphic fungi including *B. dermatitidis*, *C. immitis*, and *H. capsulatum*. However, against *S. schenckii* it is less active. Voriconazole is active against many amphotericin resistant molds, including certain strains of *Pseudoallescheria boydii*, *Fusarium* sp., and other less common opportunistic pathogens (30). Similar to the other azoles, voriconazole is devoid of activity against the agents of Zygomycetes (30).

C. Pharmacodynamic Properties

The study of pharmacodynamics examines relationships among drug concentrations, time, and pharmacological effects (46). These relationships can be elucidated in vivo through the use of infected animal models, which incorporate pharmacokinetic measurements and antibiotic effect. In-vitro, these relationships are characterized by the use of classic time-kill assays, the study of persistent and subinhibitory effects, and in vitro models or cell cultures (40). While these methods have been employed in the study of antibacterials for many years, they have only recently been applied to antifungal agents, specifically the azoles. Consequently, the pharmacodynamic behavior of ketoconazole has not been well characterized.

The pharmacodynamic characteristics of an antimicrobial are defined by two important relationships. The first relationship is the effect of drug concentration on the rate and extent of organism killing (46). When increasing drug concentrations enhance the rate and extent of killing, the relationship is referred to as “concentration-dependent” killing. In this case optimal killing is achieved by maximizing the peak drug concentration in relation to the MIC of the organism. Thus, this relationship is defined by the pharmacodynamic parameter known as the “peak-to-MIC ratio” (peak/MIC). When the rate and extent of killing is enhanced by the time course of drug exposure rather than by increasing drug concentration, the relationship is referred to as “concentration independent” killing. In this case optimal killing is achieved by maximizing the time the drug concentration remains above the MIC by some factor over the course of the dosing interval. Thus, this relationship is defined by the two pharmacodynamic parameters known as the “percentage of time that serum drug concentration exceeds the MIC” (T > MIC), and “the area under the serum concentration–time curve in relation to the MIC” (AUC/MIC). The second relationship that defines the pharmacodynamic characteristics of an antimicrobial is the ability of the organism to grow after drug exposure. In terms of antifungals, the key parameter describing this relationship is the so-called “postantifungal effect” (PAE). This parameter is a measure of time during which there is continued suppression of growth at concentrations below the MIC.

1. Fluconazole

Pharmacodynamic behavior of fluconazole is well characterized. Classic in vitro time-kill studies and an in vitro dynamic bloodstream infection model reveal that against *C. albicans* fluconazole exhibits concentration-independent fungistatic activity (47–49). Similarly, in vitro time-kill assays revealed that against *C. neoformans*, fluconazole exhibits concentration-independent fungistatic activity (50,51). Studies using incubation periods longer than 72 hr and non-proliferating growth conditions have suggested fluconazole may also exert a direct fungicidal effect on *C. albicans*.
In vivo animal infection models have confirmed that the pharmacodynamic value of fluconazole that best predicts outcome is the AUC:MIC ratio, which indicates concentration-independent behavior (40). Moreover, the infection models revealed that fungal burdens were similarly reduced regardless of the frequency of administration of a given total dose, and the optimal AUC:MIC was similar regardless of strain susceptibility (40). Fluconazole pharmacodynamic relationships have not been examined in infected patients. The in vitro persistent effects of fluconazole are apparently dependent upon the presence of human serum. In serum-free growth media there was no measurable PAE against C. albicans and C. neoformans (53,54). However, in the presence of fresh serum a short PAE was observed against C. albicans (53).

2. Itraconazole

The pharmacodynamic behavior of itraconazole is genus dependent. Classic in vitro time-kill experiments in conventional media reveal that against Candida sp. and C. neoformans, itraconazole exhibits concentration-independent fungistatic activity (49,51,55). In these studies maximum effectiveness occurred when concentrations were maintained at two times the MIC for Candida species, and 4–8 times above the MIC of C. neoformans (51,55). Similar pharmacodynamic behavior of itraconazole against Candida species was observed in time-kill assays in the presence of 80% human serum (56). In contrast to the yeasts, the pharmacodynamic behavior of itraconazole against Aspergillus sp., demonstrated both concentration- and time-dependent cidal activity (57).

The relationship between itraconazole serum concentration and efficacy has been assessed in a model of invasive pulmonary aspergillosis in immunocompromised rabbits. Like humans, peak serum concentrations were highly variable, however, a pharmacodynamic relationship between itraconazole serum concentrations, and efficacy as a function of fungal burden in lung tissue was observed. Higher plasma concentrations were associated with decreases in tissue burden of A. fumigatus (58). This study suggested that a threshold value of serum-drug concentration maybe required for optimal effectiveness. However, whether the results of this study can be extrapolated to humans is questionable. The study used an extemporaneous prepared suspension using the capsules, and a recent study has found that the rabbit is not an ideal animal model in predicting the oral absorption of itraconazole in humans (59).

Given the above animal data, the erratic pharmacokinetics and the differential pharmacodynamic behavior of itraconazole several studies have tried to establish a threshold concentration in humans that is predicative of response. The results of these studies vary, but in general, for optimal effectiveness, investigators advocate itraconazole plasma trough concentration of at least 0.25 μg/mL (measured by HPLC) (2). However, recent preliminary work suggests that 0.5 μg/mL is the minimal desirable target concentration for the prevention and treatment of invasive fungal infections, especially in neutropenic hosts (60).

3. Voriconazole

Pharmacodynamic relationships for voriconazole not been fully characterized, and have only been studied in vitro using time-kill assays. These methods indicate that voriconazole exhibits concentration-independent fungistatic activity C. albicans, C. glabrata, C. tropicalis, and C. neoformans (57,61). In contrast, voriconazole
displayed concentration-independent fungicidal activity against *A. fumigatus*, further suggesting that the fungicidal activity of the azoles may be organisms specific (57). Similar to fluconazole, the in vitro persistent effects of voriconazole are apparently dependent upon the presence of human serum. In serum-free growth media there was no measurable PAE against *C. albicans*, but in the presence of fresh serum a relatively short PAE was observed (62).

IV. MECHANISMS OF RESISTANCE TO AZOLES

A comprehensive discussion of the specific mechanisms of resistance employed by fungal pathogens against the azoles is beyond the scope of this chapter. The reader is referred to two excellent comprehensive reviews that were recently published on the subject (5,63).

In general microbes resist the effects of antimicrobials by producing enzymes to modify the antibiotic; by qualitative or quantitative modifications in the cellular target; or reducing access to the cellular target. Fungi resist the effects of the azoles by modifying the target or reducing the access to the target or a combination of both (5). The underlying mechanism of fluconazole resistance has been examined by comparing sterol composition, fluconazole accumulation, and inhibition of 14α-demethylase in clinical isolates of *Candida* sp., that express intrinsic fluconazole resistance (*C. krusei*), and a fluconazole-susceptible *C. albicans* isolate (64). Sterol content did not differ between the species, but the affinity of 14α-demethylase for fluconazole was greater in *C. albicans* (64). Moreover, over-time fluconazole accumulation in *C. krusei* was less than that in *C. albicans* (64). Thus, in this study, qualitative modifications and active efflux were implicated as coexisting resistance mechanisms in the strains of *C. krusei*. Yet, other studies examining clinical isolates have implicated only a single mechanism, (i.e., qualitative alterations in the target enzyme) (5).

Over expression of 14α-demethylase represents a quantitative modification of the target and it has also been identified as a mechanism of resistance to the azoles in a strain of *C. glabrata*. In this strain an increase in the ergosterol content led to a corresponding decrease in susceptibility to fluconazole, itraconazole, and amphotericin B (65). The increased ergosterol content was attributed to over expression of 14α-demethylase, but the strain also had evidence of active efflux of fluconazole (65). Interestingly, there was no evidence of itraconazole efflux in this strain, indicating that the azole cross-resistance was likely due to over expression of 14α-demethylase. The importance of over expression of 14α-demethylase as a mechanism of resistance is difficult to ascertain since it has only been observed in *C. glabrata*, and at least for fluconazole, other mechanisms may contribute to the resistance phenotype.

Active efflux is likely a primary method by which fungi resist the effects of the azoles. Fungi possess two types of efflux pumps. These transporters belong to either the “major facilitator” (MF) or “ATP-binding cassette” (ABC) superfamilies of proteins. Both superfamilies transport a diverse array of substrates and are involved in transport of a variety of toxic compounds out of the cell (5). Resistance to azoles is rare, and the understanding of the mechanisms involved and how they manifest clinically are evolving. Although the primary mechanism of resistance to the azoles is active efflux, high-level resistance is in fact due to multiple mechanisms acting in concert. In contrast over-expression of a target enzyme may result in cross-resistance to other azoles.
Most clinical reports of azole resistance, specifically fluconazole, involve *Candida* sp. Azole resistance is commonly observed in HIV infected patients. Estimates suggest overall, approximately one third of patients with advanced HIV will develop an azole-resistant infection (66). Azole resistant *C. albicans* are commonly isolated in AIDS patients with oropharyngeal candidiasis (67). However, azole resistance to non-*albicans* *Candida* has also been observed in this population (67). In these cases the same strain is repeatedly isolated and over time, its MIC increases with subsequentazole therapy. As the MIC climbs, clinicians often increase the dose of fluconazole. This is approach is generally successful up to an MIC of 64 μg/mL (67), at higher MIC values even high dose fluconazole therapy (>800 mg/day) frequently fails, but other azoles maybe effective. Risk factors for the development of azole resistance in this population includes patients with CD4+ cell counts of <50/mm³, prior exposure to azoles and a cumulative azole dose ≥10 g (68).

Azole resistant infections among non-HIV infected patients are most often due to non-*albicans* *Candida* sp., particularly *C. krusei* and *C. glabrata* (67), and occur in patients with malignancies. Risk factors for the development of azole resistance include leukemia, lymphoma, solid tumor, BMT, neutropenia, diabetes, and high-dose steroid therapy. Although azole resistance, if observed, occurs most often in *Candida* sp., it has also been observed in a variety of clinical isolates, including fluconazole resistant *C. neoformans*, and *H. capsulatum* and itraconazole resistant *A. fumigatus* and with more use it will likely surface with voriconazole (10,68–70).

V. IN VIVO EXPERIMENTAL ACTIVITY

A number of animal models, both immunosuppressed and non-immunosuppressed have been developed and used in the study of the pathogenesis of invasive mycoses, antifungal pharmacodynamics, and toxicities. These models have provided invaluable information on the activity and dose–response relationship of a drug or drug combination against a pathogen. In addition, in contrast to the clinical setting, these models allow for control of confounding variables, and thus provide for more specific outcome measures. A comprehensive review of the many infection models for a number of life-threatening systemic mycoses is beyond the scope of this chapter. Since most work has been directed at studying the effectiveness of an azole in the treatment of disseminated candidiasis, or invasive aspergillosis, models of these infections are discussed below.

A. *Candida* Species

The effectiveness of the azoles in the treatment of disseminated candidiasis and other serious candidal infections has been evaluated in a variety of neutropenic and non-neutropenic animal models including mice, rats, guinea pigs, and rabbits. When evaluating the results of these studies clinicians should be cognizant of interspecies differences in azole pharmacokinetics, and the route of drug administration as both may affect the performance of the model. This consideration is particularly germane when evaluating the experimental efficacy of itraconazole in fungal infection models. Itraconazole efficacy in an infection model may vary with dose, administration route, formulation, diet, and fungal species. In addition, the results will vary among animal models.
The effective itraconazole dose for the treatment of experimental disseminated candidiasis in murine models is quite variable (71). Some investigators even noted no effect with oral itraconazole despite administration of doses up to 80 mg/kg (72). Consequently, itraconazole has been associated with poor activity in mice. This lack of activity likely reflects differences in murine pharmacokinetics, or is related to the difficulty of formulating an oral-dosage itraconazole form for animal consumption. Significantly higher itraconazole plasma concentrations are achieved in DBA/2 mice than in BALB/c mice, regardless of whether the drug is administered intraperitoneally (i.p.) or orally (73). Additionally, prior to the advent of the oral HP-βCD solution form of itraconazole, non-standardized preparations were studied, and bioavailability data for these products in animals were often lacking. Such formulations have been studied in humans and they are poorly absorbed (74).

In contrast, to murine models, oral or parenterally administered itraconazole in guinea pigs has good activity and significantly enhances survival in candidiasis and several other fungal pathogen models (75,76). Whether this is due to differences in pharmacokinetics or the itraconazole formulation used is unclear. Itraconazole plasma concentrations are lower in guinea pigs than in mice, but the oral HP-βCD solution form of itraconazole, rather than non-standardized preparations have been used in the guinea pig models (73). Itraconazole efficacy in a given animal model may ultimately depend upon the formulation employed. In contrast to oral administration, IV itraconazole prolonged the survival of mice, but had little effect on reducing fungal burden in the end organs (71). Moreover, diet may influence efficacy by altering the pharmacokinetics of specific azoles in certain animals. Substituting grapefruit juice for water in the animal’s diet has been shown to enhance serum voriconazole concentrations in mice (77). However, the effect varies across animals and between animals. Grapefruit juice had no effect on serum itraconazole concentrations in DBA/2 or BALB/c mice regardless of whether the drug was administered orally or parenterally, yet in the same study it increased plasma itraconazole concentrations following oral administration in guinea pigs (73). Collectively, the experimental efficacy data for itraconazole in the treatment of candidiasis are consistent with the experience in humans. That is, because of its unpredictable pharmacokinetics, oral itraconazole is not appropriate for the treatment of disseminated candidiasis. Rather, if itraconazole is to be used for the treatment of disseminated candidiasis, then it should be administered parenterally so that adequate serum concentrations are achieved.

Fluconazole for the treatment of disseminated candidiasis has been extensively studied in a variety of animal models. In general, fluconazole is significantly more active in the prevention (i.e., prophylaxis) and early (i.e., empirical) treatment of disseminated candidiasis than in the treatment of established (i.e., chronic) infection (78). In comparisons using granulocytopenic rabbit models of disseminated candidiasis, fluconazole was as effective as amphotericin B with or without flucytosine when used for preventative or early treatment. However, in a chronic disseminated candidiasis model, fluconazole was less active than amphotericin B plus flucytosine (78).

Similar to itraconazole, there are unique interspecies differences in voriconazole that affect its activity in certain animal models. In early experimental infection models it was noted that serum voriconazole concentrations are negligible in mice, consequently other models, namely the guinea pig, were used for the study of thisazole (77). In a model of disseminated candidiasis in non-neutropenic and neutropenic guinea pigs, voriconazole produced efficacy similar to that of fluconazole or itraconazole, and was more active than amphotericin B (79). Voriconazole produced
good activity in animals infected with fluconazole resistant strains of *C. glabrata*, *C. krusei*, and *C. albicans* (79). In a neutropenic guinea-pig model of hematogenously disseminated *C. krusei* infection voriconazole was significantly more effective than either amphotericin B or fluconazole in eradicating *C. krusei* from brain, liver, and kidney tissue (80).

**B. Invasive Aspergillosis**

Similar to disseminated candidiasis models, studies of oral itraconazole in murine aspergillosis models usually demonstrate little activity. In contrast, in an immunosuppressed, temporarily leukopenic rabbit model of invasive aspergillosis the oral HP-βCD solution form of itraconazole improved animal survival. At high doses (40 mg/kg/day) it also significantly reduced antigen levels, significantly eradicated *A. fumigatus* from tissues and was as effective as amphotericin B (81). Oral itraconazole is highly effective as prophylaxis or treatment of established invasive aspergillosis in guinea-pig infection models (82,83). In addition, IV itraconazole has produced significant activity in a invasive aspergillosis guinea-pig model (76).

Voriconazole has been studied extensively in a variety of experimental infection models of invasive aspergillosis. Compared to control, and to itraconazole oral voriconazole significantly improved survival in a rat model of invasive pulmonary aspergillosis (84). Similar data were observed in a model of disseminated aspergillosis due to *A. fumigatus*, in an immunosuppressed, leukopenic rabbit and guinea pig (75,85). Because of their fungistatic activity, azoles have not had good activity in experimental models of fungal endocarditis. However, voriconazole has demonstrated unique activity in the prevention and treatment of experimental *A. fumigatus* endocarditis in guinea pigs (86).

**VI. CLINICAL EFFICACY OF THE AZOLES**

The azoles have been evaluated in large prospective randomized trials, key clinical trials, focused primarily in neutropenic hosts. The studies that demonstrate the efficacy of the azoles in the treatment or prevention of systemic infections due to opportunistic fungal pathogens are summarized below. Ketoconazole has been supplanted by the newer azoles and is primarily considered only as an alternative agent, and therefore will not be discussed further.

**A. Fluconazole**

Currently fluconazole is the most widely used azole for the treatment of infections due to *Candida* sp.. Fluconazole is devoid of activity against *Aspergillus* sp., therefore, it is studied in clinical trials for its use in the treatment of invasive or disseminated candidiasis. The results of two large prospective studies indicate that fluconazole 400 mg/day as effective as amphotericin B 0.5-0.6 mg/kg/day in the treatment of invasive candidiasis in non-neutropenic hosts. In these studies response rates for fluconazole and amphotericin B were similar, (57–70% vs. 62–79%, respectively), (87,88). In both studies the incidence of non-*albicans Candida* sp., was low. Moreover, observed mortality rates were consistent with that associated with candidemia and there was no difference in mortality rates between the treatments. When
the source of the infection is related to a vascular catheter, removal and replacement of the catheter was an important therapeutic modality (89).

There are no large prospective studies comparing the efficacy of fluconazole to amphotericin B in neutropenic hosts, but data from observational studies suggest the two treatments may be similarly effective (42). Based upon these data in non-neutropenic patients who are medically stable, or in whom the infecting pathogen is known fluconazole is a safe and effective alternative to amphotericin B in the treatment of candidemia.

Several large clinical trials have evaluated fluconazole as prophylactic therapy in the immunocompromised hosts (i.e., neutropenic cancer patients, BMT, etc.). Administering antifungal therapy to persistently febrile neutropenic patients several days after the initiation of antibacterial therapy has become standard practice. Although this practice stems from the results of two classic prospective trials performed nearly two decades ago, some have suggested that this practice has evolved largely based on theoretical principles rather than clinical science (90). The first randomized trial of fluconazole in this setting demonstrated that it as effective as amphotericin B in patients at low risk for invasive aspergillosis (91). Subsequently a larger trial also demonstrated that fluconazole was not inferior to amphotericin B in this setting (90). In patients who are at high risk for amphotericin B nephrotoxicity, these studies indicate that fluconazole is a reasonable alternative.

Two large-randomized, placebo-controlled trials established that fluconazole 400 mg administered as prophylaxis decreased the risk of superficial and invasive fungal infections after allogeneic and autologous blood and marrow transplantation (91,92). In one study, fluconazole was administered to primarily autologous graft recipients and only during neutropenia, while in the other it was used primarily in allogeneic graft recipients and administered for 75 days. Only one trial demonstrated a decrease in overall mortality (92). Whether fluconazole prophylaxis needs to be continued for 75 days in all blood and marrow transplant recipients or only allogeneic graft recipients has been debated. Data from an 8-year long-term follow up study demonstrate that the survival benefit of prophylactic fluconazole administered for 75-days is realized only by allogeneic graft recipients. Furthermore the study demonstrated that the survival benefit is a result of a reduction in candidiasis related deaths, which frequently occur during acute graft-versus-host disease (93).

B. Itraconazole

Although itraconazole is active against Candida sp., there are no prospective data from large clinical trials describing the efficacy of itraconazole for the treatment of candidemia. In addition, there have been no large, randomized clinical trials comparing the efficacy of itraconazole to other agents with activity against Aspergillus sp., However, because of its activity against Aspergillus sp., itraconazole has been evaluated in large clinical trials for antifungal therapy in persistently febrile neutropenic patients, and as long-term antifungal prophylaxis in allogeneic hematopoietic stem-cell transplant recipients.

In a randomized controlled trial IV and oral itraconazole was compared to amphotericin B deoxycholate as empiric antifungal therapy in persistently febrile neutropenic patients, who had not responded to antibacterial therapy. Patients were randomized to receive amphotericin B deoxycholate 0.7–1.0 mg/kg/day, or IV itraconazole 200 mg/day for 1–2 weeks, at which time they were switched to itraconazole oral solution 400 mg/day (94). The intent-to-treat analysis revealed that overall 47%
of patients randomized to itraconazole responded, in contrast to only 38% of the patients who received amphotericin B. Most of the patients had received prior recent antifungal prophylaxis, and when this was considered the difference in response rate was maintained (94). Significantly fewer drug-related toxicities were seen in patients receiving itraconazole. Consequently, significantly fewer patients receiving itraconazole were withdrawn from the study due to toxicities (94). The study suggests that itraconazole is safer than, and at least as effective as amphotericin B as empirical antifungal therapy in persistently febrile neutropenic patients (94). However, this study was unblinded, which may have introduced bias into the study, particularly in the decision to discontinue the investigational drug due to toxicity (90).

As described above, fluconazole prophylaxis in allogeneic blood and marrow transplant recipients has been shown to reduce the incidence of invasive infections and in allogeneic graft recipients its long-term use is associated with a demonstrable survival benefit (92,93). Infections due to *Aspergillus* sp., are a significant concern throughout the transplant process. Given the safety profile of itraconazole, and its activity against *Aspergillus* sp., it is an attractive alternative to amphotericin B deoxycholate and fluconazole in this setting. IV and oral itraconazole were compared to IV and oral fluconazole as long-term prophylaxis in allogeneic hematopoietic stem-cell transplant recipients. Patients were randomized to receive itraconazole 200 mg/day or fluconazole 400 mg/day IV and then orally for 100 days after transplantation (95). Proven invasive fungal infections were significantly less common among patients receiving itraconazole (9%), compared to those receiving fluconazole (25%) (95). Infections due to *C. albicans* occurred in both groups, but patients receiving itraconazole had fewer invasive fungal infections due to *C. glabrata*, *C. krusei*, and *Aspergillus* sp. (95). Adverse events were less common with fluconazole (95). Based upon the data it was concluded that itraconazole is more effective than fluconazole for long-term prophylaxis in allogeneic hematopoietic stem-cell transplant recipients. However, this study was unblinded, which may have introduced bias into the analysis of the study. In centers with a high incidence of fungal isolates with reduced susceptibility towards fluconazole, itraconazole administered as the IV and oral solution may be a reasonable alternative to fluconazole. However, clinicians must balance its potential benefits with the uncertainty of using the IV formulation in patients with reduced renal function, and the drugs' inherent potential to interact with other medications.

### C. Voriconazole

Voriconazole was compared to liposomal amphotericin B as empiric antifungal therapy in persistently febrile neutropenic patients in a large, randomized trial (96). This study was unblinded, which may have introduced bias into the study, particularly in the decision to discontinue the investigational drug (90). The study was also designed to demonstrate non-inferiority of voriconazole by a difference in success rate not in excess of 10% for the composite endpoint. A modified intent-to-treat analysis revealed the overall success rate for voriconazole was 26% compared to 30.6% for liposomal amphotericin B. However, the lower bounds of the 95% CI for the differences in treatment groups slightly exceeded the 10% bounds set in the non-inferiority definition. A secondary analysis of the individual components of the composite endpoint revealed that there were significantly fewer proven or probable breakthrough fungal infections with voriconazole (96). The authors concluded
that voriconazole is a suitable alternative to amphotericin B preparations for empiric antifungal therapy in persistently febrile neutropenic patients (96).

However, in light of the study design the analysis was questioned and the subsequent conclusion was somewhat disputed (97). The analysis presented was unstratified, yet a stratified analysis was initially planned and given the design of the study, it was deemed the more appropriate primary analysis (97). Second, voriconazole did not fulfill that criterion for non-inferiority to liposomal amphotericin B. In addition, even voriconazole treatment was associated with breakthrough fungal infections, the analyses of the other four components of the composite end point favored liposomal amphotericin B (97). Therefore, whether voriconazole is not as good as liposomal amphotericin B as empiric therapy in persistently febrile neutropenic hosts is still a matter of debate. Clinicians should interpret the results of the study in terms of their own clinical experience and determine what difference in success rate is acceptable to them.

VII. DRUG INTERACTIONS

Drug interactions involving the azoles are discussed in Chapter 17. In general, drug interactions occur primarily in the GI tract, liver, and kidneys by a variety of mechanisms. In the GI tract azole drug interactions result from alterations in pH, complexation with ions, or interference with transport and enzymatic processes involved in gut wall (i.e., presystemic) drug metabolism. In the liver they occur as a result of interference with phase I or II drug metabolism, or interactions with transport proteins. In the kidney–drug interactions can occur through interference with glomerular filtration, active tubular excretion, or interactions with transport proteins. The azoles are one of the few drug classes that can cause or be involved in drug interactions at all of these sites, by one or more of the above mechanisms. Many of the drug–drug interactions involving the azoles occur class-wide. Therefore, when using the azoles, the clinician must be aware of the many drug–drug interactions, both real and potential, associated with this class. For a comprehensive review of drug interactions specifically involving the azoles, the reader is referred to Chapter 17 and two recent reviews on this topic (4,13).

VIII. CONCLUSION

The systemically acting azoles are one of the largest classes of systemically acting antifungals. The chemical properties of these drugs influence their pharmacokinetics, and are the basis of the many drug interactions associated with these agents. They act at a key step in ergosterol synthesis. Unlike other classes of antifungals they are available as oral and IV dosage forms. Each agent has a unique pharmacokinetic profile and among existing antifungals as a class they among the safest agents. Additionally they are broad spectrum in activity, and they typically exhibit concentration-independent, static pharmacodynamic effects. As a class these agents possess potent yet diverse spectrum of activity. Fluconazole possesses perhaps the narrowest spectrum of activity and is primarily active against *Candida* and *Cryptococcus* sp., In contrast itraconazole, and voriconazole possesses activity against yeasts and molds including *Candida* and *Aspergillus* species, and other opportunistic pathogens. Although antifungal susceptibility testing is still in its
infancy, the susceptibility testing methods and breakpoints for *Candida* species are well defined. Resistance to the azoles is relatively uncommon, but when it occurs it is most likely due to either alterations in the target enzyme, or active efflux or a combination of both. Clinically, resistance is most often observed in the setting of HIV infection and AIDS.

The azoles have demonstrated potency in against many pathogens in a variety of experimental animal models of infection. However, in order to interpret the results obtained in these models clinicians should be cognizant of the complex pharmacokinetics displayed by itraconazole and voriconazole, and the impact the dosage form may have on the function of the model. Lastly, in large clinical trials in immunocompromised hosts the azoles have demonstrated efficacy as prophylactic and empirical therapy. Thus, they are alternative treatments to amphotericin B for a variety of systemic mycoses. As the azoles continue to grow as a class, so too will the options available to clinicians in selecting therapy for life-threatening mycoses in immunocompromised hosts. Future agents will likely continue to expand the spectrum of activity and offer improved pharmacokinetics.

REFERENCES


I. A NEW ANTIFUNGAL TARGET

The fungal cell wall is composed largely of chitins, mannoproteins, and glucans. The cell wall fulfills a number of critical functions for the fungal cell, including defining its shape, providing docking sites for fungal enzymes, filtering the passage of multiple ions and compounds into the cell, and functioning as the site for adherence of fungi to the vascular endothelium and other structures (1). The fungal cell wall is a highly desirable target for drug design because there is no equivalent in mammalian cells; thus, there may be less drug toxicity.

Disruption of the fungal cell wall was attempted some years ago by inhibiting chitin synthase with Nikkomycin Z. Although this drug was effective in vitro and in vivo against Coccidioides immitis, and to lesser degree Candida, Histoplasma capsulatum, and Aspergillus, and reached Phase I clinical trials, it was not further developed, in part because of bankruptcy of the parent company, Shaman (2–6). Efforts were also directed at attack on the fungal mannoproteins, primarily with development of pradimycin. This compound was highly effective in animals (7,8). However, in phase I clinical trials there was hepatotoxicity and the drug was withdrawn.

The third class of cell wall constituents, the glucans, was more amenable to antifungal drug development. Acułaein A and other related cyclic lipopeptides were initially tested against fungi. They showed some activity, but were not suitable for further development (1). The first compound of significant medical interest was cilofungin, developed by Lilly (Fig. 1) (9,10). However, cilofungin as a first start was quickly aborted in phase I trials. The drug was poorly soluble in water, and the vehicle used in the initial clinical trials was nephrotoxic. Nevertheless, before it was abandoned, cilofungin provided some very useful information. First, the drug acts against FKS1 synthase, an enzyme responsible for synthesis of beta-1,3-D-glucans (11). Second, it is highly effective in vitro against Candida albicans (12). Third, while
ineffective in vitro against *Aspergillus fumigatus* (using NCCLS methods), the drug was highly effective in vivo in murine aspergillosis (13,14). Fourth, its non-linear kinetics and the saturable clearance of the drug in animals suggested hepatic metabolism of this class of drugs (15,16). Fifth, cilofungin was active against *C. immitis*, an organism very rich in glucans (17). Sixth, in a series of studies which may predict clinical use of this class, cilofungin was additive or synergistic with amphotericin B against several fungal pathogens (18–21).

Despite the limitations of cilofungin, by the 1990s interest in the echinocandins had broadened considerably. Lilly continued development of drugs, and eventually produced what is now anidulafungin (Versicor). Fujisawa independently developed micafungin, and Merck developed caspofungin. At the time of this writing, caspofungin is licensed, micafungin is on the verge of licensure, and anidulafungin is well into clinical trials. Drugs focused on this new antifungal target had reached the clinic.

II. MECHANISM OF ACTION

Although the cell wall has multiple components, beta-1,3-D-glucans are critical components for many (but not all) fungal species. Double deletion mutants of FKS1 (glucan synthase) are non-viable (11). Echinocandin-induced inhibition of glucan synthesis causes *Candida* cells to lose their rigidity, become deformed, and ultimately form a protoplast-like mass (Fig. 2) (22). Echinocandin binding may occur within moments, and an exposure of just minutes is sufficient to reduce cell viability sharply (Fig. 3) (23).

One advantage of the echinocandins is that they act independently of the fungal cell membrane. Thus, as expected, triazole-resistant *Candida* are susceptible

![Figure 1](https://example.com/figure1.png) Structures of echinocandins.
in vitro to echinocandins (24–27). The echinocandins have a long postantifungal effect, and are considered fungicidal to *Candida* (28,29). Unfortunately, this broad susceptibility does not apply to all fungi. *Cryptococcus neoformans*, also a yeast, does not contain high quantities of beta-1,3-D-glucans, and thus is resistant both in vitro and in animal models (30). Other investigators have found that overexpression of a gene coding for synthesis of a Golgi protein also confers resistance to caspofungin in *Saccharomyces cerevisiae* (31).

Another potential advantage of echinocandins is that they, and lipid preparations of amphotericin B, appear unusually potent in reducing *Candida* in biofilms (32). This is of potential importance, as *Candida* are particularly difficult to treat when associated with prosthetic devices such as catheters and artificial heart valves (33,34). Whether this in vitro phenomenon can be translated into the clinical arena is unclear.

Further, glucan synthesis occurs relatively evenly in yeast cell walls, but occurs primarily at new growth tips of hyphae in mycelial fungi (35,36). Thus, we see a different picture in *A. fumigatus* exposed to echinocandins. The growing tips of fungi are damaged by the drug, and killed, but older more sessile mycelial structures remain viable (Fig. 4) (37). If susceptibilities are performed by NCCLS methods for hyphae, the resulting MIC is extremely high, (above 64 µg/mL) as fungal colonies are blunted in growth but there are few clear end points (36,38). However, if the fungal colonies are examined using a change from diffuse hyphal growth to a clumped pattern, with little new hyphal extension, then end points fall within the range of ≤2 µg/mL or less. This
end point, based on the “minimum effective concentration (MEC)” may accurately predict the efficacy of echinocandins against mycelial pathogens. The MEC may be generalizable to other moulds as well, with the mycelial phase of *C. immitis* showing the same pattern (39). Although *Aspergillus* fares well with the MEC method, other mycelial pathogens such as *Fusarium* appear resistant using even this optimized method (40,41). There is some variability in results with other mycelial fungi. Interpretation is difficult, as “resistance” may have been measured as an artifact of turbidimetric methods rather than the change in morphology which is a preferable end point (41,42). Disc diffusion has also been utilized to measure antifungal efficacy (Table 1) (9). If the antifungal drugs are removed, *Aspergillus* begin to grow rather quickly, showing very little postantifungal effect. The echinocandins are thus only considered fungistatic to mycelial pathogens (43).

III. CORRELATING IN VITRO AND IN VIVO ACTIVITY

As with other antifungal drugs, in vitro activity of the echinocandins must be proven in vivo using animal models, before the drug is taken to clinical evaluation. Here the
Echinocandins gave some surprising results. As expected, in an animal model studied with fluconazole-susceptible and -resistant *C. albicans* and non-*albicans* species, the echinocandins showed high efficacy, down to as low as <0.1 mg/kg/dose. *C. neoformans* is predictably resistant in vitro and in vivo (27,30,45–47). However, in vitro surveys showed up a number of isolates of *Candida parapsilosis* with relatively high in vitro MIC values, up to 8 mg/mL or more. Because there were no good animal models for this low virulence species, correlation of in vitro and in vivo results had to await clinical experience.

**Figure 4** Effect of extended drug treatment on *A. fumigatus* fluorescent dye staining. (A) Untreated, DiBAC-stained germlings harvested 24-hour after vehicle addition. Magnification 400×. Arrows indicate conidiophores. Caspofungin treated germlings (0.3 µg/mL) harvested 72 hr after drug addition were stained with CFDA(B) or DiBAC(C) (magnification 800×). In panels B and C, arrows with single tails indicate lysed apical cells and arrows with double tails indicate lysed conidophores. The fluorescent micrographs in panel A required a 20-sec exposure. Left panels, Normarski optics; right panels, epifluorescence with an FITC filter set (35).
More impressive surprises awaited those working with mycelial fungi. The early model was aspergillosis. In mice and rats, echinocandins prolonged the survival of treated mice, though the effects on tissue counts were variable (30,46,48,49). Further, micafungin appears highly effective in murine aspergillosis caused by *A. fumigatus* (50). In this model, survival was prolonged and lung tissue burden was reduced sharply. However, when chronically immunosuppressed rabbits were infected with *A. fumigatus* and treated with echinocandins, survival was prolonged, size of pulmonary infarct lesions was reduced, and yet there was no reduction of colony counts of infected pulmonary tissues (Fig. 5) (51).

<table>
<thead>
<tr>
<th>Species (N)</th>
<th>IZ Range in mm (Mean)</th>
<th>MEC Range in Conc’n (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. flavus</em> (27)</td>
<td>16–25 (21.2)</td>
<td>0.125–0.5 (0.26)</td>
</tr>
<tr>
<td><em>A. fumigatus</em> (26)</td>
<td>12–23 (16)</td>
<td>0.25–0.5 (0.31)</td>
</tr>
<tr>
<td><em>A. niger</em> (16)</td>
<td>15–24 (18)</td>
<td>0.125–0.25 (0.2)</td>
</tr>
<tr>
<td><em>A. terreus</em> (9)</td>
<td>12–26 (19.6)</td>
<td>0.25 (0.25)</td>
</tr>
<tr>
<td><em>F. solani</em> (18)</td>
<td>No zone</td>
<td>&gt;16</td>
</tr>
<tr>
<td><em>F. oxysporum</em> (4)</td>
<td>No zone</td>
<td>&gt;16</td>
</tr>
</tbody>
</table>

No activity is observed against *Fusarium*.

Figure 5  Effects of caspofungin treatment on survival, infarct size, and tissue burden of rabbits infected with *A. fumigatus* (52).
Furthermore, this happened not only with micafungin, but also with anidulafungin. This indicates that the effect is class specific, not drug specific (51).

The lack of effect on colony counts raises the question as to whether echinocandins actually reduce the fungal burden or somehow just hold the organism in check, with benefit in survival only. Petraitis et al. indicate that not only do fungal colony counts remain unchanged with caspofungin therapy, but galactomannan antigen, a measure of fungal biomass, also remains stable, or increases (51). The lack of effect of echinocandins on tissue burden stands in distinction from amphotericin B, where the galactomannan titer drops (Fig. 6). There is not uniform agreement on this, however, as Bowman et al. have found, with different molecular methods, that Aspergillus burden does decline on caspofungin therapy (Fig. 7) (37,53). The inference here is that the fungi are broken up into smaller particles, each of which can form a colony, though the total burden is reduced. Resolution of this difference awaits future studies.

Other mycelial and dimorphic pathogens might behave in similar ways. At present our information is incomplete. There is animal evidence of good efficacy in coccidioidomycosis, and conflicting data in histoplasmosis (39,54,55). In vitro data on other moulds need follow-up with animal studies before clinical trials are launched (56).

IV. CLINICAL EVALUATION: HOW ARE THESE DRUGS HANDLED?

Of the three echinocandins in clinical use, none are absorbed very well when administered orally. All drugs must be given intravenously. One advantage of these drugs
is that they are sufficiently water soluble that special vehicles, such as the cyclodextrins used for triazoles, are not required. Plasma protein binding is well over 90% for caspofungin, and approximately 85% for anidulafungin. However, the drugs are sufficiently potent that high plasma protein binding does not impair activity. Animal studies have shown that echinocandins are distributed well to most tissues. However, for micafungin, penetration of the brain was reduced compared to the lung, liver, and plasma (57). The caspofungin plasma concentration 1 hr after infusion of 10 mg was 1.6 mg/mL, and after 100 mg was 14.03 mg/mL (58). Clearance occurred slowly and was similar over a broad range of doses. The caspofungin plasma half-life is 9–10 hr, and is linear from 5 to 100 mg doses. The micafungin half-life is 10–16 hr, and the anidulafungin half-life is as long as 50 hr (59,60). This allows for once daily dosing for all three drugs. Chemical hydrolysis in plasma to inactive metabolites may be the primary route of clearance (61). With anidulafungin, this occurs at the same rate in saline as in serum, with a half-life at body temperature and pH 7.4 of approximately 24 hr. These drugs may be further cleared hepatically, but not via the cytochrome oxidase system used by the triazoles (61). A third advantage of the echinocandins is that there are virtually none of the complex drug interactions which plague the triazoles (62,63). One unresolved question is whether the combination of caspofungin and cyclosporine A causes liver toxicity (elevated liver enzymes were noted in a small number of healthy volunteers). Studies with anidulafungin in vitro showed that up to 30 µg/mL did not affect hepatic microsomal degradation of cyclosporin (64). Tacrolimus and mycophenolate do not affect any of these drugs. Despite secondary hepatic metabolism of some components, clearance is almost linear over the dose ranges explored thus far. Liver failure significantly impedes clearance (Table 2) (65–67). Peak serum concentrations after a single 50-mg dose in normal patients were 2.07 µg/mL vs. 2.24 µg/mL in those on dialysis. Peaks of 1.57 µg/mL vs. 2.87 µg/mL were observed in those with severe hepatic impairment. Anidulafungin is not dialyzable. A fourth advantage of the echinocandins is that all three drugs appear to have a very broad therapeutic index, and significant dose-dependent toxicity has not been a problem for any of these drugs (Table 3). In effect,
### Table 2  Clearance of Anidulafungin in Patients with Severe Liver or Renal Failure (65,67)

<table>
<thead>
<tr>
<th></th>
<th>$N$</th>
<th>$C_{\text{max}}$ (μg/mL)</th>
<th>Clearance (L/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hepatic Failure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>1.57 ± 0.4</td>
<td>1.23 ± 0.3</td>
</tr>
<tr>
<td>Severe</td>
<td>5</td>
<td>2.87 ± 0.7</td>
<td>0.74 ± 0.2</td>
</tr>
<tr>
<td><strong>Renal Failure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>2.07 ± 0.2</td>
<td>0.99 ± 0.1</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>3</td>
<td>2.24 ± 0.7</td>
<td>1.00 ± 0.2</td>
</tr>
</tbody>
</table>

The daily dose was 50 mg/day for 7 days.
The half-life is increased by hepatic failure.

### Table 3  Selected Clinical and Laboratory Adverse Reactions

<table>
<thead>
<tr>
<th></th>
<th>Caspofungin</th>
<th>Micafungin</th>
<th>Anidulafungin$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systemic (63)</td>
<td>Mucosal (68)</td>
<td>(69)</td>
</tr>
<tr>
<td>Patients ($N$)</td>
<td>Patients ($N$)</td>
<td>Patients ($N$)</td>
<td>Patients ($N$)</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------</td>
<td>--------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Total</td>
<td>114</td>
<td>83</td>
<td>67</td>
</tr>
<tr>
<td>Fever/chills</td>
<td>8</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>NS</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Phlebitis</td>
<td>4</td>
<td>3.5</td>
<td>2</td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
<td>1.8</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4</td>
<td>3.5</td>
<td>NS</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>NS</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Headache</td>
<td>NS</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Rash</td>
<td>1</td>
<td>0.9</td>
<td>2</td>
</tr>
<tr>
<td>Dizziness</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cough</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Increased SGOT</td>
<td>4</td>
<td>24.3</td>
<td>1</td>
</tr>
<tr>
<td>Increased SGPT</td>
<td>2</td>
<td>1.9</td>
<td>1</td>
</tr>
<tr>
<td>Increased bilirubin</td>
<td>3</td>
<td>2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Increased alkaline Phosphatase</td>
<td>9</td>
<td>8.3</td>
<td>3</td>
</tr>
<tr>
<td>Decreased hemoglobin</td>
<td>1</td>
<td>0.9</td>
<td>11</td>
</tr>
<tr>
<td>Leukopenia and/or neutropenia</td>
<td>NS</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Decreased albumin</td>
<td>NS</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Increased creatinine or Urea nitrogen</td>
<td>4</td>
<td>3.7</td>
<td>3</td>
</tr>
<tr>
<td>Polyarthritis</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$J. Schranz, personal communication, Versicor.
$^b$sum of patients with any liver function test abnormal.

**Abbreviation:** NS, not specified.
the maximum tolerated dose for none of these drugs has been determined. Courses of >1 week in adults have been used with caspofungin at 140 mg/day, micafungin up to 8 mg/kg/day, and anidulafungin at up to 130 mg/day (60). However, at the 100 mg anidulafungin/day dose, three of 10 healthy subjects had >2× normal elevations of the alanine or aspartate aminotransferase.

There are fewer data available for children. Micafungin has been administered at 4 mg/kg/day. Pharmacokinetics in children aged 2–17-year old were evaluated by Walsh et al. (70). The geometric mean ratios of AUC and 1-hour serum concentrations in children/adults were 0.62 and 0.87 on day-1 of treatment, and 0.52 and 0.80 on day-4 of treatment. The β-phase half-life was reduced 32–43% in children vs. adults.

With such a broad safety range and so few signs of intolerance, how are these drugs administered clinically? At this relatively early phase of experience, it appears that the potency of each echinocandin appears to be quite similar to the other, and one might almost interchange recommendations. In the initial studies of caspofungin, mouse studies were used to estimate human doses. The desired goal was a plasma concentration which consistently exceeded the MIC for _C. albicans_ at a 24-hour postdose trough, and was effective in mice. Because of the long half-life, an initial loading dose of caspofungin was used at 70 mg. Maintenance doses in initial clinical studies of _Candida_ esophagitis bracketed 50 mg/day (71).

Another advantage for use in the intensive care unit is the striking absence of severe adverse reactions (Table 3). The echinocandins have virtually no effect on the kidneys, the liver, or the myelopoietic system. Initial concerns about potential anaphylaxis have proven unfounded. Indeed, in the phase III trial in candidemia, there were 16 cases of anaphylaxis with amphotericin B and only one with caspofungin.

All of the above considerations, plus the ease of administration, suggest that the echinocandins will be excellent alternatives in patients with liver or renal failure, and in patients with known or suspected fluconazole resistance. Indeed, the cost of these new drugs may be the only reason they do not replace amphotericin B completely in hospitalized patients.

V. CLINICAL EFFICACY

A. _Candida_

Although animal studies suggest that efficacy may be broader, at present our clinical data are limited to experience with _Candida_ and _Aspergillus_ species. Uniformity of in vitro resistance and animal study failures in cryptococcal disease suggests that we should proceed cautiously in expanding clinical experience beyond _Candida_ and _Aspergillus_ (72).

For all three echinocandins in clinical use, the first studies were conducted in mucosal candidiasis, particularly in patients with HIV infection and _Candida_ esophagitis. Initial open dose ranging studies were followed by randomized clinical trials. It is remarkably how similarly the three echinocandins behaved. Using a modified intent-to-treat analysis, the endoscopic response rates were 85% for 81 recipients of caspofungin at 50 mg/day, and 86% for 94 recipients of fluconazole at 200 mg/day (68). A large randomized trial comparing anidulafungin at 50 mg/day (249 patients evaluable with endoscopy) with fluconazole at 100 mg/day (255 patients evaluable with endoscopy) and using 1 grade reduction of endoscopy score, showed 97% success for anidulafungin and 99% for fluconazole (J Schranz, personal
A dose finding study of micafungin for esophageal candidiasis had 120 patients divided into cohorts treated with 12.5, 25, 50, 75, or 100 mg/day (Protocol 97–7-003, Monograph Echinocandins in the Management of Invasive Fungal Infections, 2001, C. Brown, Project Coordinator Marketing, Fuji-sawa Healthcare Inc., Deerfield, IL). The clinical responses at end of therapy were above 80% at ≥50 mg/day, and <80% at lower doses. However, the endoscopic responses showed a dose-response all the way up to 100 mg/day (mean reduction of endoscopy score from 2.5 to 0.2 at the highest dose, vs. reduction from 1.8 to 1.1 at 50 mg/day dose) (Fig. 8). This study, while having an average of only 20 patients in each treatment group, suggested a dose-dependent response all the way from 12.5 to 100 mg/day. The study with micafungin in humans is reminiscent of a trial in rabbits, in which micafungin responses were clearly dose-dependent in fluconazole-resistant esophageal candidiasis (73). In a follow-up open label study, 97 patients were treated with at least five doses of micafungin, 50 mg/day, for esophageal candidiasis (74). Of those with end-of-treatment esophagoscopy, 91% were cured or improved compared to the baseline.

As shown in Table 4, responses of patients with thrush (complete clearing required and resolution of symptoms) and esophagitis (two grades of improvement required) were evaluated for different antifungal agents. The table shows the efficacy of caspofungin, amphotericin B, and fluconazole in the treatment of oropharyngeal and esophageal candidiasis.

Table 4 Efficacy of Caspofungin in Oropharyngeal and Esophageal Candidiasis

<table>
<thead>
<tr>
<th></th>
<th>Caspofungin</th>
<th>Amphotericin B</th>
<th>Fluconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>35 mg</td>
<td>50 mg</td>
</tr>
<tr>
<td>Thrush (71)</td>
<td>128</td>
<td>ND</td>
<td>(74)/46</td>
</tr>
<tr>
<td>Mucosal (62)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrush</td>
<td>52</td>
<td>(85)/13</td>
<td>(93)/14</td>
</tr>
<tr>
<td>Esophagitis</td>
<td>86</td>
<td>(67)/21</td>
<td>(90)/20</td>
</tr>
<tr>
<td>Esophagitis (43)</td>
<td>175</td>
<td>ND</td>
<td>(81)/82</td>
</tr>
</tbody>
</table>

Responses given as (%)/N in group.
Source: Modified from Ref. 78.
required on endoscopy, and resolution of symptoms) were as effective with caspofungin at 50 mg/day as with amphotericin B (62). Because there was slightly lower response at 35 mg/day, the 50 mg/day maintenance dose was accepted for caspofungin for further study in systemic candidiasis. In another study of patients with fluconazole-resistant mucosal disease, caspofungin was found to be no less effective than with fluconazole for susceptible disease (77).

The reason initial trials were conducted with mucosal candidiasis was that a failure of therapy simply meant persistent esophagitis, while failure of therapy for disseminated disease could be lethal. Armed with the initial data from mucosal disease, Merck and Fujisawa proceeded in different directions. Caspofungin was evaluated in a large phase III blinded randomized trial for documented systemic disease (63). The trial was sufficiently powered to give results for an overall group with systemic disease and also for the subgroup with candidemia. Patients were stratified for presence or absence of neutropenia, APACHE II score over or under 20, and randomized to caspofungin at 70 mg load then 50 mg/day vs. amphotericin B at 0.6–1 mg/kg/day. In the modified intent-to-treat analysis (MITT—each patient was randomized and received at least one dose of study drug), the caspofungin response rate in 109 patients was 73.4%, while the amphotericin B response rate in 115 patients with 61.7% (Table 5). This was not quite statistically significant \( p = 0.09 \). Of 185 patients who could be evaluated clinically for cure, the response to caspofungin was 80.7% vs. 64.9%. The comparison in this group significantly favored caspofungin over amphotericin B for efficacy \( p = 0.03 \). Overall mortality was 34% in the caspofungin recipients, and 30% in the amphotericin B recipients (not statistically significant).

Also of interest, there were no dramatic differences between caspofungin and amphotericin B for treatment of patients with \( C. \) parapsilosis infection (high MIC to caspofungin, \( >4 \mu g/mL \)) or \( C. \) glabrata or \( C. \) krusei infection (traditionally resistant to fluconazole), or in patients with neutropenia (<500 or 100 neutrophils/mm\(^3\)) or high APACHE scores (over 20) (Table 6). However, it must be emphasized that none of these subgroups is large enough for statistical comparison.

Micafungin was subjected to a phase III trial of prevention rather than the treatment of systemic candidiasis in hematopoietic stem cell transplant recipients (80).

### Table 5 Response to Treatment of Disseminated Candidiasis; Caspofungin Vs. Amphotericin B

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Overall response at end of IV therapy (test of cure) number with a favorable response/total number (%)</th>
<th>Estimated difference adjusted for strata (%) (95.6 CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caspofungin 70 mg induction 50 mg maintainence Amphotericin B 0.6–1.0 mg/kg</td>
<td></td>
</tr>
<tr>
<td>MITT(^a) (( n = 224 ))</td>
<td>80/109 (73.4)</td>
<td>12.7 (-0.7, 26.0)</td>
</tr>
<tr>
<td>Evaluable patients ( n = 185 )</td>
<td>71/88 (80.7)</td>
<td>15.4 (1.1, 29.7)</td>
</tr>
</tbody>
</table>

\(^a\)Modified Intent-to-Treat (each patient was randomized and received at least one dose of study drug).

\(^p = 0.09.\)

\(^'p = 0.03.\)

*Source: Modified from Ref. 63.*
In a large study of 882 patients, van Burik et al. found similar efficacy of micafungin and fluconazole in prevention of systemic fungal infections in both adults and children. They used 50 mg/day for adults and 1 mg/kg/day for children. Fewer micafungin recipients (15%) than fluconazole recipients (21%, \( p = 0.018 \)) required systemic antifungal therapy. Seven breakthrough infections with Aspergillus occurred in patients receiving fluconazole, vs. one in a patient receiving micafungin. The difference was almost significant (\( p = 0.07 \)). Thus, micafungin was at least as effective as fluconazole in prevention of overall deep and superficial mycoses.

From the above information one can make some tentative recommendations for the use of echinocandins for treatment of Candida infection. For mucosal candidiasis, and for prophylaxis of Candida deep infection in predisposed patients, it is clear that echinocandins are as effective as fluconazole. Most of these patients are infected with \( C. \) albicans susceptible to fluconazole. However, because fluconazole is effective, it is hard to justify the markedly increased cost and IV delivery of echinocandins. However, in a small group of patients with fluconazole-resistant \( C. \) albicans esophagitis, the echinocandins clearly are successful, and reasonable, non-toxic alternatives to amphotericin B. A daily dose of 50–100 mg/day would seem sufficient. In a study of four patients with mucosal candidiasis refractory to fluconazole, itraconazole, and amphotericin B, all four had dramatic improvements with caspofungin, with no evidence of candidiasis within 7–10 days (44). Nevertheless, treatment needs to be done daily to prevent relapse, and this is a limitation. A recent presentation found that four patients relapsed when caspofungin was reduced to 3–5 times weekly after an initial successful course (81).

For deep candidiasis, caspofungin is clearly effective, better tolerated than amphotericin B, and likely more effective than amphotericin B (63). However, the current drug of choice is fluconazole, and there is no evidence that caspofungin, as initial therapy, would be sufficiently superior to fluconazole to merit its much higher costs (82). However, there are several circumstances which would warrant the use of caspofungin. One of these is nephrotoxicity and another similar reason is concurrent administration of nephrotoxic drugs (tacrolimus, gentamicin, etc.). In these cases, echinocandins would be preferred over amphotericin B desoxycholate.

### Table 6  Response by Organism; Caspofungin Vs. Amphotericin B

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Caspofungin 70 mg induction 50 mg maintenance</th>
<th>Amphotericin B 0.6–1.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C. ) albicans</td>
<td>23/36 (63.9)</td>
<td>34/59 (57.6)</td>
</tr>
<tr>
<td>( C. ) parasiolopsis</td>
<td>14/20 (70.0)</td>
<td>13/20 (65.5)</td>
</tr>
<tr>
<td>( C. ) tropicalis</td>
<td>17/20 (85.0)</td>
<td>10/14 (71.4)</td>
</tr>
<tr>
<td>( C. ) glabrata</td>
<td>10/13 (76.9)</td>
<td>8/10 (80.0)</td>
</tr>
<tr>
<td>( C. ) guilliermondii</td>
<td>3/3 (100.0)</td>
<td>1/1 (100.0)</td>
</tr>
<tr>
<td>( C. ) krusei</td>
<td>4/4 (100.0)</td>
<td>0/1 (0.0)</td>
</tr>
<tr>
<td>Mixed Infection</td>
<td>3/3 (100.0)</td>
<td>2/4 (100.0)</td>
</tr>
<tr>
<td>Neutropenia (&lt;500 cells/mm(^3))</td>
<td>7/14 (50.0)</td>
<td>4/10 (40.0)</td>
</tr>
<tr>
<td>Apache II Score &gt;20</td>
<td>12/21 (957.1)</td>
<td>10/23 (43.5)</td>
</tr>
</tbody>
</table>

*Source: Modified from Refs. 63 and 79.*
or lipid formulations, but not fluconazole. Another circumstance is intolerance of fluconazole or other triazoles, either from toxicity (usually hepatic) or drug interactions. The latter are usually more problematic in the broad-spectrum triazoles itraconazole, voriconazole, and potentially posaconazole (83, 84). Yet another circumstance is the suspicion or documentation of a Candida species (usually C. glabrata) resistant to fluconazole. C. glabrata may have up to 20% high-level resistance to fluconazole, and tends to emerge in oncology populations, where the use of fluconazole as a prophylactic agent is common (85, 86). A similar circumstance would be in the patient who has developed candidemia while receiving or shortly after receiving fluconazole or another triazole. While the above are supported by evidence, there is one more circumstance in which I would instinctively utilize echinocandins as first line drugs. This would be in the intensive care unit patient who has fungemia and who is critically ill and hypotensive. In such a patient it is reasonable to prefer a fungicidal agent with very little toxicity and great potency and echinocandins seem to best fit those characteristics.

Taken together, these data support the contention that echinocandins are highly efficacious against both mucocutaneous (vs. fluconazole) and disseminated (vs. amphotericin B) Candida infection, that they are similarly effective against fluconazole-susceptible and -resistant organisms, and that are at least as effective as fluconazole in prevention of mycotic infections in patients undergoing allogeneic stem cell transplantation. There is also the suggestion that they may not be antagonistic to AmBisome when used in combinations. However, because of poor penetration into the urine as the active drug, the echinocandins are not likely to be useful for the treatment of Candida urinary tract infection. Finally, efficacy in severely ill patients, and the requirement for intravenous administration, suggest that these agents may find their optimal use in the intensive care unit.

B. Aspergillus and Other Moulds

Invasive mould infections are generally acquired by inhalation of conidia, and development of fungal pneumonia or sinusitis. A major problem has been in defining which pneumonias are likely to be fungal and which are not. This has led to problems comparing present results of recent results with prior experience. Prior experience used varied definitions of invasive filamentous fungal infections. More recently, a consensus on redefinition of invasive mould infection has been reached by investigators in the United States and Europe. They have established criteria for “documented” and “probable” mycelial invasive disease, but have considered “possible” mycelial infection (essentially pneumonia in a predisposed person, with no specific indicators for filamentous fungal pathogens) as too nonspecific (87). Recent studies of Aspergillus galactomannan detection have suggested that “possible” mycoses are likely not mycoses at all (88, 89). Using the EORTC/MSG definitions for documented and probable invasive aspergillosis, response rates have been in the order of 10–20% in bone marrow transplant recipients, and 40–60% in lung transplant recipients (87, 90). Most of these patients had received amphotericin B in its various forms. Amphotericin B lipid formulations appear similar in efficacy to the desoxycholate preparation (91). In this discouraging setting, the recent publication of a multi-center trial found voriconazole is markedly superior to amphotericin B for treatment of documented invasive aspergillosis (84). The data significantly favored voriconazole in terms of drug tolerance, complete and partial
responses, and survival. Voriconazole is now clearly the drug of choice for acute invasive aspergillosis.

Nevertheless, many patients still fail primary therapy with voriconazole or amphotericin B preparations. For these patients, physicians have often had salvage therapy success rates of 40–50% at best. Given the limited efficacy of the polyenes and itraconazole, and the efficacy of echinocandins in animal models, all three echinocandins are undergoing clinical evaluation in invasive aspergillosis. Preliminary data are available for caspofungin. At present there are no phase III comparative trials with echinocandins, as there are for voriconazole. The clearest data come from Merck’s open study of caspofungin in acute invasive aspergillosis. This study, which was the basis for FDA approval for caspofungin, involved salvage therapy with caspofungin for patients with either progressive disease after a week on primary therapy with another drug, or with intolerance of other therapy. This was primarily renal failure from amphotericin B. The initial experience with 56 patients was enlarged to 83 patients (79,92). Caspofungin was given at 70 mg initial loading dose, then 50 mg/day. Patients had severe predisposing diseases, including 48% with hematologic malignancy and 25% with allogeneic bone marrow transplant. The complete and partial response rates were 50% of 32 patients with pulmonary disease, 23% of 13 patients with disseminated disease, and 33% of six patients with single organ extra-pulmonary disease. Unlike candidiasis, in which neutropenia did not affect outcome, only 26% of 19 neutropenic patients had a complete or partial response. The overall response rate was 45%.

Of interest, in the initial series, 34% of 44 patients responded to caspofungin, vs. 70% of 10 patients treated because of intolerance to other antifungal drugs (79). This suggests that primary therapy of invasive aspergillosis, undertaken at an earlier time in the course of disease, might show a more favorable response than one sees in those given salvage for progressive disease.

There is a small unreported experience collected with micafungin therapy of aspergillosis. Micafungin was administered to some patients alone, and to some patients combined with liposomal amphotericin B. There are no data yet available in the public arena. The prophylaxis study by van Burik et al. found nearly significant (7 vs. 1) protection by micafungin against invasive aspergillosis (80). This suggests a role for micafungin against aspergillosis. Similarly, information on anidulafungin for invasive aspergillosis are not yet available. Although animal experience supports activity in coccidioidomycosis, there are no clinical data available. A single patient treated by us for refractory disease received caspofungin for less than 2 weeks before the drug supply was exhausted. We were unable to ascertain a change in her course.

VI. COMBINATION THERAPY OF MYCOSES

Combinations of echinocandins and other antifungals have been evaluated in numerous in vitro and some animal studies. The strongest data supporting combination therapy have come from in vitro studies. Perhaps the strongest interaction effects occur between two cell wall active agents, micafungin and anidulafungin, which inhibit glucan synthesis, and Nikkomycin Z, which inhibits chitin synthesis (93,94). While the interaction was additive to indifferent against *Fusarium solanae* and *Rhizopus oryzae*, against *A. fumigatus* there was marked synergistic activity. The hyphae
showed extensive damage over a wide range of drug concentrations. Unfortunately, Nikkomycin Z is not under active development at present.

Other studies have shown a favorable interaction of triazoles and echinocandins against \textit{C. neoformans} (95). However, Roling et al. found only indifference of caspofungin or anidulafungin and fluconazole against \textit{C. albicans}, \textit{C. tropicalis}, \textit{C. glabrata}, \textit{C. krusei}, and \textit{C. neoformans} (96). Against \textit{Aspergillus} and \textit{Fusarium}, Arikan et al. found synergistic activity in a few and additive activity in more isolates (97). Bartizal et al. have also found additive activity of echinocandin combinations (98).

Likewise, animal studies have been mixed. Early studies with cilofungin (not in clinical use) and amphotericin B showed additive effects in mice with candidiasis (20,21). Another study showed antagonism of cilofungin and amphotericin B in murine aspergillosis (19). In contrast, Nakajima et al. found additive effects of micafungin and amphotericin B in murine aspergillosis (99). Perhaps of greatest interest, Kirkpatrick et al. found “additive” effects of voriconazole and caspofungin in a guinea-pig model of aspergillosis (100). In the same laboratory, synergistic effects were found in vitro. However, there is some controversy on whether the effects of the combination were truly superior to voriconazole alone.

Given the limited successes of single drug therapy, and the in vitro/animal studies which show mixed results, clinicians have interpreted these data in the best positive terms possible, and are beginning to use caspofungin plus either amphotericin B or voriconazole clinically. Patients are treated usually for progressing disease, and in general the salvage rate for alternative antifungals has been in the 30–40% range (101,102). The combinations used seem to have responses also in this range, and it is very unclear what, if anything, these costly regimens add to improvement of the clinical outcome. In one “positive” study, 20 of 30 patients treated with caspofungin and liposomal amphotericin B had “possible” rather than “probable” or “documented” infection (103). Thus, the authors of the recent study of mainly “possible” mycoses, in which the patients may not really have had fungal infection, attribute good responses to caspofungin and voriconazole combined. In another study, 28 patients with documented invasive aspergillosis and 22 patients with possible invasive aspergillosis were treated with caspofungin added to liposomal amphotericin B, as salvage therapy (104). The response rates were 21% in documented disease and 77% in possible disease, or similar to prior response rates of 16% in documented invasive aspergillosis. The investigators at MD Anderson were not impressed that caspofungin addition improved outcome of patients on liposomal amphotericin B. In yet another study, combined liposomal amphotericin B and caspofungin or voriconazole and caspofungin were given to six patients with definite or probable invasive aspergillosis “refractory” to lipid formulations of amphotericin B (105). All patients improved, and none of the three deaths were attributed to aspergillosis. Finally, Thebaut et al. treated nine refractory patients with caspofungin and amphotericin B, four patients with caspofungin and voriconazole, and one patient with all three drugs (103). Overall, five patients (36%) had a favorable response.

From the above studies, there does not appear to emerge a clear and dramatic message that the echinocandins add significantly to either amphotericin B or to triazoles. Although there appears to be no added toxicity, these regimens are very costly. Accordingly, in the absence of Phase III studies, the anecdotes reported may be of interest but should not be taken as conclusive evidence of improved outcome in seriously ill patients. More deliberate comparative studies should be done before aggressive practitioners utilize these combinations as standard therapy.
In summary, we can glean that caspofungin (and likely micafungin and possibly anidulafungin as well) have activity against *Aspergillus* species. Data are fewer than for *Candida*, only apply to secondary or salvage therapy, and do not give us any indication of relative potency vs. other antifungals. Merck has recommended that caspofungin daily doses be raised to 70 mg in patients with aspergillosis. There are no clinical data on dose dependency of clinical responses. However, the absence of a maximal tolerable dose suggests that higher doses may be explored. Clinical data on other mycoses await presentation/publication of further information.

VII. A FOOTNOTE ON *PNEUMOCYSTIS*

In addition to classic fungi, *Pneumocystis carinii* also includes β-1,3-glucans in the walls of its cysts, though not in the trophozoites (106,107). When rats with *P. carinii* infection were treated with anidulafungin, the cyst walls were deformed and lacked cyst wall β-1,3-glucans. Echinocandins have been effective against *P. carinii* in infected rats and mice (108). Micafungin has been found effective for prophylaxis of *P. carinii* in mice (109). Although the cysts are rapidly eliminated in mice, trophozoites, which do not contain glucans, persist after caspofungin treatment (110). For this reason, and despite the success of animal treatment regimens for infected animals, the echinocandins have not been developed clinically for use in *P. carinii* pneumonia.

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Antifungal Agents: Other Classes and Compounds

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I. AGENTS FOR SYSTEMIC TREATMENT OF INVASIVE MYCOSES

A. Inhibitors of Protein Synthesis

1. Flucytosine

Flucytosine (5-fluorocytosine; 5-FC) is low-molecular weight water-soluble synthetic fluorinated pyrimidine-analogue (Fig. 1). It is taken up into the fungal cell by the fungus-specific enzyme cytosine permease and converted in the cytoplasm by cytosine deaminase to 5-fluorouracil which causes RNA miscoding and inhibits DNA-synthesis (1). The 5-FC is relatively non-toxic to mammalian cells because of the absence or very low level of activity of cytosine deaminase. In the United States, 5-FC is available only as oral formulation; outside the United States, an IV formulation is available in select countries.

a. Antifungal Activity. The antifungal spectrum of 5-FC in vitro includes Candida spp., Cryptococcus neoformans, Saccharomyces cerevisiae, and selected dematiaceous molds. The 5-FC has no or weak activity against Aspergillus spp., and other hyaline molds (1–3). Synergistic or additive effects in combination with amphotericin B (AmB) have been observed against Candida spp., and in combination with AmB or fluconazole against C. neoformans (4). Resistance to 5-FC in susceptible species may involve either mutations in enzymes necessary for cellular uptake, transport, or metabolism, or competitive upregulation of pyrimidine synthesis (1). While the exact prevalence is unclear, primary resistance has been reported in up to 8% and 22% of clinical Candida albicans and non-albicans Candida spp., respectively, and in ≤ 2% of C. neoformans isolates (5). Secondary resistance, predominantly by selection of resistant clones, can evolve rapidly, at least in C. albicans
As a consequence, 5-FC is rarely given alone but in combination with AmB, or more recently, fluconazole.

b. Pharmacodynamics. The 5-FC has demonstrated predominantly concentration-independent fungistatic activity against Candida species and C. neoformans in time-kill assays and prolonged concentration- and exposure-dependent postantifungal effects of up to 10 hr; pharmacodynamic studies in mice with experimental disseminated candidiasis revealed that both the time above the MIC and AUC/MIC were important in predicting efficacy. Maximum efficacy was observed when levels exceeded the MIC for only 20–25% of the 24-hour dosing interval (6). Thus, lower dosages or less-frequent dosing may yield identical antifungal efficacy while further reducing potential toxicities, which are mostly dose-dependent (3).

c. Pharmacokinetics. The 5-FC is readily absorbed from the gastrointestinal tract, has negligible protein binding and distributes evenly into tissues and body fluids, including the CSF, peritoneal fluid, inflamed joints, and the eye. At usual dosages, the drug undergoes negligible hepatic metabolism and is eliminated predominantly in active form by glomerular filtration into the urine with a half-life in plasma of 3–6 hr. Individual dosage adjustment is necessary in patients with impaired renal function and those undergoing hemofiltration. In patients undergoing hemodialysis, a dose of 37.5 mg/kg is recommended following dialysis; in peritoneal dialysis, the compound can be administered systemically or intraperitoneally. Although the data are limited, impaired liver function does not appear to alter the disposition of 5-FC (2,4). The pharmacokinetics of 5-FC in pediatric patients has not been systematically characterized, and uniform dosing recommendations in this population cannot be made (7).

d. Adverse Effects. Common adverse effects of 5-FC that occur in 5–6% of patients include gastrointestinal intolerance and reversible elevations of hepatic transaminases and alkaline phosphatase. More rare side effects are skin rashes, blood eosinophilia, and crystalluria (4). Hematological adverse effects have been reported in overall 6% of patients receiving oral 5-FC, and may include neutropenia, thrombocytopenia, or pancytopenia. While they are usually reversible following discontinuation of the drug or dosage reduction, fatal outcomes have been reported (3). Some of the adverse effects of 5-FC may be due to the compound’s conversion to 5-fluorouracil by the gastrointestinal bacterial flora (8) or toxic effects of endogenous metabolites. Hematological adverse effects are less frequent if plasma levels of 5-FC do not exceed 100 µg/mL (3,9).

e. Drug Interactions. Orally administered, non-resorbable antibiotics and aluminum/magnesium hydroxide-based antacids may delay absorption of 5-FC.

![Chemical structures of cytosine, flucytosine, and fluorouracil.](510 Groll and Walsh)
from the gastrointestinal tract (4). The 5-FC is not known to interfere with CYP450 enzyme system. However, any drug that can cause a reduction in the glomerular filtration rate may lead to increased 5-FC serum levels and thereby has the potential to enhance 5-FC-associated toxicity. This includes AmB as well as number of antimicrobial agents, anticancer drugs, and cyclosporin (2). Cytosine arabinoside (ARA-C) competitively inhibits 5-FC and both drugs should not be given concomitantly.

**f. Indications and Dosing.** Due to the propensity for secondary resistance (10), 5-FC is generally not administered as a single agent. An established indication for its use in combination with amphotericin B deoxycholate (DAMB) is that for induction therapy of cryptococcal meningitis (11,12). The combination of DAMB with 5-FC can also be recommended for the treatment of *Candida* infections involving deep tissues, particularly in critically ill patients and when non- *albicans* *Candida* species are involved (3). This includes *Candida* meningitis, endophthalmitis, endocarditis, vasculitis, and peritonitis, as well as osteoarticular, renal, and chronic disseminated candidiasis (4). The combination of 5-FC with fluconazole may be used for cryptococcal meningitis, when treatment with AmB is not feasible. In addition, this combination may also be useful as second-line therapy for individual patients with invasive *Candida* infections involving aqueous body compartments. Currently, we recommend a starting dosage for both adults and children of 100 mg/kg daily divided in three or four doses.

**g. Therapeutic Monitoring.** Monitoring of plasma concentrations is essential to adjust dosage to changing renal function and to avoid toxicity. Following oral administration, near peak levels 2 hr postdosing overlap with trough levels as patients reach steady state and are thus sufficient for therapeutic monitoring (3). Peak plasma levels between 40 and 60 \( \mu g/mL \) correlate with antifungal efficacy and are seldom associated with hematological adverse effects (3,9).

2. **Sordarins**

The sordarins (Fig. 2) are a distinct class of investigational semisynthetic antifungal agents that selectively inhibit fungal protein synthesis by an interaction with translation elongation factor 2 and the large ribosomal subunit stalk rpP0 (13,14). More

![Figure 2 Sordarin and the semisynthetic sordarin derivative GM193663. The sordarins are composed of a pyranose sugar and a variable ring structure attached to C3'-C4'. The parent sordarin is a natural product of the fungus *Sordaria oraneosa.*](image)
recently, several novel derivatives (GM-193663, GM-211676, GM-222712, and GM-327354) have undergone preclinical investigation and have demonstrated the potential of this class for clinical development (15).

**a. Spectrum and Pharmacodynamics in Vitro.** The sordarins have potent antifungal activity in vitro against *C. albicans* and non-*albicans* Candida spp., *(C. krusei* exempted), *C. neoformans*, other yeast-like fungi and endemic molds. With few exceptions, they appear to have lesser or no activity against opportunistic filamentous fungi and dermatophytes (16). In time-kill assays, consistent with their mechanism of action, the sordarins exhibited time-dependent fungicidal dynamics against *C. albicans* (17). Using an in vitro one compartment pharmacodynamic model, unbound plasma concentrations of the lead compound GM 237354 were simulated and antifungal activity in vitro compared to antifungal efficacy in an in vivo murine model of systemic candidiasis. The compound displayed concordant fungicidal activity in vitro and in vivo; the in vitro model predicted accurately the in vivo antifungal efficacy of GM 237354 and might thus be useful to forecast in vivo outcome in conjunction with clinical trials (18).

**b. Pharmacokinetics.** The plasma pharmacokinetics of GM-237354 have been investigated in rodents and *Cynomolgus* monkeys. GM-237354 achieved considerable exposure as measured by the plasma AUC and a half-life ranging from 0.45 hr in mice to 1.75 hr in monkeys. The compound exhibited high (> 95%) protein binding and exhibited linear pharmacokinetics. No information is available on metabolism, routes of excretion as well as safety and pharmacokinetics in humans. Based on interspecies-scaling, the half-life in humans was estimated to be around 9 hr (19,20).

**c. Antifungal Efficacy and Pharmacodynamics in Vivo.** Promising therapeutic efficacy of sordarins has been demonstrated in non-immunocompromised murine models of disseminated and mucosal candidiasis (21,22), histoplasmosis (23) and coccidioidomycosis (24). Efficacy in non-immunocompromised murine models of disseminated aspergillosis was limited (21,25). Sordarins have shown strong inhibitory activity on protein synthesis and replication of *Pneumocystis carinii* in vitro that translated into promising in vivo efficacy in murine models of pneumocystosis (21,26). Relationship between pharmacokinetic parameters of GM-237354 and outcome were investigated in a lethal murine kidney target model of systemic *C. albicans* candidiasis (27). Across all dosing regimens, the AUC correlated best with survival and clearance of the organism from the kidney, while C<sub>max</sub> and the T<sub>tau</sub> > MIC were not predictive. Using pharmacodynamic modeling and E<sub>max</sub> functions, the predicted AUC for an efficacy target of 90% survival was 67 μg/hr/mL.

**d. State of Development.** The example of the sordarins clearly reflects the progress in antifungal drug development with pharmacodynamic studies being incorporated in early steps of the preclinical drug development process. Although preclinical safety data were favorable, sordarin compounds have not yet entered the stages of clinical development.

3. Cispentacin Derivatives: PLD-118 (BAY 10–8888)

PLD-118[(-) (1R,2S)-2-amino-4-methylene-cyclopentane carboxylic acid] is a novel synthetic, water-soluble orally bio-available antifungal agent of the beta-amino acid class that is structurally related to the naturally occurring beta-amino acid cispentacin and that is currently undergoing phase II clinical trials for oral treatment of mucocutaneous *Candida*-infections. The PLD acts by a dual mode of action (Fig. 3): first, it is accumulated in yeast cells by active transport via permeases specific for branched amino acids. Inside the cell, PLD-118 specifically inhibits
isoleucyl t-RNA synthetase, resulting in inhibition of protein synthesis and cell growth (28,29). Of note, efflux of PLD-118 from the cell occurs by diffusion and is not carrier mediated (28). *C. albicans* and *C. tropicalis* isolates resistant to PLD-118 showed either decreased accumulation or increased isoleucyl-tRNA synthetase activity (30).

**a. Antifungal Spectrum and In Vivo Efficacy.** The in vitro activity of PLD-118 depends strongly on assay conditions, mainly due to the expression of the transporter for active transport (31,32). Using a microtiter plate dilution assay and YPG medium, PLD-118 showed antifungal activity against *C. albicans* (32), including azole-resistant isolates (31), *C. glabrata, C. krusei*, and to a lesser extent, *C. tropicalis* and *C. parapsilosis* (33). Time-kill assays against fluconazole-resistant *C. albicans* showed concentration-dependent pharmacodynamics in vitro (34). In vivo, oral PLD achieved complete survival in lethal models of disseminated azole-susceptible and azole-resistant *C. albicans* infections in mice and rats at dosages of 10 and 4 mg/kg BID, respectively (35,31). Against experimental esophageal candidiasis caused by fluconazole-resistant *C. albicans* in corticosteroid-immunosuppressed rabbits, PLD-118 demonstrated significant dose-dependent antifungal efficacy (34).

**b. Preclinical Pharmacokinetics and Safety.** Plasma pharmacokinetics in rats, rabbits, and dogs revealed linear pharmacokinetics following single oral dosing over a concentration range of 1-100 mg/kg. The half-life increased from 3 hr in mice to 10 hr in dogs. Plasma protein binding was less than 1%, and studies with radiolabeled compound showed a homogeneous distribution within all tissues. Almost complete renal elimination of unchanged compound was observed in rats and dogs, and no major metabolites were detected. Using pooled liver microsomes and specific probe substrates, PLD did not inhibit the most prevalent human liver cytochrome P450 isoenzymes. The PLD was well tolerated in experimental animals with an LD50 exceeding 2000 mg/kg in mice and rats and a no effect level of 30 mg/kg in rats and dogs after 4 weeks of administration (36,37).

**c. Clinical Trials.** In healthy volunteers, tolerance, safety, and pharmacokinetics of oral PLD-118 were investigated in a double-blind, randomized, and placebo-controlled cross-over study at dosages ranging from 17.5 to 280 mg. The PLD exhibited linear plasma pharmacokinetics. Peak plasma concentrations ranged...
from 0.5 to 8.5 μg/mL and were achieved at approximately 1 hr after dosing. Elimination half-life was 6–7 hr and 70–90% of unchanged compound was recovered in urine within 72 hr after dosing. The PLD appeared to be well tolerated without significant acute adverse events (38). Following multiple dosing at 50–200 mg q8h and 150 or 300 mg ql2h for 7 days, steady-state plasma levels were dose-proportional and achieved within 2–3 days of multiple dosing. Similar to single dosing, half-life was around 7 hr, and more than 80% of compound were recovered in urine in unchanged form. The PLD was well tolerated. The most common adverse event was a dry mouth, occurring in six subjects receiving PLD (39).

d. Perspectives. Based on its unique mechanism of action, promising activity against Candida spp., in vitro and in vivo, favorable pharmacokinetic and safety profiles, and the potential for oral and IV administration of PLD-118 warrants further preclinical and clinical investigation as therapeutic agent against superficial and perhaps, invasive Candida infections.

II. CELL WALL ACTIVE AGENTS

A. Nikkomycin Z

The nikkomycins and the structurally closely related polyoxins are antifungal antibiotics produced by Streptomyces and were discovered in the 1970s through programs searching for new fungicides and insecticides for agricultural use (40). The nikkomycins and polyoxins are pyrimidine nucleosides that are linked to a di- or tripeptide moiety (Fig. 4). They are structurally similar to UDP-N-acetyl-glucosamine, the precursor substrate for chitin, a linear polymer of β-(1-4)-linked N-acetylglucosamine residues.

The nikkomycins and polyoxins act as competitive inhibitors of chitin synthesis, leading to inhibition of septation, chaining, and osmotic swelling of the fungal cell (40,41). In C. albicans, three different membrane-bound isoenzymes of chitin synthase have been described. Although the absence of all three isoenzymes is uniformly lethal, no single one is essential, and each may be inhibited to different degrees by different compounds. Of note, the chitin-synthase inhibitors are required

![Figure 4 Chemical structure of nikkomycin Z.](https://example.com/figure4.png)
to be transported into the cell via one or more permeases. This transport system, however, is subject to antagonism by extracellular peptides. In addition, various proteases can inactivate the nucleoside-peptide compounds. This results in a wide range of susceptibility of intact fungi, even though their isolated enzyme preparations are uniformly sensitive (4,40,41).

1. Antifungal Spectrum and In Vivo Efficacy
Among the nucleoside-peptide chitin-synthase inhibitors, only nikkomycin Z has been investigated to a greater extent. Nikkomycin Z has been shown to have particularly good activity against the chitinous dimorphic fungi *Coccidioides immitis* and *Blastomyces dermatitidis*, both in vitro and in vivo (42,43). Its in vitro activity against *Histoplasma capsulatum*, *C. albicans*, and *C. neoformans* was only moderate, and non- *albicans* *Candida* spp., and filamentous fungi appear essentially resistant (44). However, the compound had inhibitory activity in murine models of systemic candidiasis and histoplasmosis, respectively (45–47).

Notably, synergy between nikkomycin Z and antifungal triazoles against *C. albicans*, *C. neoformans*, and *Aspergillus fumigatus* has been observed in vitro (45,48,49), and synergy with fluconazole could be demonstrated in a mouse model of systemic candidiasis (45). Whereas synergy with glucan synthesis inhibitors against *C. albicans* in vitro has been described early on (50,51), more recent in vitro studies also provided strong evidence for additive or synergistic cooperation against *A. fumigatus* and, more variably, against other filamentous fungi (52–54).

2. State of Clinical Development
Apart from a phase I study of safety and pharmacokinetics of single oral doses of 0.25–2.0 mg/kg in human volunteers, nikkomycin Z is being evaluated for treatment of coccidioidomycosis. The compound may be useful for other mycoses as well, particularly in combination with other antifungals such as triazoles and echinocandins and remains a candidate for clinical development as well as natural peptidyl nucleoside lead compounds that continue to be investigated.

III. CELL MEMBRANE ACTIVE AGENTS

A. Pradimicins
The pradimicins and the structurally similar benanomycins constitute a unique class of antifungal antibiotics derived from *Actinomycetes* with broad-spectrum fungicidal activity (55,56). The chemical structure of these compounds is characterized by a benzonaphthacene quinone skeleton substituted by a D-amino acid and a disaccharide side chain (Fig. 5). Since their discovery in the late 1980s, numerous natural and semisynthetic congeners have been developed, which differ from one another by virtue of substitutents and type of D-amino acid and hexose sugar (57). The pradimicins and benanomycins possess a novel mechanism of action, consisting of a calcium-dependent complexing with the saccharide portion of cell surface mannoproteins, leading to perturbation of the cell membrane, leakage of intracellular contents, disintegration of intracellular organelles, and ultimately, cell death (58).

1. Antifungal Spectrum and In Vivo Efficacy
Both pradimicins and benanomycins possess broad-spectrum antifungal activity in vitro and in vivo, including *Candida* spp., *C. neoformans*, *Aspergillus* spp.,
dematiaceous molds and dermatophytic fungi without cross-resistance to polyenes, antifungal azoles and 5-FC (57,59–61). Pradimicins also have antiviral activity, possibly due to an interaction with mannose-containing glycoproteins on the surface of those viruses (57).

The pradimicins have demonstrated potent efficacy in murine models of systemic candidiasis, cryptococcosis and aspergillosis, both in immunocompetent and immunosuppressed animals (57). BMS 181184, a 4’hydroxy analog of the earlier BMY 28864 with excellent water solubility, was the first pradimicin targeted for clinical development. The compound had promising antifungal efficacy in rabbit models of subacute disseminated candidiasis, disseminated aspergillosis, and invasive pulmonary aspergillosis in profoundly neutropenic rabbits (62–64). Among the benanomycins, benanomycin A exhibited the best activity. Apart from therapeutic efficacy against disseminated candidiasis, cryptococcosis, and aspergillosis in non-immunocompromised mice (65) the compound also demonstrated activity in a murine model of *P. carinii* pneumonia (66).

2. State of Development
A high-therapeutic index permitted the administration of large doses of BMS 181184 to animals with minimal toxicity. Significant findings in unpublished preclinical toxicity studies were red discoloration of body fluids and numerous tissues associated with reversible granulomatous inflammation in these tissues; renal tubular degeneration at high dosages and dose-dependent, species specific hemolysis in *Cynomolgus* monkeys. Unexpected hepatic toxicity in early phase I studies led to the stop of
further development of this compound; no new pradimicin or benenomycin lead compounds have been reported in the interim.

B. Antimicrobial Peptides

Potentially promising approaches to antifungal therapy are naturally occurring cationic peptides or their synthetic derivatives. These molecules are part of the mammalian oxygen independent microbial host defense mechanisms [histatins, indolicidin, bactericidal/permeability increasing (BPI) factor, lactoferrin, and defensins] or belong to the antimicrobial response of insects (cecropins), bees, amphibes, plants, and various other species. Cationic peptides may bind to the lipid bilayer of biological membranes, form pores and ultimately produce cell death; others may traverse the membrane and interact with intracellular molecules. They may possess broad-spectrum, non-crossresistant antifungal activity, and probably do not induce resistance at measurable frequencies. While some of these peptides are quite toxic, others have only weak activity against mammalian cells (67–69).

Naturally occurring, cationic peptides with potentially exploitable antifungal activity in vitro include, among others, the defensins, the protegrins, gallinacin I, cecropin A, thanatin, and the dermaseptins. Cecropin, an antimicrobial lytic peptide not lethal to mammalian cells, is derived from the silk moth and appears to bind to fungal ergosterol. Its antifungal properties are genus and species-dependent, and include prevention of spore germination, reduced hyphal viability, and potential lethality to hyphae (70). In vivo, a liposomal formulation of indolicin was effective against experimental systemic aspergillosis (71). Similarly, synthetic derivatives of the BPI protein demonstrated dose-dependent effects on survival and fungal burden in mouse models of systemic candidiasis and disseminated aspergillosis (72); in the latter study, combination with AmB significantly increased survival when compared with either agent alone. Finally, genes encoding cationic peptides with antifungal activity have been successfully transferred to the salivary glands of laboratory animals, thus allowing the investigation of their effect on mucosal candidiasis in permanently immunosuppressed subjects (73).

First clinical trials involving antimicrobial therapy with cationic peptides are under way (68,69). However, the potential usefulness of these peptides in treating human diseases of infectious origin remains to be determined.

IV. AGENTS FOR SYSTEMIC TREATMENT OF MYCOSES OF THE SKIN AND ITS APPENDAGES

A. Griseofulvin

Griseofulvin (Fig. 6) was originally isolated in 1939 as a natural product of Penicillium griseofulvum. Griseofulvin interferes with fungal microtubule formation, disrupting the cell’s mitotic spindle formation and arresting the metaphase of cell division. Griseofulvin is a fungistatic compound. It is active against Trichophyton, Microsporon, and Epidermophyton species. The drug has no activity against yeast-like organisms, opportunistic hyaline and dematiaceous molds, and the dimorphic (endemic) molds. Of note, in-vitro resistance of dermatophytes to griseofulvin has been reported and may be the cause for therapeutic failure (74,75).
1. Pharmacokinetics

Griseofulvin is commercially available for oral administration only as griseofulvin microsize (4 μm particle size) and griseofulvin ultramicrosize (1 μm particle size). Oral bioavailability of the micronized formulation is variable and ranges from 25% to 70%; ultramicronized griseofulvin, in contrast, is almost completely absorbed (76). Peak plasma concentrations occur approximately 4 hr after dosing. Griseofulvin distributes to keratin precursor cells and is concentrated in skin, hair, nails, liver, adipose tissue, and skeletal muscles. In skin, over time, a concentration gradient is established, with the highest concentrations in the outermost stratum corneum. However, within 48–72 hr after discontinuation, plasma concentrations of griseofulvin are markedly reduced and the compound is no longer detectable in the stratum corneum (75,76). The elimination of griseofulvin from plasma is bi-exponential with a terminal elimination half-life of 9–21 hr (77). The compound is oxidatively demethylated and conjugated with glucuronic acid primarily in the liver; its major metabolite, 6-desmethylgriseofulvin, is microbiologically inactive; within 5 days, approximately one third of a single dose of micronized griseofulvin is excreted in feces, and 50% in urine, predominantly as glucuronized 6-desmethylgriseofulvin (78).

2. Adverse Effects and Drug Interactions

Griseofulvin is generally well tolerated. More common adverse effects include headaches and a variety of gastrointestinal symptoms. Griseofulvin can cause photosensitivity and exacerbate lupus and porphyria. Cases of erythema multiforme-like reactions, toxic epidermal necrolysis, and a reaction resembling serum sickness have been reported. Proteinuria, nephrosis, hepatotoxicity, leukopenia, menstrual irregularities, estrogen-like effects, and reversible diminution of hearing have been reported rarely in association with griseofulvin therapy (75,76). The compound has been shown to be teratogenic in animals. Griseofulvin also has mutagenic and carcinogenic potential; the significance of these observations for humans, however, is unclear. Griseofulvin has been noted to enhance the clearance of oral contraceptives, cyclosporine, theophylline, aspirin, and warfarin. Concurrent use of phenobarbital may lead to decreased griseofulvin levels. Finally, concurrent alcohol ingestion may lead to a disulfiram-like reaction (74).

3. Clinical Indications and Dosing

Griseofulvin remains an important agent for the treatment of tinea capitis and refractory tinea corporis caused by dermatophytes. For tinea capitis, 6–8 weeks of
treatment are usually required; the usual duration of therapy for refractory tinea corporis is 4 weeks (75,79). Nail infections usually fail to respond to therapy with griseofulvin and are better treated with itraconazole or terbinafine. The recommended dosage in adults ranges from 500 to 1000 mg (microsize) and 330 to 750 mg (ultramicrosize) as a single daily dose or in 2–4 equally divided doses. The compound is approved for children older than 2 years of age. The recommended pediatric dosage of microsize griseofulvin is 10–20 mg/kg/day (maximum 1 g), and that of ultramicronized griseofulvin is 5–10 mg/kg/day (maximum 750 mg), respectively, administered in two equally divided doses (7).

B. Terbinafine

The synthetic allylamine terbinafine (Fig. 6) is a newer antifungal agent that is useful for topical and systemic (oral) treatment of superficial infections of the skin and its appendages by dermatophytes and yeasts, and possibly, for cutaneous sporotrichosis. It acts by inhibiting the biosynthesis of fungal ergosterol at the level of squalene epoxidase (Fig. 2), leading to depletion of ergosterol and accumulation of toxic squalenes in the fungal cell membrane (80). Terbinafine has potent, fungicidal in vitro activity against dermatophytes. It is also highly active against *Aspergillus* species, *Fusarium* spp. dematiaceous and dimorphic fungi and *P. carinii*. Its in vitro activity against yeasts appears more variable (4,81). Of note, synergy with triazoles, and more variable, with AmB, has been reported against yeasts and filamentous fungi in vitro (82–84).

1. Pharmacokinetics

Terbinafine displays linear plasma pharmacokinetics over the current dosage range. Independent of food, oral bioavailability is 70–80%, and peak plasma concentrations of are measured within 2 hr (85). Steady-state in plasma is reached after 10–14 days after only twofold accumulation. As a lipophilic drug, terbinafine is strongly bound to plasma proteins. The compound is extensively distributed to tissues, accumulating throughout adipose tissues, dermis, epidermis, and nail. It exhibits a triphasic distribution pattern in plasma with a terminal half-life of up to 3 weeks; microbiologically active concentrations can be measured in plasma for weeks to months after the last dose, which is consistent with a slow redistribution from peripheral tissue and adipose tissue sites (85,86). Terbinafine undergoes extensive and complex hepatic biotransformation that involves at least seven CYP450 enzymes (87); none of its metabolites has been shown to be mycologically active (86). Urinary excretion accounts for more than 70% and fecal elimination for 10% of total excretion; the extent of enterohepatic recycling is yet unknown. Due to the compound’s extensive hepatic metabolism and urinary excretion, caution is warranted in patients with severe hepatic and renal impairment (80). The plasma pharmacokinetics of terbinafine in pediatric patients have been comparatively well investigated (88). Whereas no apparent differences in metabolism were observed, on a mg/kg and mg/m² basis, children had a shorter B half-life, a lower mean AUC, and a higher volume of distribution, reflecting the higher proportion of lipophilic tissues in pediatric age groups.

2. Adverse Effects

At dosages of up to 500 mg/day, terbinafine is usually well tolerated. The most common adverse effects include gastrointestinal upsets and skin reactions in 2–7%
of patients. Terbinafine can cause hepatitis and liver failure; potentially severe hepatotoxicity is estimated to occur in 1:120,000 patients, and asymptomatic rises in liver enzyme activities are likely to occur at a frequency of 1:200. The drug should not be administered in patients with an underlying liver problem, and liver function tests should be obtained prior to the prescription of terbinafine. Less common significant adverse effects have included reversible loss of taste, severe skin eruptions, Stevens–Johnson syndrome, and blood dyscrasias (4).

3. Drug Interactions

With the possible exception of CYP2D6 substrates, in vitro studies revealed little or no effect of terbinafine on the metabolism of many characteristic CYP substrates (87). Inhibition of CYP2D6-mediated metabolism may be relevant with the concomitant use of tricyclic antidepressants, beta-blockers, selective serotonin reuptake inhibitors, and type-B monoaminooxidase inhibitors. Terbinafine can reduce the clearance of theophylline, increase levels of nortryptiline, increase or reduce warfarin exposure, and can reduce the trough cyclosporine concentration in transplant patients. The metabolism of terbinafine may be decreased by cimetidine and increased by rifampin (4).

4. Clinical Indications and Dosing

Terbinafine is indicated for treatment of superficial infections of the skin and its appendages by dermatophytes (80), and possibly, for cutaneous sporotrichosis (89). The recommended dosage range in adults is 250–500 mg once daily, and the recommended durations of treatment for tinea capitis, tinea corporis and pedis, fingernail onychomycosis, and toenail onychomycosis are 4, 2, 6, and 12 weeks, respectively (80,90). Several clinical studies have documented the safety of terbinafine in pediatric patients aged 2–17 years (90); based on the experience with dosages of 10 mg/kg and less in adults and the described pharmacokinetic profile of the compound in children, a dose of 250 mg/day has been proposed for children weighing >40 kg, a dose of 125 mg/day for children weighing 20–40 kg, and 62.5 mg/day for children weighing less than 20 kg (90).

The broad-spectrum, fungicidal in vitro activity, systemic availability, and the lack of significant side effects suggested potential against selected deep-seated fungal infections. Unfortunately, terbinafine was ineffective in rodent models of pulmonary aspergillosis (91), systemic sporotrichosis (92), cerebral phaeohyphomycosis (93), systemic Candida infection, and pulmonary cryptococcosis (94). This ineffectiveness is likely due to the compound’s non-saturable protein binding in serum (91). Of note, terbinafine was as effective as TMP/SMZ in a rat model of pulmonary pneumocystosis, as rated by survival, lung weight, infection rate, and microbial tissue burden (95,96), indicating potential usefulness for treatment of selected compartments.

V. TOPICAL ANTIFUNGAL AGENTS

Apart from fungal keratitis, the use of topical agents is confined to superficial infections of the skin and mucosal surfaces. The decision to use a topical or a systemic agent depends mainly on the site and extent of the infection. Immunocompromised patients, however, usually require systemic therapies as do patients with tinea capitis and onychomycosis (97).
A. **Superficial Skin Infections**

Dermatophytosis is caused by the filamentous fungi *Microsporon* spp., *Trichophyton* spp., and *Epidermophyton floccosum*. A large variety of agents and formulations are available for topical treatment of dermatophytic skin infections (tinea corporis, facialis, or pedis), including allylamines, benzylamines, thiocarbamates, morpholines, and azoles. Agents for treatment of *Candida* dermatitis and Tinea ( pityriasis) versicolor (caused by *Malassezia furfur* or *M. pachydermatidis*) include various topical azoles and topical polyenes. Most topical agents are usually applied twice daily well beyond the clinical resolution of the infection. A detailed review of the treatment of cutaneous mycoses is beyond the scope of this chapter and can be found elsewhere (75,76,79).

B. **Mucosal Candidiasis**

Agents for the topical treatment of vulvovaginal candidiasis include a large variety of antifungal azoles and the polyene nystatin. Of note, azole agents maybe absorbed to a minor extent and can potentially interfere with the metabolism of concomitant drugs. For example, potentiation of the anticoagulatory effects of acenocoumarol has been noted after vaginal administration of miconazole capsules and after oral administration of miconazole gel (7).

Antifungal azoles such as clotrimazole and miconazole and antifungal polyenes such as AmB and nystatin are effective in the treatment of oropharyngeal candidiasis. Many clinical trials have evaluated the usefulness of these agents for prevention of fungal infections in immunocompromised cancer or hematopoietic stem-cell transplant patients. While most agents have documented efficacy in the prevention of oropharyngeal candidiasis, they are not effective in preventing invasive mycoses and improving infection related and overall mortality in this setting (98–100).

**REFERENCES**


Clinically Relevant Drug Interactions with Systemic Antifungal Therapy

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I. INTRODUCTION

A drug interaction is best defined as “the possibility that one drug may alter the intensity of pharmacological effects of another drug that is given concurrently.” This results in either enhanced activity of the affected drug, which may lead to toxicity, or reduced activity of the affected drug, leading to therapeutic failure. It is also possible that there may be the appearance of a new effect that is not seen with either drug alone, such as caspofungin and cyclosporine coadministration leading to elevated liver function tests. Drug interactions can be divided into either pharmacokinetic (PK) or pharmacodynamic (PD) interactions.

A. Pharmacokinetic Drug Interactions

Pharmacokinetic drug interactions influence the disposition of the drug in the body. When drugs are ingested, they undergo several processes to produce an end-effect, including absorption, distribution, metabolism, and excretion. Interactions between two or more drugs may affect any of these phases, including modulation of hepatic drug biotransformation, renal clearance, altered distribution, or changes in plasma protein binding.

The most clinically relevant mechanisms of PK drug interactions relate to metabolism and elimination of drugs from the body, and involve the cytochrome P450 (CYP) enzymes and the transporter P-glycoprotein at the hepatic and intestinal levels, and glomerular filtration and tubular secretion at the renal level. The CYP microsomal enzyme system is a family of hemoproteins that is responsible for the oxidative biotransformation of endogenous substrates and xenobiotics. CYP’s are expressed in several tissues, with the main drug metabolizing CYP’s concentrated in the smooth endoplasmic reticulum in the liver, with lower expression in the lungs, kidneys, intestines, and brain (1). Of the several different CYP isoforms identified, CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 have the greatest involvement in drug metabolism. An extensive list of drugs metabolized by the various isoenzymes is outlined in Table 1 (2). CYP1A2 accounts for approximately 15% of
<table>
<thead>
<tr>
<th>Cytochrome P450 Isoenzyme</th>
<th>Substrates</th>
<th>Inhibitors</th>
<th>Inducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP 1A2</td>
<td>Amitriptyline, caffeine, haloperidol, ondansetron, propranolol, theophylline, verapamil, (R) warfarin</td>
<td>Amiodarone, cimetidine, fluoroquinolones, ticlopidine</td>
<td>Broccoli, brussel sprouts, chargrilled meat, insulin, tobacco</td>
</tr>
<tr>
<td>CYP 2C9</td>
<td>Oral hypoglycemics, celecoxib, fluoxetine, fluvastatin, NSAID’s, phenytoin, tamoxifen, S-warfarin, voriconazole</td>
<td>Fluconazole, fluvastatin, isoniazid, lovastatin, paroxetine, sertraline, teniposide, trimethoprim, voriconazole</td>
<td>Rifampin</td>
</tr>
<tr>
<td>CYP 2C19</td>
<td>Cyclophosphamide, diazepam, nelfinavir, phenobarbitone, phenytoin, primidone, proton pump inhibitors, teniposide, voriconazole</td>
<td>Cimetidine, fluconazole, fluoxetine, ketoconazole, omeprazole, voriconazole</td>
<td>Carbamazepine, prednisone, rifampin</td>
</tr>
<tr>
<td>CYP 2D6</td>
<td>Fluoxetine, haloperidol, paroxetine, methadone, metoclopramide, morphine, ondansetron, propranolol, risperidone, tamoxifen, tricyclic antidepressants</td>
<td>Celecoxib, doxorubicin, fluoxetine, lopinavir, metoclopramide, paroxetine, ritonavir, sertraline</td>
<td>Dexamethasone? Rifampin</td>
</tr>
<tr>
<td>CYP 3A4/5</td>
<td>Antifungals: itraconazole, Antihistamines (astemizole, terfenadine) Benzodiazepines (alprazolam, diazepam, midazolam, triazolam) Calcium channel antagonists (amlodipine, diltiazem, nifedipine, felodipine, verapamil) HIV antivirals (indinavir, nelfinavir, ritonavir, saquinavir) HMG Co A reductase inhibitors (atorvastatin, lovastatin, simvastatin) Immunosuppressants (cyclosporine, sirolimus, tacrolimus) Macrolides (clarithromycin, erythromycin) Others (cisapride, dapsone, irinotecan, ondansetron, tamoxifen, thiopeta, vincristine, voriconazole)</td>
<td>Ampronavir, cimetidine, ciprofloxacin, clarithromycin, delavirdine, diltiazem, efavirenz, erythromycin, fluconazole, grapefruit juice, indinavir, itraconazole, ketoconazole, lopinavir, nelfinavir, posaconazole, ritonavir, saquinavir, voriconazole</td>
<td>Ampronavir, barbiturates, carbamazepine, efavirenz, glucocorticoids, lopinavir, saquinavir, phenobarbital, phenytoin, rifampin, rifabutin, ritonavir, St John’s wort, troglitazone</td>
</tr>
</tbody>
</table>
hepatic CYP and CYP2D6 represents 1.5% of total hepatic CYP. The CYP2C isoforms are abundant in the liver, second only to CYP3A4. There are two isoforms of CYP2C, namely 2C9 and 2C19, which share 91% identity in amino acid sequences, therefore, most substrates of CYP2C9 are metabolized by CYP2C19 as well (1). The majority of oxidatively biotransformed drugs are metabolized, at least in part, by the CYP3A4 isoenzyme, which is responsible for drug metabolism both in the liver and the small intestine.

CYP-mediated drug interactions can occur by two separate mechanisms, enzyme inhibition and enzyme induction. Inhibition is the process whereby there is either enzyme inactivation or mutual competition of substrates at a catalytic site. The net response is inhibition of drug metabolism leading to increased serum concentrations, increased trough concentrations, and a prolongation of half-life. This causes potentiation of the pharmacodynamic effect, and often leads to enhanced toxicity, particularly among those agents with a very narrow therapeutic index, such as cyclosporine and tacrolimus. Examples of substrates whose bioavailability is increased by inhibition includes benzodiazepines (midazolam, triazolam), immunosuppressants (cyclosporine, tacrolimus, sirolimus), nonsedating antihistamines (astemizole, terfenadine), calcium channel blockers (amlodipine, diltiazem, nifedipine, verapamil), HMG CoA-reductase inhibitors (atorvastatin, lovastatin, simvastatin), and many of the antineoplastic agents (etoposide, ifosfamide, cyclophosphamide, vinca alkaloids). Examples of inhibitors include the azole antifungals (fluconazole, itraconazole, ketoconazole, voriconazole), and the macrolide antibiotics (clarithromycin, erythromycin). Grapefruit juice, a commonly ingested “health” drink, is also a potent inhibitor of CYP enzymes and must be considered when reviewing drug interactions.

Induction is a process whereby there is increased synthesis or decreased degradation of CYP enzymes, resulting in decreased plasma levels of the substrate, and a decrease in its pharmacodynamic effects. Examples of enzyme inducers are rifampin, rifabutin, phenytoin, carbamazepine, phenobarbital, and St John’s wort.

Drugs that require metabolism by the same CYP enzyme compete for binding to, and metabolism by CYP (3). Therefore, in theory, any two drugs metabolized by identical CYP enzymes have a potential for interaction, although the clinical significance of the interaction will rely on the drug’s relative affinities for binding to these enzymes, concentrations achieved in the endoplasmic reticulum after therapeutic doses, dependence on CYP for elimination, and therapeutic ratios (3).

A second potential mechanism of pharmacokinetic drug interactions is thought to occur by modulation of P-glycoprotein. P-glycoprotein, the product of the multidrug resistance gene (MDR1), is an ATP-dependent plasma membrane transporter. It is present in the proximal tubular cells of the kidneys, the bile cannalicular membrane of hepatocytes, endothelial cells of the blood–brain barrier and blood–testis barriers. P-glycoprotein is best known for its role in resistance of cancer cells to antineoplastic agents, namely paclitaxel, vinca alkaloids, the epipodophyllotoxins, and the anthracycline antibiotics. P-glycoprotein also promotes the excretion of many other drugs, including digoxin, HIV protease inhibitors, and cyclosporine from renal tubule and intestinal cells (4). Administration of an agent that inhibits or induces P-glycoprotein activity can increase or decrease the clearance of P-glycoprotein substrates at the renal level, and increase or decrease bioavailability at the intestinal level (4).
B. Pharmacodynamic Drug Interactions

In addition to PK mediated drug-interactions, there are also pharmacodynamic interactions. These occur when a medication induces a change in the patient’s response to a drug without altering the pharmacokinetics of the object drug, e.g., amphotericin B-induced hypokalemia in a patient on digoxin, leading to enhanced digoxin toxicity. Pharmacodynamic interactions are primarily seen with the polyene antifungal agents, e.g., amphotericin B. Examples of pharmacodynamic interactions are listed in Table 2.

II. DRUG–DRUG INTERACTIONS

With the large number of pharmaceuticals available today, it is not surprising that drug interactions occur commonly, particularly, in diseases that require polypharmacy. Immunocompromised patients, including recipients of solid organ transplant (SOT), hematopoietic stem cell transplantation (HSCT), neonates, surgical candidates, and patients infected with the human immunodeficiency virus (HIV), frequently receive complex medication regimens, with the potential for numerous drug interactions. Due to the vast number of potential drug interactions that can occur in a patient, only PK and PD drug interactions involving antifungal therapy is discussed in detail in this chapter.

A. Polyenes

1. Amphotericin B

Amphotericin B is a potent nephrotoxin, causing toxicity by arteriolar vasoconstriction, which leads to renal ischemia and a decreased glomerular filtration rate. Nephrotoxicity is one of the main mechanisms by which pharmacodynamic drug interactions occur. Table 2 summarizes some of the pharmacodynamic interactions involving Amphotericin B.

### Table 2  Pharmacodynamic Drug Interactions with Amphotericin B

<table>
<thead>
<tr>
<th>Drug A</th>
<th>DrugB</th>
<th>Clinical Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>Aminoglycosides</td>
<td>Additive or synergistic nephrotoxicity</td>
</tr>
<tr>
<td></td>
<td>Antineoplastic</td>
<td>Additive or synergistic nephrotoxicity (i.e., cisplatin)</td>
</tr>
<tr>
<td></td>
<td>agents</td>
<td>Delayed clearance of other renally excreted drugs leading to enhanced toxicity (i.e., bleomycin)</td>
</tr>
<tr>
<td></td>
<td>(e.g., cisplatin,</td>
<td></td>
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<tr>
<td></td>
<td>bleomycin)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cidofovir</td>
<td>Additive or synergistic nephrotoxicity</td>
</tr>
<tr>
<td></td>
<td>Cyclosporine</td>
<td>Additive or synergistic nephrotoxicity</td>
</tr>
<tr>
<td></td>
<td>Digoxin</td>
<td>Hypokalemia induced by amphotericin B promotes inhibition of Na(^+), K(^+)ATPase by digoxin</td>
</tr>
<tr>
<td></td>
<td>Flucytosine [5-FC]</td>
<td>Enhanced bone marrow suppression due to delayed clearance of 5-FC in the presence of renal dysfunction induced by amphotericin B</td>
</tr>
<tr>
<td></td>
<td>Foscarnet</td>
<td>Additive or synergistic nephrotoxicity</td>
</tr>
<tr>
<td></td>
<td>Ganciclovir</td>
<td>Hematological toxicity due to delayed clearance; dose reduce ganciclovir</td>
</tr>
<tr>
<td></td>
<td>Tacrolimus</td>
<td>Additive or synergistic nephrotoxicity</td>
</tr>
<tr>
<td></td>
<td>Zidovudine</td>
<td>Hematological toxicity</td>
</tr>
<tr>
<td>5-Flucytosine</td>
<td>Zidovudine</td>
<td>Hematological toxicity</td>
</tr>
<tr>
<td></td>
<td>Ganciclovir</td>
<td>Potentially greater hematological toxicity</td>
</tr>
</tbody>
</table>
interactions occur with amphotericin B (and its analogues). Pharmacodynamic interactions of importance are listed in Table 2, along with the likely clinical effects. Another type of pharmacodynamic interaction that can occur is via the electrolyte imbalances that result from amphotericin B administration. The third type of pharmacodynamic interaction that may occur results from additive myelotoxic interactions, e.g., enhanced myelosuppressive toxicity in patients who are coadministered zidovudine and amphotericin B products.

The main interaction of concern with the polyene products arises when combination antifungal therapy with amphotericin B and an azole is considered. Theoretically these two classes of drug may interact negatively. Amphotericin B exerts its effects by binding to ergosterol whereas azole antifungals block the enzyme 14-α demethylase, a necessary enzyme to convert lanosterol to ergosterol. By depleting ergosterol, the target of amphotericin B, clinical efficacy of amphotericin B may be diminished, potentially leading to higher mortality rates when combination therapy is used. Based on the potential for theoretical antagonism, several in vitro and animal studies have been conducted to address this issue. In vitro and animal models suggest an attenuation of response in animals with prior exposure to an azole before receiving a polyene (5–13). Increasing the dose of amphotericin B does not overcome the noted antagonism. There does appear to be some sequence dependence to the interaction. Pretreatment with an azole, as would be a common practice in immunocompromised patients that were receiving fungal prophylaxis decreases the ability of amphotericin B to prolong survival. Similarly, the same effect is seen when a polyene and an azole are administered simultaneously. The interaction is not noted when a polyene is administered first, followed by an azole. There are limited clinical data supporting these animal and in vitro models. The only available clinical data is in the setting of candidemia, in a trial conducted by the National Institute of Allergy and Infectious Diseases Mycoses Study Group (14). This trial compared high-dose fluconazole (800 mg/day) in combination with either placebo or amphotericin B deoxycholate (0.7 mg/kg/day, with the placebo/amphotericin B component given only for the first 5–6 days). Overall success rates were 56% and 69%, respectively ($p = 0.043$), with failure to clear the bloodstream occurring in 17% and 6% of patients, respectively ($p = 0.02$) (14). Based on these results, one can conclude that the combination of fluconazole and amphotericin B was not antagonistic when compared to fluconazole monotherapy, and that the combination trended towards improved success and more rapid bloodstream clearance. Whether there are differences in outcome, based on the underlying fungal pathogen, remains to be determined.

B. Azoles

When one agent within a class of drugs is reported to interact with a medication, a frequent assumption is that it is a “class effect.” This assumption must not be made when considering clinically relevant drug interactions with azole antifungals. Drugs within the class have distinct differences, some with very different metabolic pathways. As a consequence, the presence of a drug interaction with one agent cannot be extrapolated to others within the class, nor can be the magnitude of the interaction, as the azoles have different affinities for the CYP isoenzymes.
1. **Ketoconazole**

In 1981, ketoconazole was the first azole to be introduced into clinical practice, and was used widely for a variety of fungal infections. Its use is now relegated to second-line therapy, behind the newer azoles that possess more favorable side effect profiles and a broader spectrum of activity. Ketoconazole is metabolized primarily by the CYP3A4 isoenzyme system, with other CYP enzymes involved to a lesser extent. Ketoconazole has also been reported to interact with the transport protein P-glycoprotein, another mechanism by which drug–drug interactions can occur.

Drug interactions of most concern in immunocompromised patients include the calcineurin inhibitors cyclosporine, tacrolimus and sirolimus, and the antiretrovirals and antineoplastic agents. Ketoconazole was first noticed to reduce the daily cyclosporine requirements in renal transplant patients in 1982 (15). Since that time it has been widely accepted as a method to reduce costs associated with organ transplantation. Concomitant administration of ketoconazole and cyclosporine leads to a reduction in the cyclosporine dose of 70–80% (16–18). This then reduces the overall cost of the transplant immunosuppression by this percentage. Initially, many centers were concerned about introducing another drug that had a known side-effect profile to reduce the dose of another agent purely for financial reasons. Long-term follow-up of patients treated with combination ketoconazole and cyclosporine have not shown any detrimental effects, with a similar number of acute rejection episodes and chronic graft dysfunction (17), a similar rate of hepatotoxicity to those not receiving ketoconazole, and had better metabolic profiles in the ketoconazole/cyclosporine recipients. The dose of ketoconazole required to induce the cyclosporine sparing effect is 87 mg. A similar interaction has been observed among recipients of tacrolimus (19,20), although it has not been studied formally. Similar dose reductions should be made to tacrolimus when initiating ketoconazole therapy.

Patients infected with HIV are immunocompromised and may develop either topical or systemic fungal infections requiring antifungal therapy. Ketoconazole is known to alter the metabolism of several of the protease inhibitors (PI) and the non-nucleoside reverse transcriptase inhibitors (NNRTI’s), and many drugs within these classes in turn affect the PK of ketoconazole. Importantly, the drug interactions between ketoconazole and each of the agents within these classes are not the same. For example, within the PI class of drugs, only indinavir requires a dosage adjustment whereas no dosage modifications are necessary for amprenavir and nelfinavir. The lopinavir/ritonavir combination, ritonavir, and saquinavir have all been shown to increase the exposure of ketoconazole several fold, and as a consequence, a maximum dose of 200 mg/day of ketoconazole is recommended. Clinically significant interactions between ketoconazole and the PI’s and the NNRTI’s are summarized in Table 3 (21–35).

Lastly, interactions between ketoconazole and antineoplastic chemotherapy must be considered. These interactions are poorly characterized with the majority of data arising from animal models or the use of test systems in vitro. As a consequence limited data is available. Ketoconazole is a model CYP3A4 inhibitor, and has been studied in combination with irinotecan, a commonly used agent in colorectal cancer. It is also a partial substrate of CYP3A4 isoenzymes (25). Patients were initially exposed to irinotecan monotherapy, at a standard dose of 350 mg/m², and then 3 weeks later were exposed to a lower dose of irinotecan (100 mg/m²) in combination with ketoconazole 200 mg/day. Irinotecan is metabolized to several metabolites, including the pharmacologically active metabolite SN-38. Following concomitant
<table>
<thead>
<tr>
<th>Drug</th>
<th>Data type</th>
<th>Proposed Mechanism of Interaction by Ketoconazole (K) or Drug (D)</th>
<th>Clinical Effect (Potential or Actual)</th>
<th>Recommended Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiarrhythmics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dofetilide (21)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by K Inhibition of tubular secretion via the cation transport system</td>
<td>↑ Dofetilide [ ] by 53% males ↑ Dofetilide [ ] by 97% females</td>
<td>Stop dofeclide 2 days prior to starting ketoconazole</td>
</tr>
<tr>
<td>Antihistamines (astemizole, terfenadine) (22)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by K Prostaglandin D2 inhibition</td>
<td>↑ Astemizole and Terfenadine [ ] → QTc prolongation</td>
<td>Contraindicated. Avoid</td>
</tr>
<tr>
<td>Benzodiazepines (midazolam, triazolam) (22)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by K</td>
<td>↑ Midazolam/Triazolam [ ]</td>
<td>Prolonged sedative and hypnotic effects</td>
</tr>
<tr>
<td>Bosentan (23)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by K</td>
<td>↑ C&lt;sub&gt;max&lt;/sub&gt; Bosentan 2.1-fold ↑ AUC Bosentan 2.3-fold</td>
<td>Reduce the dose of cyclosporine or tacrolimus by 75–80% when starting ketoconazole</td>
</tr>
<tr>
<td>Calcineurin Inhibitors</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by K</td>
<td>↑ C&lt;sub&gt;min&lt;/sub&gt; ↑ AUC cyclosporine</td>
<td>Reduce the dose of cyclosporine or tacrolimus by 75–80% when starting ketoconazole</td>
</tr>
<tr>
<td>Cyclosporine (16–18,24)</td>
<td>PK studies</td>
<td>CYP3A4 inhibition by K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus (19,20)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by K</td>
<td>↑ C&lt;sub&gt;min&lt;/sub&gt; AUC tacrolimus ↑ cisparide AUC 8-fold</td>
<td>Contraindicated. Avoid</td>
</tr>
<tr>
<td>Cisparide (22)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by K</td>
<td>↑ AUC SN-38 (pharmacologically active metabolite of irinotecan) 109%</td>
<td>↓ Dose irinotecan by 75% Monitor for hematological toxicity</td>
</tr>
<tr>
<td>Cytotoxic chemotherapy</td>
<td>PK study</td>
<td>CYP3A4 inhibition by K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irinotecan (25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMG-CoA reductase inhibitors (26)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by K</td>
<td>↑ Simvastatin [ ] - &gt;toxicity</td>
<td>Monitor for signs of rhabdomyolysis</td>
</tr>
</tbody>
</table>

(Continued)
**Table 3** Clinically Relevant Pharmacokinetic Drug Interactions with Ketoconazole (21–35) (Continued)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Data type</th>
<th>Proposed Mechanism of Interaction by Ketoconazole (K) or Drug (D)</th>
<th>Clinical Effect (Potential or Actual)</th>
<th>Recommended Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNRTI’s Nevirapine (27)</td>
<td>PK study</td>
<td>CYP3A4 induction by D</td>
<td>↓ Ketoconazole AUC 72% ↓ Ketoconazole C&lt;sub&gt;max&lt;/sub&gt; 44%</td>
<td>Avoid</td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>PK study</td>
<td>CYP3A4 inhibition by D</td>
<td>↑ Ketoconazole AUC 44%</td>
<td>No dose modification</td>
</tr>
<tr>
<td>Amprenavir (28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indinavir (29)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by K</td>
<td>↑ Indinavir AUC by 68%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↓ Indinavir AUC to 600mg TTD</td>
</tr>
<tr>
<td>Lopinavir/Ritonavir (30)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by D</td>
<td>↑ Ketoconazole AUC 3-fold</td>
<td>Do not exceed 200 mg/day ketoconazole</td>
</tr>
<tr>
<td>Nelfinavir (31)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by K</td>
<td>↑ Nelfinavir AUC 35%; C&lt;sub&gt;max&lt;/sub&gt; 25%</td>
<td>No dose adjustments necessary. Monitor for PI toxicity</td>
</tr>
<tr>
<td>Ritonavir (32)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by D</td>
<td>↑ Ketoconazole AUC 3.4-fold ↑ Ketoconazole C&lt;sub&gt;max&lt;/sub&gt; 55%</td>
<td>Use with caution. Do not exceed ketoconazole 200 mg/day</td>
</tr>
<tr>
<td>Saquinavir (33)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by K</td>
<td>↑ Saquinavir AUC&lt;sub&gt;0.8&lt;/sub&gt; by 190%</td>
<td>If ketoconazole dose is &gt; 200 mg monitor for saquinavir toxicity</td>
</tr>
<tr>
<td>Rifampin (22)</td>
<td>PK study</td>
<td>CYP3A4 induction by D</td>
<td>↓ AUC Ketoconazole</td>
<td>Avoid concomitant use where possible. If essential, increase dose</td>
</tr>
<tr>
<td>Telithromycin (34)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by K</td>
<td>↑ Telithromycin C&lt;sub&gt;max&lt;/sub&gt; and AUC by 51.3% and 94.5%</td>
<td>Unknown</td>
</tr>
<tr>
<td>Warfarin (35)</td>
<td></td>
<td>CYP2C9 inhibition by D</td>
<td>↑ INR → bleeding</td>
<td>Monitor INR</td>
</tr>
</tbody>
</table>

<sup>a</sup>Single dose study. In multiple dosing study, 18% decrease in AUC of indinavir seen.

---

| Concentration, ↑, increased; →, leads to; ↓, decreased; AUC, area under the curve; C<sub>min</sub>, trough concentration; C<sub>max</sub>, peak concentration; PK, pharmacokinetic; CYP3A4, cytochrome P450 3A4 isoenzyme; NNRTI’s, non-nucleoside reverse transcriptase inhibitors.

<sup>a</sup>Single dose study. In multiple dosing study, 18% decrease in AUC of indinavir seen.
administration of ketoconazole, circulating SN-38 concentrations are increased by approximately 109%. Similarly, the principal oxidative metabolite, 7-ethyl-10-{4-N-(5-aminopentanoic acid)-l-piperidinol-carbonyloxycampothecin (APC), was reduced by 87%. A paired analysis of hematologic toxicity demonstrated a similar degree of myelosuppression, despite a 3.5-fold reduction in irinotecan dose, when administered in combination with ketoconazole compared to irinotecan monotherapy. This study highlights the importance of understanding metabolic pathways prior to administering medications. Irinotecan is not the only antineoplastic that is likely to be subject to such an interaction; potentially docetaxel, etoposide, cyclophosphamide, and ifosfamide may interact similarly.

2. Fluconazole

Fluconazole, a bis-triazole antifungal, is widely used in high-risk population (neonates, surgical candidates, HIV, HSCT, and SOT patients) to prevent superficial and systemic fungal infections. Recent studies have shown that fluconazole can reduce systemic and superficial fungal infections, reduce fungal colonization, and reduce the use of empiric amphotericin B in high-risk populations (36–45). Fluconazole is metabolized by CYP3A4 isoenzymes, and is also a potent inhibitor of CYP2C9 isoenzymes, leading to several potential drug interactions (see Table 4) (46–87). One of the interactions of most concern, related to the transplantation community, is that of fluconazole and the calcineurin inhibitors, cyclosporine, and tacrolimus. Many centers have reported a clinically significant drug interaction between these two agents, although the magnitude of the interaction is smaller than seen with itraconazole, ketoconazole, and voriconazole. Two issues warrant consideration: first, does the interaction occur when the agents are administered intravenously as well as orally, and second, does the interaction occur at all doses of fluconazole, or is it a dose-dependent phenomenon?

Several case reports in the literature document a drug interaction between fluconazole and the calcineurin inhibitors (52–69), although these are retrospective and uncontrolled observations. Osowski and colleagues performed a controlled PK study of the interaction between intravenous cyclosporine/tacrolimus and intravenous fluconazole 400 mg daily in HSCT recipients (52). They observed no statistically or clinically significant differences in steady-state concentration or clearance of tacrolimus, and a statistically but not clinically significant difference in the clearance of cyclosporine A. These results did not reflect the experience seen with oral administration of both agents in many of the earlier case reports.

CYP3A4 isoenzymes are the most abundant isoforms of CYP, accounting for nearly 30% of the total P450 content in the human liver, and as much as 70% in the gut wall (88). It has been postulated that tacrolimus and cyclosporine are also metabolized in the intestine by gut CYP3A4 isoenzymes. If this does occur, it would explain why a drug interaction is seen betweenazole antifungals and tacrolimus/cyclosporine when administered orally, and not when administered intravenously. In addition to tacrolimus and cyclosporine being metabolized in this manner, there is also evidence that fluconazole inhibits CYP3A4 substrate, which could result in increased serum tacrolimus or cyclosporine concentrations, with resultant toxicities. These differing observations lead to the conclusion that the interaction between tacrolimus and fluconazole is because of the inhibition of gut metabolism of tacrolimus by fluconazole, resulting in increased absorption, an increased AUC, and increased trough concentrations when these agents are administered orally.
Table 4  Clinically Relevant Pharmacokinetic Drug Interactions with Fluconazole (FLU) (46–87)

<table>
<thead>
<tr>
<th>Drug (D)</th>
<th>Data type</th>
<th>Proposed mechanism of interaction by FLU (F) or (D)</th>
<th>Clinical effect (potential or actual)</th>
<th>Recommended Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-trans retinoic acid (ATRA) (46,47)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by F</td>
<td>↑ ATRA [ ] → ↑ CNS toxicity</td>
<td>Monitor closely for toxicities associated with ATRA</td>
</tr>
<tr>
<td>Antihistamines (astemizole, terfenadine) (48)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by F</td>
<td>↑ Astemizole/Terfenadine [ ] → prolonged QTc interval</td>
<td>Avoid. Contraindicated</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midazolam (49,50)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by F</td>
<td>↑ Midazolam [ ] 0–4 fold → ↑ sedation</td>
<td>Monitor sedation; ↓ Midazolam dose; &gt; interaction with oral Rx and high dose/continuous infusion ↑ Sedation; monitor</td>
</tr>
<tr>
<td>Triazolam (51)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by F</td>
<td>↑ Triazolam AUC and t1/2 (fluconazole dose dependent increases)</td>
<td></td>
</tr>
<tr>
<td>Calcineurin inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus (52–54)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by F</td>
<td>↑ Tacrolimus Cmin → ↑ toxicitya</td>
<td>Monitor levels; ↓ Tacrolimus dosea</td>
</tr>
<tr>
<td>Cyclosporine (55–68)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by F</td>
<td>↑ Cyclosporine Cmin → ↑ toxicity</td>
<td>Monitor levels; ↓ Cyclosporine dosec</td>
</tr>
<tr>
<td>Sirolimus (69)</td>
<td>Case report</td>
<td>CYP3A4 inhibition by F</td>
<td>↑ Sirolimus Cmin → ↑ toxicity</td>
<td>Monitor levels; ↓ Sirolimus dose</td>
</tr>
<tr>
<td>Carbamazepine (70,71)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by F</td>
<td>↑ Carbamazepine [ ] → ↑ toxicity</td>
<td>Monitor levels; ↓ Carbamazepine dose</td>
</tr>
<tr>
<td>HMGCoA reductase inhibitor (simvastatin) (72)</td>
<td>Case report</td>
<td>CYP3A4 inhibition by F</td>
<td>↑ Simvastatin [ ] → ↑ musculo skeletal toxicity</td>
<td>Monitor patient closely for symptoms of rhabdomyolysis</td>
</tr>
<tr>
<td>Opioids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfentanil (73)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by F</td>
<td>↓ Alfentanil CL 55%; ↓ respiratory rate</td>
<td>Monitor sedation and respiratory rate</td>
</tr>
<tr>
<td>Phenytoin (74–77)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by F</td>
<td>↓ Phenytoin [ ]</td>
<td>Monitor phenytoin [ ]</td>
</tr>
<tr>
<td>Rifamycins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifabutin (78)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by F</td>
<td>↑ Rifabutin exposure</td>
<td>Treatment failure of antifungal; monitor for response</td>
</tr>
<tr>
<td>Rifampin (79,80)</td>
<td>Case reports</td>
<td>CYP3A4 induction by D</td>
<td>↓ Fluconazole AUC; t1/2 ↓ CL</td>
<td></td>
</tr>
<tr>
<td>Sulfonylureas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glimepiride (81)</td>
<td>Case report</td>
<td>CYP2C9 inhibition by F</td>
<td>↑ Glimepiride AUC; t1/2</td>
<td>Monitor blood sugar; ↓ glimepiride dose</td>
</tr>
<tr>
<td>Warfarin (82–87)</td>
<td>Case reports</td>
<td>CYP2C9 and CYP3A4 inhibition by F</td>
<td>↑ Warfarin [ ] → ↑ bleeding</td>
<td>Monitor INR; ↓ Warfarin dose</td>
</tr>
</tbody>
</table>

[C], concentration; ↑, increased; →, leads to; ↓, decreased; AUC, area under the curve; Cmin, trough concentration; F, bioavailability; t1/2, half-life; CL, clearance; GVHD, graft vs. host disease; PK, pharmacokinetic; CYP3A4, cytochrome P450 3A4, isoenzyme; CYP2C9, cytochrome P450 2C9 isoenzyme.

*aDrug interaction occurs when both agents administered orally and where fluconazole dose is > 200 mg/day.
Therefore, appropriate dose reductions in cyclosporine and tacrolimus should be made when administered orally, but are unnecessary when given intravenously.

At the University of Florida HSCT program, lower doses of fluconazole (100 mg for autologous HSCT recipients were used; 200 mg for allogeneic HSCT recipients) as part of our antifungal prophylaxis strategy. When the calcineurin inhibitors are concomitantly administered with fluconazole at these doses, appreciable increases in cyclosporine or tacrolimus serum concentrations are not noted. This suggests that the drug interaction is dose-dependent, and that at doses of fluconazole of greater than 200 mg per day the interaction is much more likely to occur, requiring reductions in cyclosporine/tacrolimus doses (60,62,66). Fluconazole doses < 200 mg daily typically do not require a dose adjustment of the calcineurin inhibitor. Other investigators have made similar observations (60,62,64,68).

The other drugs with which fluconazole has been reported to interact include rifampin, phenytoin, and warfarin. Rifampin, a potent CYP3A4, CYP2C9, and CYP2C19 isoenzyme inducer, was shown to significantly lower the AUC of fluconazole by 52%, increase clearance by 93%, and shorten the half-life of fluconazole when concomitantly prescribed (79). In this case series, the dose of rifampin was higher than the doses used for HSCT antibiotic prophylaxis (1200 mg daily vs. 600 mg daily), and may account for the differences seen in this trial compared to earlier healthy volunteer studies, where the mean decrease in exposure was 23% (80). Relapse or failure of fluconazole therapy in cryptococcal meningitis has been reported due to this enzyme induction.

Phenytoin, a known potent inducer of CYP3A4 enzymes, is itself metabolized by CYP2C9 and CYP2C19. Fluconazole is a potent inhibitor of these isoenzymes, leading to increased serum concentrations of phenytoin and clinical toxicity. Several case reports have indicated clinical phenytoin toxicity when phenytoin is administered with fluconazole at doses of 200 mg or greater per day (74–77). In healthy volunteers, fluconazole administration increases phenytoin trough concentrations and AUC by 128% and 75%, respectively. The onset of the interaction is relatively quick, within 2–6 days of concomitant administration. Phenytoin dosage should be reduced, and serum concentrations closely monitored. Warfarin also requires close monitoring and potential dosage adjustment when prescribed with fluconazole. Fluconazole inhibits the metabolism of warfarin by CYP2C9, thus potentiating the hypoprothrombinemic response of warfarin, usually in the first few days of concomitant administration. Frequent monitoring of the INR should occur in this population. In healthy volunteer studies, the mean change in the INR was 38% (range 16–64%) (84–87).

3. Itraconazole

Itraconazole, a triazole compound with activity against yeasts and molds, has been used as prophylaxis against fungal infections in immunocompromised patients (89,90), in the empiric setting for fever unresponsive to broad-spectrum antibiotics (91), and in the treatment of documented fungal infections (92–94). Like other members of theazole family, it undergoes extensive metabolism via the CYP3A4 enzyme system, and is subject to drug interactions with agents metabolized by a similar pathway (95–134).

Itraconazole differs from fluconazole in that there is a relationship between drug dose, serum drug concentration, and efficacy. Serum concentrations of itraconazole greater than 500 ng/mL are required to prevent invasive fungal infection.
If enzyme inducers are coadministered, there is potential for increased metabolism of itraconazole leading to reduced serum concentrations and potential drug failure. Drugs that decrease plasma concentrations of itraconazole include carbamazepine (106,107), phenobarbital, phenytoin (116), isoniazid, rifampin (117,118), and rifabutin (119). Whenever possible, these should not be prescribed concomitantly with itraconazole, although if no suitable alternative exists, routine drug monitoring of itraconazole levels is recommended to ensure that therapeutic levels are achieved (i.e., >500 ng/mL).

Itraconazole also has the ability to increase concentrations of many other medications (see Table 5) (95–134). Numerous case reports in the literature document an interaction between oral itraconazole and oral tacrolimus (122,125–129), and oral itraconazole and oral cyclosporine (122–124). These cases demonstrate that the addition of itraconazole to calcineurin inhibitor therapy increases the trough concentrations of the calcineurin inhibitors by two-to three-fold. In each of these case reports, additional factors could affect outcome, so the frequency of occurrence and the magnitude of the drug interaction is not clear. Despite these uncertainties, the dose of cyclosporine and tacrolimus must be reduced, and based on available data, the reduction should be at least 50% at the time itraconazole is added to therapy. Evaluation of the pharmacokinetic drug interaction between intravenous itraconazole and intravenous calcineurin inhibitors has also been conducted (122). In a controlled environment, allogeneic HSCT recipients were stabilized on intravenous calcineurin inhibitors. Once steady state was reached, intravenous itraconazole was added to therapy. Among the 16 patients studied, the mean steady-state trough concentration of cyclosporine and tacrolimus increased by 123–249% and 126–207%, respectively. This is accompanied by a corresponding mean decrease in clearance of 46.4% and 44%, respectively. The increase in serum cyclosporine and tacrolimus concentrations is evident at the completion of the intravenous loading dose, when steady state is achieved. A 50% reduction in dose of cyclosporine and tacrolimus doses should occur when starting intravenous itraconazole therapy.

Many critically ill patients require narcotic analgesics for pain relief. Phenylpiperidine opiates, which include fentanyl, sufentanil, and alfentanil, are extensively metabolized by CYP3A4 isoenzymes. There is potential for elevated serum concentrations of these opioids if coadministered with medications that inhibit CYP3A4 isoenzymes. When administration of both agents is essential, patients should be monitored closely for excessive sedation and for low respiratory rates.

The other major class of drugs that requires consideration when prescribing itraconazole is the antineoplastic agents. Numerous case reports exist in the literature documenting greater neurotoxicity when itraconazole is prescribed during vinca alkaloid administration (98–102). Cyclophosphamide, a cytotoxic agent used in many chemotherapy regimens, is a prodrug that must undergo metabolism by CYP enzymes to produce the alkylator species required for its antineoplastic effect. The CYP’s most involved in this process are CYP3A4 and CP2C9 (135). Coadministration of inhibitors of these isoenzymes may lead to increased exposure of cyclophosphamide, and increased toxicity. It has been suggested that concomitant administration of itraconazole with cyclophosphamide preparative regimens may lead to greater toxicity. Itraconazole has also been reported to reduce the clearance of busulfan when concomitantly prescribed with itraconazole. Clearance was reduced by 20%, and was accompanied by increased toxicity (136). Similarly, other antineoplastic agents that are metabolized by the CYP enzymes may have their serum concentrations enhanced leading to greater toxicity. Metabolic pathways of
### Table 5  Clinically Relevant Pharmacokinetic Drug Interactions with Itraconazole (96–134)

<table>
<thead>
<tr>
<th>Drug (D)</th>
<th>Data type</th>
<th>Proposed mechanism of interaction by Itraconazole (I) or drug (D)</th>
<th>Clinical effect (potential or actual)</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| Antiarrhythmics  
Dofetilide (121) | Case report | CYP3A4 inhibition by I; inhibition of tubular secretion via the cation transport system | ↑ Dofetilide [ ] | Contraindicated together. Stop dofetilide 2 days prior to starting at interacting drugs |
| Antihistamines  
Terfenadine (96,97)  
Antemizole | PK study  
PK study | CYP3A4 inhibition by I  
CYP3A4 inhibition by I | ↑ [ ] unmetabolized terfenadine; ↑ $C_{\text{max}}$, $t_{1/2}$  
↑ Antineoplastic [ ] due to ↓ metabolism → ↑ toxicity e.g., neurotoxicity with vinca alkaloids | Contraindicated—Avoid  
Avoid concomitant therapy. Use alternative nonazole antifungal during chemotherapy administration and until antineoplastic elimination complete |
| Antineoplastics e.g., vinca alkaloids, busulfan, ifosfamide, cyclophosphamide, docetaxel; epipodophyllotoxins (98–102) | Case reports/  
Theoretical | CYP3A4 inhibition by I  
CYP3A4 inhibition by I | ↑ BZD $C_{\text{max}}$, ↑ AUC, $t_{1/2}$  
→ prolonged hypnotic and sedative effects; prolonged psychomotor impairment | Avoid where possible. Use alternative BZD e.g., lorazepam, temazepam |
| Benzodiazepines (BZD)  
(alprazolam, midazolam—oral, triazolam) (103,104) | Case reports | CYP3A4 inhibition by I | ↑ $C_{\text{min}}$ 1.23–2.49-fold  
→ nephrotoxicity | Decrease dose of CyA by 50% when starting intravenous itraconazole |
| Calcineurin Inhibitors  
Cyclosporine (CyA) IV (122)  
Cyclosporine (oral) (123,124) | PK study  
Case reports | CYP3A4 inhibition by I  
CYP3A4 inhibition by I | ↑ $C_{\text{min}}$ by 33–85%  
→ potential nephrotoxicity | Decrease dose of CyA by 50% when starting itraconazole (Kramer). |

(Continued)
<table>
<thead>
<tr>
<th>Drug (D)</th>
<th>Data type</th>
<th>Proposed mechanism of interaction by Itraconazole (I) or drug (D)</th>
<th>Clinical effect (potential or actual)</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus(IV) (122)</td>
<td>PK study</td>
<td>CYP3 A4 inhibition</td>
<td>↓ Tacrolimus C_{min} \text{1.26–2.07-fold} → ↑ toxicity</td>
<td>Reduce tacrolimus dose by 50% at the time of initiating itraconazole therapy</td>
</tr>
<tr>
<td>Tacrolimus (oral) (125–129)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition</td>
<td>↑ Tacrolimus C_{min}</td>
<td>Reduce the dose of tacrolimus by 50–67% when initiating itraconazole therapy</td>
</tr>
<tr>
<td>Cisapride (121)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by I</td>
<td>↓ Tacrolimus CL → ↑ toxicity</td>
<td>Contraindicated—Avoid</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous MP (130)</td>
<td>PK studies</td>
<td>CYP3A4 inhibition by I</td>
<td>↓ CL MP; [↑ 2-fold]</td>
<td>Enhanced steroid side effects</td>
</tr>
<tr>
<td>Inhaled (131,132)</td>
<td>PK studies</td>
<td>CYP3A4 inhibition by I</td>
<td>↑ AUC Methylprednisolone (no effect on prednisone);</td>
<td>Enhanced steroid side effects</td>
</tr>
<tr>
<td>Oral</td>
<td>PK studies</td>
<td>CYP3A4 inhibition by I</td>
<td>↑ t_{1/2}</td>
<td>Enhanced suppression of exogenous cortisol secretion</td>
</tr>
<tr>
<td>Digoxin (108–111)</td>
<td>Case reports</td>
<td>↓ renal CL due to inhibition of digoxin p-glycoprotein pump</td>
<td>↑ Digoxin [ ] 2–4 fold → toxicity</td>
<td>Decrease dose of digoxin 60–75%. Monitor digoxin levels closely. Note: interaction occurs 7–13 days after starting itraconazole</td>
</tr>
<tr>
<td>HMG Co-A reductase inhibitors (lovastatin (112), atorvastatin (113), simvastatin (114,115))</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by I</td>
<td>L: ↑ C_{max} 22-fold; ↑ AUC 13-fold; 97% ↓ clearance S: ↑ C_{max} &gt; 10-fold; ↑ AUC 19-fold; &gt;90% ↓ clearance A: ↑ AUC 4-fold; 70% ↓ clearance → ↑ risk of rhabdomyolysis</td>
<td>Avoid atorvastatin, lovastatin, and simvastatin = contraindicated Fluvastatin and pravastatin are suitable alternatives, as they do not interact with itraconazole.</td>
</tr>
<tr>
<td>Drug Class</td>
<td>Drug</td>
<td>Case report/Study Type</td>
<td>CYP Isoenzyme</td>
<td>Effect</td>
</tr>
<tr>
<td>------------</td>
<td>------</td>
<td>------------------------</td>
<td>---------------</td>
<td>--------</td>
</tr>
<tr>
<td>Opioids</td>
<td>Fentanyl (133)</td>
<td>Case report</td>
<td>CYP3A4 inhibition by I</td>
<td>↑ Fentanyl [ ]</td>
</tr>
<tr>
<td></td>
<td>Phenytoin (116)</td>
<td>Case reports</td>
<td>CYP3A4 induction by D</td>
<td>↓ Itraconazole $C_{\text{max}}$ 95%</td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td></td>
<td></td>
<td>$C_{\text{max}}$ 95% &amp; AUC 93%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&amp; $t_1/2$ 83%</td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>Amprenavir</td>
<td>Theoretical</td>
<td>CYP3A4 inhibition by D</td>
<td>↑ Itraconazole [ ], AUC</td>
</tr>
<tr>
<td></td>
<td>Indinavir</td>
<td>PK study</td>
<td>CYP3A4 inhibition by D</td>
<td>↑ Itraconazole [ ], AUC</td>
</tr>
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<td></td>
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<td>↓ Itraconazole [ ], AUC</td>
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<tr>
<td></td>
<td>Lopinavir/ritonavir (134)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by D</td>
<td>↑ Itraconazole [ ] → treatment failure</td>
</tr>
<tr>
<td></td>
<td>Rifampin (117,118)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by D</td>
<td>↑ Itraconazole [ ] → treatment failure</td>
</tr>
<tr>
<td></td>
<td>Rifabutin (119)</td>
<td>Case report</td>
<td>CYP3A4 induction by D</td>
<td>↑ Itraconazole [ ] → treatment failure</td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>Amprenavir</td>
<td>Theoretical</td>
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<td></td>
<td>Indinavir</td>
<td>PK study</td>
<td>CYP3A4 inhibition by D</td>
<td>↑ Itraconazole [ ], AUC</td>
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<td>↓ Itraconazole [ ], AUC</td>
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<td></td>
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<td>↓ Itraconazole [ ], AUC</td>
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<td>↓ Itraconazole [ ], AUC</td>
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<td>Case reports</td>
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<td>Case reports</td>
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<td>Case report</td>
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<tr>
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<td>Indinavir</td>
<td>PK study</td>
<td>CYP3A4 inhibition by D</td>
<td>↑ Itraconazole [ ], AUC</td>
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<td>Case reports</td>
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<td>Rifampin (117,118)</td>
<td>Case reports</td>
<td>CYP3A4 inhibition by D</td>
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</tr>
<tr>
<td></td>
<td>Rifabutin (119)</td>
<td>Case report</td>
<td>CYP3A4 induction by D</td>
<td>↑ Itraconazole [ ] → treatment failure</td>
</tr>
</tbody>
</table>

CYP, cytochrome P450; CYP3A4, cytochrome P450 3A4 isoenzyme; CYP2C9, cytochrome P450 2C9 isoenzyme ↑, increase; ↓, decrease; [ ], concentration; →, leads to; INR, international normalized ratio; PK, pharmacokinetic; BP, blood pressure; HR, heart rate; CL, clearance; $C_{\text{min}}$, trough concentration; $C_{\text{max}}$, maximum concentration; AUC, area under the curve; $t_1/2$, half-life; P-gp, P-glycoprotein; MP, methylprednisolone; INR, international normalized ratio; PT, prothrombin time; QTc, QTc interval on an EKG; F, bioavailability.

*There are literature reports of an interaction between voriconazole/sirolimus, and ketoconazole/sirolimus. Significant ↑ in AUC, $C_{\text{max}}$ seen.*
the antineoplastic agent should be verified prior to coadministering with itraconazole. Patient’s receiving antineoplastic agents metabolized by the CYP3A4 isoenzyme system should have the itraconazole therapy held during the chemotherapy administration. One must be mindful of the long half-life of itraconazole (64 hr with oral dosing when at steady state), and stop the drug early enough to permit complete excretion of itraconazole and its metabolites.

4. Voriconazole

Voriconazole, the most recent commercially availableazole, offers new challenges to the prescribing clinician. It is similar to other members of the azole family in that it is subject to several drug interactions (137–146). Voriconazole, however, is metabolized by three separate cytochrome P450 enzyme systems, namely CYP2C9, CYP2C19, and CYP3A4. This differs from the other azoles (fluconazole, itraconazole, ketoconazole, posaconazole, and ravuconazole), which are metabolized primarily by CYP3A4. In vitro metabolism studies show that voriconazole is both a substrate and an inhibitor of these three enzymes. Due to extensive hepatic microsomal metabolism, caution is needed when prescribing voriconazole to immunocompromised patients receiving multiple medications. Prior to starting any new drug, a screen of potential interactions should always be performed, and if there are no data, evaluate the metabolic pathways of the new agent to evaluate the hypothetical risk of a drug interaction occurring.

Based on data from in vitro drug metabolism studies, voriconazole was subject to extensive clinical PK drug interaction studies prior to marketing. These PK interaction studies were designed around target drugs that were likely to be coadministered to target patient populations and/or when an interaction might be expected on mechanistic grounds. The main clinically significant drug interactions that are known to occur when coprescribed with voriconazole are summarized in Table 6 (137,139,140,142–146), and the effect of other drugs on voriconazole metabolism are summarized in Table 7 (137,139,141–143). There are several agents that are contraindicated in patients receiving voriconazole, including sirolimus, ergot alkaloids, terfenadine, astemizole, cisapride, pimozide, quinidine, rifampin, and rifabutin. Coadministration of sirolimus with voriconazole results in an increased maximal concentration ($C_{\text{max}}$) by 556%, and an increased exposure, or “area-under-the curve” of sirolimus by 1014%, prohibiting dose reduction to a safe level (137). The interaction with astemizole, cisapride, pimozide, quinidine, and ergot derivatives is due to potential prolongation of the QTc interval leading to Torsades de pointes. A similar reaction is noted to occur when these agents are administered with several other members of the azole family (itraconazole, ketoconazole). Rifampin, however, induces the metabolism of voriconazole to such an extent that there is no dose of voriconazole that could be safely administered to a patient to overcome this inhibition. It should be noted that increasing the dose of voriconazole when coadministered with rifabutin can overcome the enzyme induction, although this approach should be avoided.

Again, the one therapeutic class of drugs where there is a lack of available information regarding drug interactions with voriconazole, is antineoplastic therapy. This is an area that should be addressed because immunocompromised patients will often require antifungal therapy with ongoing chemotherapy. Several antineoplastic agents are metabolized by the CYP3A4 enzyme system. The ramifications of the potential drug interaction include elevated concentrations of the antineoplastic...
<table>
<thead>
<tr>
<th>Target drug</th>
<th>Population</th>
<th>Proposed mechanism of interaction</th>
<th>Clinical effect on target drug</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antihistamines</td>
<td>Theoretical</td>
<td>CYP3A4 inhibition</td>
<td>↑ Astemizole and terfenadine → QTc prolongation</td>
<td><strong>Contraindicated. Potential for QT prolongation and <em>Torsades de pointes</em></strong></td>
</tr>
<tr>
<td>Astemizole, terfenadine</td>
<td></td>
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</tr>
<tr>
<td>Benzodiazepines (BZD) (137)</td>
<td>In vitro studies</td>
<td>CYP3A4 inhibition</td>
<td>Potential for inhibition of metabolism of BZD → ↑ sedation</td>
<td><strong>Frequent monitoring for adverse events and toxicity</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>Dose reduce BZD if symptomatic</strong></td>
</tr>
<tr>
<td>Calcineurin inhibitors</td>
<td>PK study</td>
<td>CYP3A4 inhibition</td>
<td>↑ CyA AUC 70%</td>
<td><strong>When initiating VORI in patients already on CyA, ↑ CyA dose by 50% and closely monitor blood levels Interaction evident on day 4 of coadministration ↑ CyA dose when VORI discontinued</strong></td>
</tr>
<tr>
<td>Cyclosporine (144)</td>
<td>Stable renal transplant patients</td>
<td></td>
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</tr>
<tr>
<td>Tacrolimus (FK) (145,146)</td>
<td>PK study</td>
<td>CYP3A4 inhibition</td>
<td>↑ Tacrolimus AUC 321%</td>
<td><strong>When initiating VORI in patients on FK, ↓ dose of FK by 67–90%, ↑ FK dose when VORI is discontinued</strong></td>
</tr>
<tr>
<td></td>
<td>Healthy volunteers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sirolimus (solution) (137)</td>
<td>PK study</td>
<td>CYP3A4 inhibition</td>
<td>↑Cmax 217% → ↑ toxicity</td>
<td><strong>Contraindicated</strong></td>
</tr>
<tr>
<td></td>
<td>Healthy volunteers</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Calcium channel blockers (CCB)</td>
<td>In vitro studies</td>
<td>CYP3A4 inhibition</td>
<td>Potential for inhibition of metabolism of CCB → ↓ toxicity</td>
<td><strong>Monitor blood pressure and pulse. Dose reduction of CCB may be needed if symptomatic</strong></td>
</tr>
<tr>
<td>(dihydropyridine class) (137)</td>
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</tr>
<tr>
<td>Chemotherapy/ cytotoxics</td>
<td>Hypothesized interaction</td>
<td>CYP3A4, 2C9, 2C19 inhibition</td>
<td>Potentially ↑ exposure to chemotherapeutic agent and enhanced toxicity</td>
<td><strong>Avoid coadministration with cytotoxic agents metabolized by the CYP 3A4, 2C9, 2C19 pathways. If fungal coverage necessary, change to alternative nonazole antifungal with similar spectrum</strong></td>
</tr>
<tr>
<td>(metabolized by CYP 3A4, 2C19, or 2C9)</td>
<td></td>
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</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Target drug</th>
<th>Population</th>
<th>Proposed mechanism of interaction</th>
<th>Clinical effect on target drug</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergot alkaloids (137)</td>
<td>Hypothesized interaction</td>
<td>CYP3A4 inhibition</td>
<td>↑ Ergot alkaloid</td>
<td>Contraindicated. Potential for QT prolongation and <em>Torsades de pointes</em></td>
</tr>
<tr>
<td>HMG-CoA reductase inhibitors (Statins) (137)</td>
<td>In vitro studies</td>
<td>CYP3A4 inhibition</td>
<td>↑ Plasma exposure of the statins, → ↑ toxicity</td>
<td>Monitor for increased muscle pain/weakness and rhabdomyolysis</td>
</tr>
<tr>
<td>NNRTI's (137)</td>
<td>In vitro studies</td>
<td>CYP3A4 inhibition</td>
<td>↑ Plasma exposure of NNRTI's</td>
<td>Monitor frequently for adverse events</td>
</tr>
<tr>
<td>Phenytoin (143)</td>
<td>PK study Healthy volunteers</td>
<td>CYP2C9 inhibition</td>
<td>↑ AUC 81%</td>
<td>Monitor phenytoin concentrations and adjust the dose accordingly</td>
</tr>
<tr>
<td>Prednisolone (138)</td>
<td>Healthy volunteers</td>
<td>CYP3A4 inhibition</td>
<td>↑ AUC 34% prednisolone</td>
<td>None recommended</td>
</tr>
<tr>
<td>HIV protease inhibitors (137)</td>
<td>In vitro studies</td>
<td>CYP3A4 inhibition</td>
<td>↑ Plasma exposure HIV protease inhibitors</td>
<td>Monitor frequently for adverse events</td>
</tr>
<tr>
<td>Proton pump inhibitors</td>
<td>Healthy volunteers</td>
<td>CYP3A4 inhibition</td>
<td>↑ AUC 280%</td>
<td>Doses &gt;40 mg omeprazole per day may be reduced by 50%</td>
</tr>
<tr>
<td>Omeprazole (142)</td>
<td></td>
<td></td>
<td>↑ Cmax 116%</td>
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</tr>
<tr>
<td>Quinidine (137)</td>
<td>Theoretical</td>
<td>CYP3A4 inhibition</td>
<td>↑ Quinidine</td>
<td>Contraindicated. Potential for QT prolongation and <em>Torsades de pointes</em></td>
</tr>
<tr>
<td>Rifabutin (139)</td>
<td>Healthy volunteers</td>
<td>CYP3A4 inhibition</td>
<td>↑ Rifabutin AUC 331%</td>
<td>Contraindicated</td>
</tr>
<tr>
<td>Rifampin (139)</td>
<td>Healthy volunteers</td>
<td>CYP3A4 induction</td>
<td>↑ Rifabutin Cmax 195%</td>
<td></td>
</tr>
<tr>
<td>Sulfonylurea oral hypoglycemics (137)</td>
<td>Theoretical</td>
<td>CYP2C9 inhibition</td>
<td>↑ Plasma exposure to sulfonylurea → ↓ BSL</td>
<td>Frequent BSL monitoring → ↓ dose of sulfonylurea</td>
</tr>
<tr>
<td>Vinca alkaloids (also see chemotherapy)</td>
<td>Hypothesis</td>
<td>CYP3A4 inhibition</td>
<td>↑ Exposure to vinca’s → ↑ neurotoxicity</td>
<td>Avoid coadministration with vinca alkaloids</td>
</tr>
<tr>
<td>Warfarin (140)</td>
<td>Healthy volunteers</td>
<td>CYP2C9 inhibition</td>
<td>↑ PT, INR → bleeding</td>
<td>Monitor INR/PT closely ↓ Warfarin dose</td>
</tr>
</tbody>
</table>

CYP 3A4, Cytochrome P450 3A4 isoenzyme; CYP2C9, cytochrome P450 2C9 isoenzyme; CYP2C19, cytochrome P450 2C19 isoenzyme; AUC, area under the curve; ↑, increase; ↓, decrease; →, leads to; NNRTI's, non-nucleoside reverse transcriptase inhibitors.
<table>
<thead>
<tr>
<th>Drug Population</th>
<th>Proposed mechanism of interaction</th>
<th>Clinical effect</th>
<th>Recommended Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbiturates (long acting) (137)</td>
<td>Hypothesized interaction</td>
<td>CYP3A4 induction</td>
<td>Likely to result in ↓ VORI exposure</td>
</tr>
<tr>
<td>Carbamazepine (137)</td>
<td>Hypothesized interaction</td>
<td>CYP3A4 induction</td>
<td>Enzyme induction → enhanced VORI metabolism → potential VORI treatment failure</td>
</tr>
<tr>
<td>NNRTI’s (137)</td>
<td>In vitro</td>
<td>CYP3A4 inhibition or induction</td>
<td>↑ and ↓ VORI [ ]</td>
</tr>
<tr>
<td>Omeprazole (141,142)</td>
<td>PK study</td>
<td>CYP2C19 inhibition</td>
<td>↑ VORI AUC 41%</td>
</tr>
<tr>
<td>Phenytoin (143)</td>
<td>PK study</td>
<td>Healthy volunteers</td>
<td>CYP3A4 induction</td>
</tr>
<tr>
<td>Protease inhibitors (PI’s) (137)</td>
<td>In vitro studies</td>
<td>CYP3A4 inhibition</td>
<td>↑ VORI [ ]</td>
</tr>
<tr>
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<td>PK study</td>
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<td>CYP3A4 induction</td>
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CYP 3A4, cytochrome P450 3A4 isoenzyme; CYP2C9, cytochrome P450 2C9 isoenzyme; CYP2C19, cytochrome P450 2C19 isoenzyme; AUC, area under the curve; ↑, increase; ↓, decrease; →, leads to; VORI, voriconazole; C_{max}, maximum “peak” concentration; ADR’s, adverse drug reactions.
agent, leading to greater chemotherapy or preparative regimen associated toxicity. Increased toxicity has been noted both with itraconazole and ketoconazole when administered at the same time as antineoplastic therapy as discussed previously. These case reports and studies clearly highlight the potential theazole antifungal agents have to increase concentrations of antineoplastic agents metabolized by CYP 3A4, leading to greater toxicity if dose reductions are not made. If a patient is receiving voriconazole for a pre-existing fungal infection, and is scheduled to undergo chemotherapy metabolized by CYP2C9, CYP2C19, or CYP3A4 (whether that be a HSCT preparative regimen or conventional chemotherapy), the best course of action would be to stop the voriconazole at least 30 hr prior to the chemotherapy/preparative regimen, and continue to hold during chemotherapy. Once the antineoplastic agent has been eliminated (approximately 5 half-lives of the antineoplastic agent), voriconazole can be restarted. Other suitable nonazole antifungals can be instituted during the preparative regimen.

In some of the clinical trials, adverse events including visual hallucinations and confusion were reported. When evaluated more closely, there appeared to be a relationship between these side effects and the use of benzodiazepines and/or opioid analgesic consumption in conjunction with voriconazole. Many of the benzodiazepines are metabolized by the CYP3A4 isoenzymes, including midazolam, triazolam, and alprazolam. Diazepam is metabolized by CYP2C19 in addition to CYP3A4. If the BZD’s are coadministered with a drug that is a potent inhibitor of CYP3A4, there will be an increase in serum concentrations of the BZD, with potential for increased sedation. Lorazepam, a commonly used component of antiemetic regimens, is eliminated by conjugation, and therefore, does not undergo a PK drug interaction with CYP3A4 modulators, and can be safely used. Similarly, oxazepam and temazepam are safe to use in combination with CYP3A4 agents.

In addition to these listed interactions, it is important to note that several drug interaction studies did not demonstrate an increase in either voriconazole or the concomitant medication when coadministered. Agents studied that are safe to coadminister with voriconazole include erythromycin, azithromycin, indinavir, prednisolone, ranitidine, digoxin, and mycophenolate mofetil. It is clear that there are many other medications that may theoretically interact with voriconazole, yet have not been studied. Whenever prescribing voriconazole, it is prudent to monitor the patient very closely in the early phase of concomitant administration, looking for both toxicities as well as a lack of effect of the concomitant medication as well as voriconazole. Despite the numerous known drug interactions with voriconazole, if appropriate caution is used and modifications to target drugs are made, it is relatively safe to use voriconazole in the majority of patients.

5. Posaconazole
Posaconazole is an investigational “extended spectrum triazole” with clinical activity demonstrated in Candida spp. (147–149), Coccidiomycosis spp. (150), Aspergillus spp. (147), Fusarium spp. (147), and Mucor spp. (147) infections. Posaconazole is an inhibitor of CYP3A4, and has not been shown to affect the activity of CYP1A2, CYP2C8/9, CYP2D6, and CYP2E1 (151). Therefore, interactions are likely to occur with drugs that are metabolized via the CYP3A4 isoenzyme system. Because of the investigational status of posaconazole, there are limited data on drug interactions with this agent (Table 8) (152–157). It appears that there is an interaction with cyclosporine, although the magnitude of this interaction is not as great as that seen with
Table 8 Clinically Relevant Pharmacokinetic Drug Interactions with Posaconazole (POS) (152–157)

<table>
<thead>
<tr>
<th>Drug (D)</th>
<th>Data type</th>
<th>Proposed mechanism of interaction by Posaconazole (P) or (D)</th>
<th>Clinical effect (potential or actual)</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcineurin inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine (152)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by P</td>
<td>↑ CyA C_{min} → ↑ toxicity</td>
<td>Monitor levels. ↓ CyA dose (0-29%)</td>
</tr>
<tr>
<td>Tacrolimus (153)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by P</td>
<td>↑ AUC, 469% → toxicity</td>
<td>Monitor levels, ↓ tacrolimus dose (at least 50%)</td>
</tr>
<tr>
<td>Cimetidine (154)</td>
<td>PK study</td>
<td>CYP3A4 induction by D</td>
<td>↑ Posaconazole C_{max}, AUC</td>
<td>Increase posaconazole dose</td>
</tr>
<tr>
<td>Glipizide (155)</td>
<td>PK study</td>
<td>CYP3A4 inhibition by P</td>
<td>↓ 15% mean plasma glucose</td>
<td>Monitor blood glucose closely</td>
</tr>
<tr>
<td>Phenytoin (156)</td>
<td>PK study</td>
<td>CYP3A4 induction by D</td>
<td>↑ Posaconazole CL by 90%</td>
<td>Avoid</td>
</tr>
<tr>
<td>Rifabutin (157)</td>
<td>PK study</td>
<td>CYP3A4 induction by D</td>
<td>↑ Posaconazole C_{max}, AUC</td>
<td>Avoid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CYP3A4 inhibition by P</td>
<td>↑ Rifabutin C_{max}, AUC</td>
<td></td>
</tr>
</tbody>
</table>

[ ], concentration; ↑ increased; →, leads to; ↓, decreased; AUC, area under the curve; C_{min}, trough concentration; C_{max}, peak concentration; CL, clearance; t_{1/2}, half-life; PK, pharmacokinetic; CYP3A4, cytochrome P4503A4 isoenzyme.
voriconazole and itraconazole. In a limited number of cardiac transplant candidates, the dose reductions of cyclosporine ranged from 0% to 29% (152). Like the other azoles, the magnitude of the drug interaction with the various calcineurin inhibitors is not consistent within the class. Posaconazole has been shown to interact with tacrolimus, and the magnitude of this interaction is greater than the interaction with cyclosporine. In a healthy volunteer study, the concomitant administration of posaconazole and tacrolimus resulted in a 469% increase in the AUC and a 221% increase in the $C_{\text{max}}$ concentration (153). Clearance of tacrolimus decreased 5-fold, and there was an extension in the half-life of tacrolimus to 7.5 hr (153). While no specific dosage recommendations have been made, and since we do not have the relative $C_{\text{min}}$ concentrations, it is clear that a dose reduction of tacrolimus will be necessary, most likely in the vicinity of 50%.

The hepatic microsomal enzyme inducer, rifabutin, has been shown to increase the clearance of posaconazole two-fold, resulting in a 57% reduction in the $C_{\text{max}}$ and a 51% reduction in exposure (AUC) (157). When rifabutin and posaconazole are coadministered there is also an increase in the PK parameters of rifabutin. Plasma rifabutin $C_{\text{max}}$ and AUC are increased 31% and 72%, respectively (157). Phenytoin also reduces the bioavailability of posaconazole 2–3-fold, however, there is no effect of posaconazole on serum phenytoin concentration (156). Both phenytoin and rifabutin should be avoided in patients receiving posaconazole.

Other drugs that have been studied in combination with posaconazole include the H$_2$-antagonist cimetidine, as well as the antiretroviral agents zidovudine/lamivudine and indinavir. Concomitant administration of posaconazole and cimetidine results in a 40% reduction in the $C_{\text{max}}$ and AUC of posaconazole compared to posaconazole alone (154). The mechanism by which this interaction takes place is not clear. A similar study evaluating the effect of antacids on the PK of posaconazole did not demonstrate an effect of antacid on $C_{\text{max}}$ or AUC of posaconazole thus, suggesting that the cause of the interaction is not pH mediated (158). Similarly, administration of posaconazole with zidovudine/lamivudine and indinavir in HIV infected patients does not lead to any significant interaction and no dosage modification is required.

6. Ravuconazole

Ravuconazole is also a broad-spectrum triazole, currently under investigation, and as a consequence there are limited data on the drug interaction profile of this agent. Pharmacokinetic data are available evaluating the interaction between ravuconazole and nelfinavir (159). There were no significant changes in the $C_{\text{max}}$ and AUC of nelfinavir when both drugs were administered together compared to one drug alone, thus, it is safe for these to be coadministered. There are also data evaluating the drug interaction between ravuconazole and simvastatin in healthy subjects. Concomitant administration of these agents lead to an initial doubling of the AUC and $C_{\text{max}}$, and over repeated dosing (14 days) the $C_{\text{max}}$ increased 4-fold and the AUC increased 4-fold (160). While an interaction does occur, it does not appear to be as great as the interaction seen with other azoles and the HMG CoA reductase inhibitors. As this drug moves further along the investigational pipeline, we can anticipate more PK data.

C. Echinocandins

1. Caspofungin

Caspofungin, the first echinocandin approved by the Food and Drug Administration (FDA), is used widely in the treatment of *Aspergillus* fungal infections in immunocompromised patients (161,162). The echinocandin class of agents is unique in that
they are subject to few drug interactions. Caspofungin is neither a substrate nor an inhibitor of the cytochrome P450 enzyme system. PK studies demonstrate that caspofungin is not affected by the administration of itraconazole (163), amphotericin B (164), or mycophenolate mofetil (164), nor does it affect the pharmacokinetics of these target drugs. Caspofungin has been shown to decrease the peak plasma concentration, the 12-hr trough concentration, and AUC of tacrolimus when these two agents are concomitantly administered (165), and a corresponding increase in the dose of tacrolimus may be required. Close monitoring of tacrolimus concentrations should occur.

The interaction noted with tacrolimus is not a calcineurin inhibitor class effect, as a completely different interaction occurs when cyclosporine and caspofungin are administered concomitantly. Cyclosporine increases the AUC of caspofungin by 35% whereas cyclosporine concentrations are unaffected by caspofungin (164). In a healthy volunteer study, concomitant administration of cyclosporine and caspofungin resulted in significant elevations in liver function tests. In the first cohort of this study, healthy volunteers received caspofungin 70 mg intravenously once daily from day one to ten. Cyclosporine 3 mg/kg per dose was administered twice on day 10, each dose 12 hr apart. On day 11, transiently elevated alanine aminotransferase (ALT), approximately 2–3 times the upper limit of normal was noted in three out of four (75%) patients (164). A separate group of patients on the same study received caspofungin at a lower dose of 35 mg intravenously daily on day 1–3. Cyclosporine was administered at the same dose as the first cohort on day 1. On day 2, two out of eight patients (25%) had small increases in ALT, only slightly above the upper limit of normal (164). Based on these results, concomitant administration of caspofungin with cyclosporine is not recommended unless the potential benefit outweighs the risks. Until further information is available, the alternative is to change cyclosporine to tacrolimus.

Concomitant administration of caspofungin with inducers of drug clearance or mixed inducers/inhibitors may result in clinically meaningful reductions in serum caspofungin concentrations. Based on regression analyses of PK data, consideration should be given to increasing the daily dose of caspofungin to 70 mg when coadministering efavirenz, nelfinavir, nevirapine, phenytoin, rifampin, dexamethasone, or carbamazepine (164).

2. Micafungin

Micafungin, formerly known as FK463, is a new echinocandin antifungal that is pending final FDA approval. Micafungin differs from caspofungin in that it is not subject to the same drug interaction with cyclosporine. In the recently completed trial comparing fluconazole to micafungin in the prophylaxis of fungal infection in HSCT recipient’s (166), approximately half of the 882 patients enrolled received concomitant micafungin and cyclosporine. No elevations in cyclosporine levels were seen. Similarly, no increases in liver function tests occurred, including AST, ALT, and bilirubin over time, which included baseline assessments, evaluation at week 1, 2, 3, and 4, as well as end of therapy assessments. These data have been confirmed in a formal PK study where healthy volunteers received a single dose of cyclosporine (5 mg/kg) on study days 1, 5, and 9, and a single infusion of micafungin on days 11–15. PK data demonstrated no significant interaction effects of single or multiple doses of micafungin on cyclosporine, and similarly no PK interaction effect of single dose cyclosporine on micafungin (167). Based on these data, micafungin would be
the preferred echinocandin in patients requiring concomitant cyclosporine, once final FDA approval is granted. The effect of micafungin on the PK of tacrolimus, and the effects of tacrolimus on the PK of micafungin have also been evaluated in healthy volunteers. A similar result was seen with no drug–drug interaction effects on single-dose tacrolimus PK, and single dose tacrolimus exposure did not alter the PK of micafungin (168).

3. Anidulafungin (LY303366, V-echinocandin, VER-002)

Anidulafungin is an experimental echinocandin being evaluated for its activity against Candida and Aspergillus spp., infections. Because of the investigational nature of the drug, there is limited information on drug interactions. In preclinical murine models, coadministration of anidulafungin with glucocorticoids resulted in lethal toxicity. Deaths occurred in the DBA/2 mice treated with cortisone acetate prior to intranasal inoculation with Aspergillus, as well as the mice treated as above but with anidulafungin. Studies were also conducted with other corticosteroids, including hydrocortisone and triamcinolone, with similar outcomes. The use of dexamethasone did not result in the death of any mice. The rationale for the lethal effects of anidulafungin and corticosteroids remains unknown (169).

The effect of cyclosporine on the PK profile of anidulafungin has been evaluated. Healthy volunteers were administered anidulafungin 200 mg IV on day 1, followed by 100 mg/day on days 2–8. Oral cyclosporine solution was administered on days 5–8, at a dose of 1.25 mg/kg twice a day. The PK profile of cyclosporine A was characterized on day 4 (absence of cyclosporine) and on day 8 (presence of anidulafungin). The changes in the $C_{\text{max}}$ and AUC of anidulafungin, both in the presence and absence of cyclosporine, were clinically insignificant (170). To assess the effect of anidulafungin on cyclosporine metabolism, a small in vitro study assessed different doses of anidulafungin in combination with cyclosporine in human hepatic protein in vitro. This study concluded that anidulafungin at concentrations of up to 30 μg/mL does not affect the in vitro metabolism of cyclosporine (171). Further clinical data is necessary before conclusive recommendations can be made.

D. Miscellaneous

1. Terbinafine

Terbinafine, an allylamine derivative, is structurally unrelated to other available antifungal agents. It is a potent competitive inhibitor of CYP2D6, but at least six other CYP450 enzymes are involved in its metabolism, including CYP1A2, CYP2C9, and CYP3A4 (172). Terbinafine is weakly bound to hepatic cytochromes with its metabolism involving only 5% of the metabolizing capacity of hepatic CYP450 enzymes (173). In recent years, attention has been paid to the inhibition of CYP2D6, the isoenzyme system responsible for the metabolism of a wide variety of medications including anti depressants, neuroleptics, antihypertensives, opioids, and antiarrrhythmics (174). When prescribing warfarin (175), theophylline, nortriptyline (176), desipramine (177), cimetidine, or rifampin concomitantly with terbinafine, the prescriber must consider the potential for toxicity or lack of effect to occur. Unlike the azole antifungal agents, terbinafine can be coadministered with cyclosporine without any significant changes in cyclosporine concentrations (178).
5-Flucytosine (5-FC)

5-Flucytosine, first synthesized in 1957, is an antimetabolite antifungal agent whose spectrum of activity includes *Candida* spp., and *Cryptococcus neoformans*. 5-FC is not subject to the same interactions as the azoles and interacts with relatively few agents. When administered with cytosine arabinoside, there is a reduction in its antymycotic activity, most likely due to competitive inhibition. Inhibition is thought to result from 5-FC being taken up by susceptible cells by the same transport system as cytarabine (179). Administration of 5-FC in close proximity to antacids containing aluminum hydroxide or magnesium hydroxide results in delayed absorption, although this is clinically insignificant.

Pharmacodynamically, there is a concern that 5-FC may enhance the marrow suppressive effects of other medications that are marrow suppressive. As a consequence, caution should be exercised when prescribing this agent with medications such as zidovudine, ganciclovir, etc.

Griseofulvin

Griseofulvin is primarily restricted to the treatment of topical fungal infections. There are a couple of noteworthy drug interactions that occur, and patients should be monitored closely when concomitantly prescribing warfarin, oral contraceptives, and phenobarbital. Concomitant administration of griseofulvin and warfarin leads to a diminished anticoagulant effect (180–182). Repeated administration of griseofulvin induces the enzymes that metabolize warfarin, whereas a single dose of griseofulvin has been reported to increase the international normalized ratio and increase serum warfarin concentration (183). With prolonged treatment, the dose of warfarin required for therapeutic anticoagulation is likely to increase, and similarly, close monitoring of the INR on withdrawal of griseofulvin should occur.

The other notable drug interaction is between griseofulvin and oral contraceptive medications (184). Griseofulvin induces the metabolism of estrogens, both endogenous and exogenous, thus, leading to loss of oral contraceptive efficacy. Patients should be informed of the interaction and counseled to use alternative contraceptive measures while receiving griseofulvin.

Finally, griseofulvin has been shown to have its concentrations affected by phenobarbital. Whether this occurs by decreased absorption, or by enzyme induction is not clear, but concomitant griseofulvin and phenobarbital leads to a reduction in griseofulvin concentrations and may lead to antifungal failure.

Antifungal Combinations

The recent approval of caspofungin, an echinocandin whose target is β 1,3 glucan, and voriconazole, a second-generation triazole with an extended spectrum of action, has encouraged the infectious disease community caring for immunocompromised patients to assess the efficacy of combination antifungal therapy. This approach is similar to that of antineoplastic therapy, where using two drugs with different mechanisms of action to improve outcomes. Historically, this has not been possible mostly due to the limited antifungal armamentarium. Up until 2001, we only had the polyenes, first-generation azoles, terbinafine, and flucytosine available. Many institutions used combinations of itraconazole and amphotericin B in patients with *Aspergillus* infections who were not responding to monotherapy. The fear with this approach was that there could be antagonism between the drugs based on their
mechanisms of action, leading to a worse outcome (see azole section in this chapter). In a candidiasis model, this has not occurred (14). Whether this is an agent specific interaction requires clarification. With the availability of new classes of antifungal agents, there seems little reason to pursue the azoles in combination with a polyene with this controversy, and combinations of azoles and echinocandins, or polyenes and echinocandins make greater theoretical sense.

Researchers involved in mycology and the treatment of high-risk patients with invasive fungal infections are now evaluating several antifungal combinations for synergy, indifference, and antagonism both in vitro and in animal models (185–194). The clinical relevance of these combinations remains untested in large prospective randomized trials, although observational data in limited patients does exist (195,196). Hopefully, the most promising laboratory combination identified will be pursued in a large clinical trial to definitively conclude that this is an effective strategy. At least from a pharmacokinetic standpoint, there does not appear to be any interaction, and preliminary “observational” clinical data do not suggest any negative effects.

III. CONCLUSION

Drug interactions are common among critical care, HIV, and transplant patients due to the large number of medications and the complexity of prescribed regimens. It is important that the medical teams caring for these populations are aware of the potential for drug interactions, and screen all new medications against a full history to attempt the decrease of the probability of interaction occurring. With the availability of new medications each year, it is clear that we must ensure and understand the metabolic routes of these drugs, and in the absence of any substantive data, make out “best guess” as to the possibility of a potential drug interaction. Knowledge of the dominant CYPs involved in the metabolism of a drug makes it possible to anticipate from a list of inducers and inhibitors which drugs might cause significant interactions.

REFERENCES


Clinically Relevant Drug Interactions

122. Leather HL, Wingard JR. Characterizing the pharmacokinetic (PK) drug interaction between intravenous (IV) itraconazole (ITRA) and IV tacrolimus (FK506) or IV cyclosporine (CyA) in allogeneic bone marrow transplant (alloBMT) patients. Program and Abstracts of the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, IL, 16–19.


I. INTRODUCTION

A. Invasive Fungal Infections in Immunocompromised Patients

During the last two decades invasive fungal infections (IFIs) have emerged as important medical problems, particularly among immunocompromised patients. This trend is alarming problems, IFIs are associated with significant morbidity and high mortality, which in some cases may exceed 90% (1,2). While endemic fungi may also cause infections in immunocompromised patients in certain areas of the world, opportunistic fungi are by far the most important causes of IFIs in these patients worldwide. While infections due to *Candida* spp. remain an important problem in immunocompromised patients, infections due to filamentous fungi have become progressively more frequent and retain their extremely high mortality in susceptible patients (2,3). It is especially against these refractory infections that host defenses and immune reconstitution are very important in order to improve their dismal outcome.

B. Settings of IFIs

The underlying immunological status is the major contributor to host defense against opportunistic fungal infections. These infections occur when host defense mechanisms are absent or dysfunctional. Since innate host defense against fungi is primarily based on the antifungal activity of phagocytes, patients with deficient number or function of phagocytes are at increased risk of IFIs. Such deficiencies occur in a variety of patients (Table 1) including those with cancer and chemotherapy-related neutropenia, transplant recipients with neutropenia or receiving corticosteroids or other immunosuppressants (3–5), and other functional deficiencies of phagocytes, most importantly chronic granulomatous disease (CGD) (6). Therefore, immunotherapy aimed at augmenting host defense mechanisms may improve outcome in these patients (7).
II. OVERVIEW OF ANTIFUNGAL HOST DEFENSES

A. Innate Host Defense

The human host defense against opportunistic fungi is divided into innate and adaptive immunity. The innate immunity is served mainly by phagocytes, such as tissue macrophages, circulating monocytes (MNCs), and neutrophils (PMNs) and does not involve T-cell memory (Table 2). Its great importance in the defense against IFIs due to opportunistic pathogens is demonstrated by their high incidence in patients with deficient macrophage (i.e., corticosteroids) or PMN (i.e., neutropenia) host defense systems. Adaptive immunity appears to be less important against opportunistic fungi in immunocompromised patients.

Table 1  Diseases and Conditions Predisposing to Development of IFIs

<table>
<thead>
<tr>
<th>Diseases and Conditions Predisposing to Development of IFIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-dose chemotherapy-associated neutropenia</td>
</tr>
<tr>
<td>High-dose corticosteroid therapy</td>
</tr>
<tr>
<td>Hematological disorders (main risk factors: persistent and profound neutropenia, use of broad-spectrum antibacterial agents, indwelling central venous catheters, and damage of normal host barriers following intensive cytotoxic chemotherapy)</td>
</tr>
<tr>
<td>Hospitalization in surgical, neonatal, and intensive care units: extensive burn injury and extensive surgery operations (main risk factors: use of broad-spectrum antibacterial agents, prematurity, indwelling central venous catheters, and damage of normal host barriers)</td>
</tr>
<tr>
<td>Transplantation [bone marrow transplantation (BMT), peripheral blood stem cell transplantation (PBSCT), and solid organ transplantation]</td>
</tr>
<tr>
<td>Acquired immunodeficiency syndrome (AIDS) (main risk factors: neutropenia and high-dose corticosteroid therapy)</td>
</tr>
<tr>
<td>Chronic granulomatous disease (CGD)</td>
</tr>
</tbody>
</table>

Table 2  Innate Immune Response to Specific Groups of Fungi

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Innate Immune Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin and gut fungi</td>
<td></td>
</tr>
<tr>
<td>(mainly Candida albicans and other Candida spp.)</td>
<td>Macrophages (peritoneal, Kupffer cells, spleen adherent cells, etc.) phagocytose and kill blastoconidia intracellularly by oxidative and non-oxidative mechanisms, produce monokines (i.e., TNF-α)</td>
</tr>
<tr>
<td></td>
<td>Neutrophils (bloodstream circulating and in tissues) ingest and kill blastoconidia intracellularly, damage pseudohyphae and hyphae extracellularly by oxidative burst, and non-oxidative mechanisms</td>
</tr>
<tr>
<td></td>
<td>T lymphocytes produce lymphokines (i.e., IL-18, IFN-γ)</td>
</tr>
<tr>
<td>Airborne fungi (i.e., Aspergillus spp., Zygomycetes, Fusarium spp., Scedosporium spp.)</td>
<td>Pulmonary alveolar macrophages ingest and kill conidia intracellularly by oxidative and non-oxidative mechanisms, produce monokines (i.e., TNF-α)</td>
</tr>
<tr>
<td></td>
<td>Neutrophils damage escaping hyphae extracellularly by oxidative burst and non-oxidative mechanisms</td>
</tr>
<tr>
<td></td>
<td>T lymphocytes produce lymphokines (i.e., IL-18, IFN-γ)</td>
</tr>
<tr>
<td>Cryptococcus neoformans (and Trichosporon spp.)</td>
<td>Polysaccharide-specific antibody mediated response, phagocytosis by macrophages and neutrophils, and inhibition of immune functions</td>
</tr>
</tbody>
</table>

Source: Modified from Ref. 8.
B. Phagocytes

Depending on the fungus and the route of acquisition, specific macrophage-type phagocytes recognize particular ligands on the surface of fungi, ingest them, become activated, and destroy the intracellular forms of fungi. The roles of oxygen-dependent intermediates as well as antimicrobial peptides and other non-oxygen dependent metabolites are very important for intracellular killing (9–12). When the fungal conidia escape and germinate to hyphae, macrophages cannot handle them. Circulating PMNs and MNCs are attracted by the action of a number of cytokines and chemokines that are released at the site of infection, attach to the surface of the hyphae and inflict damage by mainly oxygen-dependent antifungal intermediates (reviewed in Refs. 9 and 13).

C. Hematopoietic Growth Factors (HGFs) and Other Cytokines

A number of hematopoietic growth factors (HGFs) and other cytokines are capable to act on immature or end-stage phagocytes and either increase their number (HGFs) or modulate their antifungal function (HGFs and cytokines). The most clinically relevant HGFs acting on phagocytes are granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and macrophage colony-stimulating factor (M-CSF) (Table 3). Cytokines that affect the function of phagocytes against fungi include Th1 cytokines, such as interferon-γ (IFN-γ), interleukin (IL) -12, IL-15, and tumor necrosis factor-α (TNF-α). Additionally, cytokines of Th2 pattern, such as IL-4 and IL-10, exert an overall suppressive effect on antifungal function of phagocytes (14).

III. RATIONALE OF ADJUNCTIVE IMMUNOTHERAPY

Despite the development of new potent and less toxic antifungal agents such as lipid formulations of amphotericin B, second generation of triazoles, and echinocandins, the mortality of IFIs in immunocompromised patients is still unacceptable reaching the frequency of 90% in some fungal syndromes (1,15). A critical factor for the increased frequency of IFIs and their resistance to therapy nowadays is the profound compromise of the immune system that is created by potent immunosuppressive therapies and the prolonged survival of patients in such profound immunocompromised state. Therefore, alternative or adjunctive therapeutic approaches have been investigated for the management of IFIs that are still a challenge for clinicians.

Table 3  Clinically Relevant Hematopoietic Growth Factors and Cytokines with Potential Utility as Adjunctive Antifungal Therapy

<table>
<thead>
<tr>
<th>Hematopoietic Growth Factors</th>
<th>Cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocyte colony-stimulating factor (G-CSF)</td>
<td>Interferon-γ (IFN-γ)</td>
</tr>
<tr>
<td>Granulocyte-macrophage colony-stimulating factor (GM-CSF)</td>
<td>Tumor necrosis factor-α (TNF-α)</td>
</tr>
<tr>
<td>Macrophage colony-stimulating factor (M-CSF)</td>
<td>Interleukin (IL)-12</td>
</tr>
<tr>
<td></td>
<td>Interleukin (IL)-15</td>
</tr>
</tbody>
</table>
Because of the importance of intact innate immune response to the fight against IFIs, and thanks to advances in understanding pathogenesis of IFIs as well as the availability of recombinant cytokines, immunotherapeutic approaches have become very appealing. These can be either reconstitution of effector cells numerically and/or functionally with cytokines and/or white blood cell transfusions (WBCTx), or manipulation of cytokine dysbalance (16).

IV. PRECLINICAL BASIS OF IMMUNOTHERAPY

A. In Vitro and In Vivo Laboratory Investigations

Many preclinical studies have shown that certain HGFs and cytokines augment the antifungal host defense by increasing the number and/or enhancing the function of phagocytes. The most important results of these studies have extensively been reviewed elsewhere (17) and are briefly summarized in this chapter. Data from experimental animal models have suggested the utility of cytokines prophylactically or as adjunctive therapy in combination with conventional antifungal chemotherapy in the setting of certain IFIs (18–22) (Table 4).

The G-CSF acts upon PMNs promoting their maturation and increasing their number in peripheral blood (43,44). The G-CSF enhances the antimicrobial activity of PMNs against a wide variety of bacteria (45). It also regulates the function of intact PMNs against fungi including *C. albicans* and *A. fumigatus* hyphae, mainly by enhancement of PMN oxidative burst (46–48). This enhancement occurs even in cases of corticosteroid-suppressed PMNs (49). In various animal models of infection, therapeutic administration of G-CSF has been shown to enhance pathogen eradication and to decrease morbidity and/or mortality (50). The G-CSF enhances host resistance to disseminated candidiasis in non-neutropenic mice through activation of PMNs and their recruitment to the site of infection (23) and also has a protective effect on bacterial and fungal infections in neutropenic mice (51). In addition, it can induce dendritic cells with a Th2 response profile (52) and, accordingly, may down-regulate the proinflammatory response having a more favorable benefit-toxicity ratio against infections for which PMNs are the predominant effector cells. The antifungal effects of G-CSF in vitro and in animal models of IFIs are reviewed in an excellent recent paper (50).

The PMNs from patients treated with G-CSF have been found to possess enhanced activity not only against *A. fumigatus* but also against *Rhizopus arrhizus*, *C. albicans* (48), and *Candida neoformans* (53). The last two fungi were killed even by PMNs from HIV-infected patients who had received G-CSF (5 μg/kg), a finding suggesting that this cytokine restores the suppressed function (53).

The effect of G-CSF also has been studied in vitro in combination with fluconazole and voriconazole where an additive effect has been found against *Rhizopus arrhizus*, *C. albicans* (48), and *Candida neoformans* (53). The last two fungi were killed even by PMNs from HIV-infected patients who had received G-CSF (5 μg/kg), a finding suggesting that this cytokine restores the suppressed function (53).

The GM-CSF promotes the differentiation and proliferation of mononuclear cells as well as PMNs (50,56). Although it is biologically similar to G-CSF, GM-CSF acts on progenitor cells capable of differentiating into both granulocytic and monocytic lineages. Consequently, it can increase the numbers of PMNs,
**Table 4 Effects of Cytokines in Animal Models of Candidiasis and Aspergillosis**

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Animal Model</th>
<th>Organism</th>
<th>Antifungal Rx</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSF</td>
<td>Immunocompetent mice</td>
<td><em>C. albicans</em></td>
<td>Fluconazole</td>
<td>Additive effect, ↑ survival, and renal clearance (24)</td>
</tr>
<tr>
<td></td>
<td>Immunocompetent and neutropenic mice</td>
<td><em>C. albicans</em></td>
<td>Fluconazole</td>
<td>Additive effect, ↑ survival, and renal clearance (24)</td>
</tr>
<tr>
<td></td>
<td>Neutropenic mice</td>
<td><em>C. albicans</em></td>
<td>Alone</td>
<td>Protective (19) or no effect (23)</td>
</tr>
<tr>
<td></td>
<td>Neutropenic mice</td>
<td><em>C. albicans</em></td>
<td>± Amphotericin B</td>
<td>↑ Survival (25)</td>
</tr>
<tr>
<td></td>
<td>Neutropenic mice</td>
<td><em>C. albicans</em></td>
<td></td>
<td>Protective effect</td>
</tr>
<tr>
<td></td>
<td>Neutropenic mice</td>
<td><em>Aspergillus fumigatus</em></td>
<td>Posaconazole</td>
<td>Additive effect (20)</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Neutropenic mice</td>
<td><em>C. albicans</em></td>
<td>+ Fluconazole + Amphotericin B</td>
<td>↑ Survival (30)</td>
</tr>
<tr>
<td>M-CSF</td>
<td>Immunocompetent mice</td>
<td><em>C. albicans</em></td>
<td>Fluconazole</td>
<td>Improved survival (29)</td>
</tr>
<tr>
<td></td>
<td>Immunocompetent rats</td>
<td><em>C. albicans</em></td>
<td>Fluconazole</td>
<td>Improved survival (29)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Neutropenic rabbits</td>
<td><em>A. fumigatus</em></td>
<td></td>
<td>↑ Survival</td>
</tr>
<tr>
<td>IFN-γ, TNF-α, GM-CSF</td>
<td>Neutropenic mice</td>
<td>Paracoccidioides brasiliensis</td>
<td>Posaconazole</td>
<td>Protective effect major mediator of resistance against paracoccidioidomycosis (33)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Immunocompetent mice</td>
<td><em>C. albicans</em></td>
<td></td>
<td>↑ Resistance (34)</td>
</tr>
<tr>
<td>TNF-α, GM-CSF</td>
<td>Immunocompetent mice</td>
<td><em>A. fumigatus</em></td>
<td></td>
<td>Role in recruitment of neutrophils (↑ fungal clearance) (35)</td>
</tr>
</tbody>
</table>

(Continued)
 eosinophils, and MNCs. The GM-CSF enhances antifungal activities of intact PMNs and/or MNCs against \textit{C. albicans} (57), \textit{A. fumigatus} (58), and other less frequently isolated fungi (59). Its effect has been tested in vitro in combination with voriconazole and PMNs and an additive effect, similar with G-CSF, has been found against \textit{A. fumigatus} (55). Such an effect has not, however, been seen with GM-CSF, voriconazole, and MNCs suggesting that there are differences in the way that phagocytes interact with antifungal drugs under cytokine-induced activation.

The GM-CSF also primes macrophages to release mediators of inflammation such as IL-1 and TNF-\(\alpha\) (56) that can overcome dexamethasone-mediated suppression of antifungal monocytic activity against \textit{Aspergillus} (60). Some forms of GM-CSF, particularly non-glycosylated preparations, have been associated with toxicity that may reflect its ability to increase proinflammatory mediators and there has also been a theoretical concern that GM-CSF therapy could hinder PMNs migration to sites of infection (16). Indeed, a deleterious GM-CSF-mediated proinflammatory response may have resulted in worsened outcome in an animal model of disseminated trichosporonosis (61).

The M-CSF promotes the differentiation, proliferation, and activation of MNCs and macrophages. It enhances the antifungal activity of MNCs and macrophages against \textit{Candida} spp. (62), \textit{A. fumigatus} (63), and other fungi (59). Animal studies have revealed complex effects with regard to the immunomodulatory activity of M-CSF (29,64). In a rabbit model of invasive aspergillosis, administration of

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Cytokines & Animal Model & Organism & Antifungal Rx & Outcome \\
\hline
TNF-\(\alpha\) agonist & Neutropenic mice & \textit{A. fumigatus} & & \uparrow Survival (22) \\
IL-1 & Neutropenic mice & \textit{C. albicans} & & \uparrow survival (36) \\
& Neutropenic mice & \textit{C. albicans} & Fluconazole & \downarrow The number of \textit{Candida} in organs (37) \\
IL-4 & Immunocompetent mice & \textit{C. albicans} & \textit{A. fumigatus} & \downarrow Survival (38) \\
& & & & \downarrow Survival IL-4-deficiency protected (39) \\
IL-10 & Immunocompetent mice & \textit{C. albicans} & \textit{A. fumigatus} & \downarrow Survival (38) \\
& & & & \downarrow IL-10-neutralization protected (40) \\
& & & & \downarrow IL-10-deficiency protected (41) \\
& & & & \downarrow Survival IL-10-deficiency protected (41) \\
IL-12 & Neutropenic mice & \textit{P. brasiliensis} & & Protects against disseminated infection but enhances pulmonary inflammation (42) \\
\hline
\end{tabular}
\caption{Effects of Cytokines in Animal Models of Candidiasis and Aspergillosis (Continued)}
\end{table}
M-CSF augmented pulmonary host defense against *A. fumigatus* suggesting potential role for this cytokine as adjunctive therapy in the treatment of pulmonary aspergillosis in the setting of profound neutropenia (31). In a murine model of candidiasis, M-CSF synergized with amphotericin B but not with fluconazole for an improved outcome of infection as this was documented by decreased mortality in the animals (30).

The IFN-γ is a potent activator of both PMN and macrophage function (65) that can enhance the phagocytic activity against a number of fungal pathogens. Depending on the experimental conditions, IFN-γ has been shown to have an enhancing effect (66) or no effect (67) on fungicidal activities of human or murine PMNs against *C. albicans* blastoconidia. While dexamethasone suppresses the fungicidal activity of human MNCs against *A. fumigatus* evidenced as oxidative burst in response to hyphae and as hyphal damage, IFN-γ is able to restore these activities (60). An up-regulatory role of IFN-γ on both PMNs and MNCs against *Aspergillus* hyphae has been shown as well as an additive effect of the combination of IFN-γ and G-CSF at high concentrations (46,58). Compared to G-CSF and GM-CSF, IFN-γ has been shown to be a very potent activator of phagocytes against opportunistic fungal pathogens (68). Synergy between IFN-γ and antifungal agents against *Candida* spp., *A. fumigatus*, *C. neoformans*, *P. brasiliensis*, and *Blastomyces dermatitidis* has been demonstrated in vitro by use of macrophages (56) and in vivo for experimental cryptococcosis (69).

The IL-12 plays a key role in promoting Thl responses and subsequent cell mediated immunity (70). It is produced primarily by antigen presenting cells. Its major biologic function is to enhance proliferation and cytolytic activity of natural killer (NK) and T cells, and stimulate their IFN-γ production (71). Thl type cellular responses are essential for protection against fungal pathogens, including *Candida* spp., *Aspergillus* spp., and *C. neoformans* (72). The IL-12 has activity in experimental murine cryptococcosis (73), histoplasmosis (74), coccidioidomycosis (75), and early in the course of aspergillosis (76). In addition, it can enhance fluconazole’s efficacy against *Candida* infections in mice with neutropenia (77). Thus, IL-12 may be useful adjunctive therapy against various IFIs in the setting of neutropenia. However, IL-12 may be detrimental in hosts that are not neutropenic because it can induce an excessive inflammatory response (78). Adjustment of dosage of IL-12 may ameliorate these adverse effects.

The IL-15 has similar biologic properties in vitro with IL-2, consistent with their shared receptor signaling components (IL-2/15Rc). However, specificity for IL-15 vs. IL-2 also exists (79). Both IL-15 and IL-15R transcripts have a much broader tissue distribution than IL-2/IL-2R. Studies to date examining the biology of IL-15 have identified several key roles, such as IL-15’s importance during NK and T-cell development and function. The IL-15 has important activity in host defense against fungi. It has been shown to enhance oxidative burst and antifungal activities of PMNs and MNCs including the abilities to ingest and inhibit growth of *C. albicans* (80,81). Similarly, it enhances PMN-induced hyphal damage in a number of filamentous fungi including *A. fumigatus*, *Scedosporium prolificans*, and *Fusarium* spp., but not *Aspergillus flavus* or *Scedosporium apiospermum* (Pseudallescheria boydii) (82). Similar to IFN-γ, IL-15 may be a candidate for adjunctive immunotherapy in cases of Th1/Th2 dysregulation.

The TNF-α is a potent immunoenhancing cytokine augmenting the production of other cytokines, such as GM-CSF as well as enhancing several PMN functions, mainly by increasing O₂⁻ and H₂O₂ release (83). This cytokine has a protective role on systemic infections by *C. albicans* (84). The TNF-α stimulates PMNs to damage
hyphae, enhances pulmonary alveolar macrophage (PAM) phagocytosis of conidia, augments PMN oxidative respiratory burst, and the degranulation induced by opsonized fungi (83). Fungicidal activity of PMNs against blastoconidia of *C. albicans* and *Candida glabrata* also has been shown to be enhanced (66,85), whereas the results with pseudohyphae of *C. albicans* have been equivocal (86). Underscoring its critical role in host response to pathogenic fungi, suppression of TNF-α by infliximab (anti-TNF-α antibody) may result in invasive aspergillosis (87,88).

The IL-4 has anti-inflammatory properties and primarily exerts a suppressive effect on immune cells. It has been shown to suppress the oxidative burst of MNCs and the killing of *C. albicans* blastoconidia (89). In the case of *A. fumigatus*, IL-4 significantly suppresses MNC-induced damage of hyphae, but it does not alter phagocytic activity or inhibition of conidial germination. In murine models of candidiasis and aspergillosis, IL-4 had detrimental effects (39,90) and its inhibition improved outcome. Its administration to patients with renal carcinoma, however, did not induce serious infections (91).

The IL-10 affects MNCs by suppressing oxidative burst and antifungal activity against *A. fumigatus* hyphae. The IFN-γ and GM-CSF may counteract suppressive effects of IL-10 (92). The IL-10 also affects PMN function against *C. albicans* by suppressing phagocytosis of blastoconidia and by reducing PMN-induced damage of *C. albicans* pseudohyphae (93). In murine models of candidiasis and aspergillosis, IL-10 had detrimental effects (40) and its inhibition improved outcome. Administration of IL-10 to patients with psoriasis or Crohn’s disease, however, did not result in serious infections probably because of production of IFN-γ that high doses of IL-10 may induce (94,95).

B. Combined Activity of Antifungal Drugs, Phagocytes, and Cytokines

Antifungal drugs such as conventional and lipid formulations of amphotericin B, triazoles, and echinocandins have been studied in combination with PMNs or other phagocytes against fungal pathogens (55,96). These studies aimed first to exclude any adverse effect of the antifungal drugs on the antifungal activity of phagocytes, and second to find whether a synergistic effect exists between the two components. In these studies, no adverse effects were documented with any of the antifungal drug classes studied. Amphotericin B formulations have been shown to exert overall additive antifungal effects (conidiocidal activity and hyphal damage) in combination with PAMs and PMNs against *A. fumigatus* (97). Similar combinational effects have been found with caspofungin and phagocytes against the same organism (98). Triazoles and PMNs are synergistic to cause increased hyphal damage to *S. prolificans* and *S. apiospermum*, two therapy-refractory fungi (99). Further, amphotericin B lipid complex (ABLC) exerts additive antifungal activity in combination with PMNs against the two *Scedosporium* spp. (100). The ABLC exerts additive antifungal activity in combination with PAMs against *Fusarium solani* (unpublished authors’ results). Taken all these data together, it appears that certain antifungal drugs, e.g., ABLC, have the capacity to be synergistic with the antifungal host response to an improved defense against the infection.

Beside the direct effects of cytokines on effector cells in inhibiting fungal growth, cytokines may collaborate with antifungal drugs in producing larger antifungal effects. As an example, when voriconazole was combined with GM-CSF or G-CSF treatment of PMNs against *A. fumigatus* hyphae, growth inhibition was
significantly increased compared to growth inhibition due to unstimulated PMNs (101). Similar phenomena were observed when voriconazole was combined with IFN-\(\gamma\) treated PMNs (102). However, such collaboration between GM-CSF and voriconazole in inhibiting \(A. fumigatus\) hyphal growth was not observed when MNCs were used.

This antifungal–cytokine collaboration may occur through the fungi, through the effector cells, or through both of them (Fig. 1). Antifungal drugs such as polyenes and azoles, which alter the fungal membrane, or echinocandins, which damage the cell wall, may render fungi more susceptible to oxidative and non-oxidative products. Furthermore, antifungal drugs may have direct immunomodulatory activity on phagocytes as it was found for amphotericin B and voriconazole, which enhanced the conidiocidal and antihyphal activity of PAMs and PMNs against \(A. fumigatus\) (97,101). While it is known that amphotericin B may induce secretion of oxidative and non-oxidative metabolites and immunoenhancing cytokines, such as TNF-\(\gamma\) and IL-1\(\beta\), and may enhance phagocytosis of fungal spores, the immunomodulatory effects of azoles and echinocandins are not well understood. Cytokines may up-regulate host antifungal mechanisms (oxidative burst or antifungal peptides), which can interact with antifungal drugs, enhance penetration of antifungals into phagocytes, where the drugs may be more effective against ingested fungi, and restore defense mechanisms, which can be depressed by an antifungal drug.

### C. Cytokine Administration

Two patient populations are at high risk for IFIs: those with neutropenia and those with functional deficiencies of lymphocytes and phagocytes. The HGFs and other cytokines have a role in prophylaxis and as adjunctive treatment of IFIs in combination with antifungal drugs in both patient categories. However, due to limitations in clinical trial design and defining patient populations at risk, definitive data on the efficacy of recombinant HGFs and other cytokines have not been well-demonstrated.
1. Prevention of IFIs by Use of Cytokines in Neutropenic Patients

Malignancies and disease- or therapy-related neutropenia constitute the broadest field of acquired defects in host defenses and the greatest need for immune reconstitution. Thus, most of the studies have focused on this patient population. Although the cytokines have been extensively evaluated in preclinical studies, there is still controversy with regard to their utility in these patients. The main reason is that the number of IFIs as sequelae of immune compromise is relatively small, and no study has had the statistical power to demonstrate significant differences in the proportions of IFIs between the patients receiving or not receiving a HGF/cytokine prophylactically or empirically. Nevertheless, there are several case reports and small, uncontrolled studies that have been published suggesting the potential beneficial effects of immunotherapy. Unfortunately, the conclusions of all these studies are limited by the caveats characterizing uncontrolled studies and case reports. These include desperate use of cytokines only in the most seriously ill patients with grave prognosis, and investigators’ as well as publications’ bias towards improved outcome that make any definitive conclusion very difficult to be drawn. Thus, conclusive clinical data are still missing, making the issue of their use in the management of IFIs not definitive.

The clinical use of G-CSF and GM-CSF has been mainly based on the ability of either factor to abbreviate the depth and duration of neutropenia as well as to enhance the antifungal function of phagocytes (43,103,104). Both are clinically used in patients with neutropenia associated with chemotherapy and/or hematopoietic stem cell transplantation (HSCT), myelodysplastic syndromes, and aplastic anemia in order to promote bone marrow recovery (43). Both HGFs have assumed a central role in the supportive care of cancer, stem cell transplant, aplastic, and congenital neutropenic patients. Since susceptibility to IFIs is proportional to the duration and degree of neutropenia (105), the outcome of neutropenic patients with IFIs who receive a HGF is expected to be better.

The HGFs/cytokines have been administered at different settings of immunosuppression for the management of IFIs. For prophylaxis from infections they have been given at the onset of neutropenia (106–108) (Table 5). Although beneficial effects were noted in some of these studies, the number of IFIs that developed was too small to evaluate any potential effect. In a retrospective study of prophylactic administration of GM-CSF to patients with autologous bone marrow transplantation (BMT) for lymphoid cancer (105), the 28-day post-BMT incidence of infections occurring in those who had taken GM-CSF was compared with those who had not. The GM-CSF resulted in a trend towards fewer IFIs and decreased use of amphotericin B.

The GM-CSF has been used in patients with malignancies undergoing chemotherapy or HSCT, and has been found to improve survival and decrease the rate of bacterial and fungal infections (108–111). A retrospective study (109) suggested that GM-CSF has some advantages compared to G-CSF as preventive therapy of IFIs in patients receiving high-dose chemotherapy, with or without autologous stem cell transplantation.

The only prospective, randomized study in which there were enough IFIs to show significant differences has been the Eastern Co-operative Oncology Group study (108). In this placebo-controlled study, GM-CSF administration to elderly patients with myelogenous leukemia resulted in a reduction in the IFI-related mortality (2% in the GM-CSF group as compared to 19% in the placebo group), and a higher rate of complete response. Among the patients with IFIs, 11 patients had aspergillosis, seven candidiasis and two other IFIs. Only one of eight patients
who had been randomized to receive GM-CSF and developed IFI died (13%) as compared to nine among 12 patients on placebo (75%). No significant difference between aspergillosis and candidiasis was noted (111).

Furthermore, lack of consistent beneficial effects to support routine use of HGFs exists in the case of administration of G- or GM-CSF to patients at the onset of febrile neutropenia empirically or pre-emptively (112,113) (Table 6). In this setting, however, significantly less usage of antifungals was observed probably due to the effect of the cytokines on the incidence and duration of febrile neutropenia. Again, the numbers of IFIs diagnosed in the treated or the control arms were too small for any meaningful comparison to be made. The G-CSF also was studied in a randomized trial where patients with hematological malignancies and febrile neutropenia received either G-CSF with antibiotics or antibiotics alone. Although only four IFIs occurred, they were all encountered in the group receiving antibiotics alone (114).

In a double-blind controlled study, the administration of M-CSF to patients with myelogenous leukemia and febrile neutropenia decreased the incidence and duration of febrile neutropenia and significantly decreased the use of systemic antifungals (115). However, no impact on disease-free survival was found, an outcome heavily dependent on many confounding factors in these high-risk patients.

According to the American Society of Clinical Oncology (ASCO) updated recommendations regarding the use of HGFs (116), when fever persists during neutropenia

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Use of Cytokines for Prophylaxis Against IFIs in Cancer Patients</th>
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<tbody>
<tr>
<td>Reference</td>
<td>Cytokine</td>
</tr>
<tr>
<td>105</td>
<td>GM-CSF</td>
</tr>
<tr>
<td>108</td>
<td>GM-CSF</td>
</tr>
<tr>
<td>109</td>
<td>GM-CSF</td>
</tr>
<tr>
<td>107</td>
<td>G-CSF</td>
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</tbody>
</table>

Table 6

<table>
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<tr>
<th>Table 6</th>
<th>Use of Cytokines as Adjunctive Management of Febrile Neutropenia (Early Treatment of a Possible or Probable IFI) in Cancer Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>Cytokine</td>
</tr>
<tr>
<td>112</td>
<td>G-CSF</td>
</tr>
<tr>
<td>114</td>
<td>G-CSF</td>
</tr>
<tr>
<td>113</td>
<td>GM-CSF</td>
</tr>
<tr>
<td>115</td>
<td>M-CSF</td>
</tr>
</tbody>
</table>

*Retrospective analysis of GM-CSF vs. non-macrophage enhancing cytokines.
and IFI is suspected, adjunctive use of a HGF with empirical antifungal therapy may be justified. The ASCO does not recommend the routine use of HGFs in afebrile neutropenia. In view of the development of new strategies for early diagnosis of IFIs such as serial high-resolution CT scans, galactomannan, and glucan assays as well as PCR, in the future one may be able to administer antifungal drugs and immunomodulators specifically to patients in whom such tests are indicative of IFI (pre-emptive therapy).

2. Adjunctive Therapy of IFIs in Neutropenic Patients

Both in vitro and experimental animal models have suggested the utility of cytokine treatment as adjunctive therapy in combination with conventional antifungal chemotherapy against refractory IFIs (7,14,117–119). There are several small studies and case reports suggesting the use of G-CSF as adjunctive therapy for certain IFIs with very poor prognosis in combination with amphotericin B or fluconazole and in some cases in addition to surgical debridement but the conclusions remain controversial (Table 7). These include reports of five children with aspergillosis (128) and patients with fungemia in the setting of hematological malignancy (132) and five patients with refractory zygomycosis (125,130). The G-CSF and GM-CSF have been also used in invasive fusariosis, another resistant IFI with mixed results (133). Similarly, some reports have suggested potential beneficial effect of the combination of GM-CSF and IFN-γ with antifungal agents (Table 7). However, statistically powered randomized clinical trials examining the utility of cytokine therapy in combination with conventional antifungal agents remain to be performed.

In a pilot study in which GM-CSF was administered to eight patients with IFIs and severe neutropenia, six had a PMN response and four of them were completely cured. However, three patients developed a capillary leak syndrome, suggesting that the dosage of GM-CSF was excessive (120). A subsequent open study of GM-CSF plus amphotericin B in 17 neutropenic cancer patients with proven IFIs did not show similar favorable results. Eight of the patients suffered from candidemia, eight from pulmonary aspergillosis and one from fusariosis (121).

The GM-CSF therapy was associated with a clinical response when administered with amphotericin B to a small number of patients with established IFIs. These patients included one with systemic infection due to B. capitatus (131), three with AIDS and oropharyngeal candidiasis (81), and one with refractory Aspergillus vertebral osteomyelitis (134). Two cases of chronic disseminated candidiasis in leukemia patients were resolved completely following six weeks of therapy with GM-CSF and IFN-γ (123). However, other clinical case reports of combination therapy with IFN-γ and conventional antifungal therapy have had mixed results. Thus, there is an urgent need of well-structured, randomized clinical trials to determine optimal dose, duration, and timing for different combinations of immunotherapy and antifungal agents in high-risk patients.

As with the other HGFs, M-CSF was used as an adjunct to antifungal therapy in patients with established IFIs. In the first clinical trial examining combination immunotherapy, M-CSF was administered to patients with proven IFIs at escalating dosages (50–2000 μg/m², IV) in combination with the appropriate antifungal agent (amphotericin B, fluconazole, or flucytosine) at maximally tolerated doses (135). There was a trend toward better survival in the patients receiving M-CSF (122,136). Moreover, this increase in survival was significant in patients with candidiasis and a Karnofsky score > 20% when compared with historical controls.
The issue of cost effective use of cytokines as adjunctive therapy in combination with antifungal agents has not been thoroughly studied and does not allow specific recommendations. In 29 neutropenic patients with IFIs following chemotherapy or BMT, combined therapy of conventional amphotericin B and G-CSF (3–5 μg/kg/day) was associated with an improved response rate (62% vs. 33% of control) (137). This study showed a greater cost-effectiveness of combination regimen, based on drug acquisition (all the failures were treated with liposomal amphotericin B), hospital stay, and treatment duration (138).

The only HGF that has been investigated in immunocompetent patients is G-CSF. In a multicenter clinical trial addressing the utility of G-CSF as adjunctive therapy of invasive candidiasis, 51 non-neutropenic patients were randomized to receive either fluconazole alone or fluconazole with G-CSF. While not statistically

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fungal Infections</th>
<th>Antifungal Therapy</th>
<th>Cytokine</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>121</td>
<td><em>Candida</em> 8, <em>Aspergillus</em> 8, <em>Fusarium</em> 1</td>
<td>Amphotericin B</td>
<td>GM-CSF</td>
<td>No effect on outcome (six deaths)</td>
</tr>
<tr>
<td>122</td>
<td><em>Candida</em> 30, <em>Aspergillus</em> 15, Other 1</td>
<td>Amphotericin B</td>
<td>M-CSF</td>
<td>Trend of ↓ IFIs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significant improvement of survival of patients with candidiasis and Karnofsky score &gt; 20%</td>
</tr>
<tr>
<td>123</td>
<td>Chronic disseminated candidiasis, <em>Trichosporon beigeli</em> bloodstream infection</td>
<td>Liposomal amphotericin B</td>
<td>GM-CSF + IFN-γ</td>
<td>2/2 Responses</td>
</tr>
<tr>
<td>124</td>
<td>Disseminated <em>zygomycosis</em></td>
<td>Amphotericin B</td>
<td>G-CSF</td>
<td>Response</td>
</tr>
<tr>
<td>125</td>
<td>Disseminated <em>Fusarium oxysporum</em> infection</td>
<td>Liposomal amphotericin B</td>
<td>G-CSF</td>
<td>Response</td>
</tr>
<tr>
<td>126</td>
<td>Disseminated <em>Fusarium oxysporum</em> infection</td>
<td>Amphotericin B + 5-FC</td>
<td>G-CSF</td>
<td>Response</td>
</tr>
<tr>
<td>127</td>
<td>Invasive thoracopulmonary mucormycosis</td>
<td>Amphotericin B</td>
<td>G-CSF</td>
<td>Response</td>
</tr>
<tr>
<td>128</td>
<td>Invasive pulmonary aspergillosis</td>
<td>Liposomal amphotericin B</td>
<td>G-CSF</td>
<td>3/5 Responses</td>
</tr>
<tr>
<td>129</td>
<td>Disseminated <em>Fusarium</em> infection</td>
<td>Amphotericin B</td>
<td>GM-CSF (WBCTX)</td>
<td>Response</td>
</tr>
<tr>
<td>130</td>
<td>Rhinocerebral mucormycosis</td>
<td>Liposomal amphotericin B</td>
<td>G-CSF</td>
<td>4/4 Responses</td>
</tr>
<tr>
<td>131</td>
<td><em>Blastoschizomyces capitatus</em> septicemia</td>
<td>Amphotericin B + 5-FC</td>
<td>GM-CSF</td>
<td>Response</td>
</tr>
</tbody>
</table>
significant, there was a trend toward an earlier resolution of infection as well as reduced mortality in the patients receiving G-CSF (139). This study supports in vitro and ex vivo results that have shown that not only number, but also function of host immune cells is of importance in recovery from IFIs (56,77).

3. **Phagocytic Dysfunction**

Certain non-neutropenic patients are characterized by phagocytic dysfunction and are also at high risk for IFIs. Among them, patients with HSCT after recovery from neutropenia and especially during corticosteroid treatment of postengraftment graft-vs.-host disease (GVHD) are the most susceptible hosts (56). Apart from a decrease in the function of circulating phagocytes, these patients present abnormal cell-mediated immunity related to defective function of macrophages, MNCs, T, and NK cells. Indeed, IFIs, particularly aspergillosis, frequently occur in HSCT patients after the resolution of neutropenia (3,140), presumably related to an existing cytokine network dysregulation (141). These high-risk patients may benefit from cytokines administered during IFIs developed in the non-neutropenic phase after transplantation.

With the exception of a major prospective, randomized, placebo-controlled clinical trial, the clinical efficacy of IFN-γ against IFIs has not been extensively studied. As mentioned above, patients with qualitative phagocytic defects (most importantly CGD) are also at increased risk of IFIs, especially of invasive aspergillosis, an important cause of mortality in these patients (6). Long-term administration of IFN-γ has been shown to significantly reduce the incidence of serious infections in CGD patients (142). Patients in this study tended to have a reduced incidence of Aspergillus pneumonia compared with controls (two episodes in one patient in the IFN-γ group as compared to four episodes in four patients in the placebo group).

In addition, there is anecdotal evidence suggesting that IFN-γ can be useful adjunctive therapy for the treatment of certain unusual IFIs (143,144). Adjunctive therapy with IFN-γ has proven to be most useful in patients with defects in their immune cell function. For example, this cytokine has successfully been used for therapy of invasive aspergillosis in CGD patients in combination with antifungal agents (145,146) (Table 8).

Other non-neutropenic patients at high risk for IFIs who might benefit from immunotherapy, such as those with acquired immunodeficiency syndrome (AIDS), lymphoma, solid organ transplant recipients, and patients receiving corticosteroids or other immunosuppressants, have dysfunctional phagocytes along with cytokine dysregulation and lymphocytic defects. For example, PMNs and MNC-derived macrophages from patients with AIDS possess decreased ability to damage hyphae and to ingest conidia of *A. fumigatus*, respectively (155). In these patients, administration of recombinant IFN-γ showed a trend of decreased incidence of oral/esophageal candidiasis compared to control subjects (156).

V. **RECOMMENDATIONS FOR THE USE OF HGFs AND CYTOKINES IN THE PREVENTION AND TREATMENT OF IFIs IN IMMUNOCOMPROMISED PATIENTS**

As insufficient clinical data exist on the use of cytokines in the management of IFIs, definite guidelines for their routine use cannot be established. Nevertheless, reconstitution of the immune response by various actions has to be taken into serious consideration (Table 9). Reversion of immunosuppression is very important and
when it is possible (i.e., by decreasing or discontinuing immunosuppressive therapies), it must be attempted. In particular, corticosteroids, a major risk factor of IFIs, must be decreased or discontinued. In addition, exogenous administration of HGFs and proinflammatory cytokines or inhibition of immunoregulatory cytokines appears to be a promising adjunct to our armamentarium against life-threatening IFIs. In its updated guidelines (103,116,157), ASCO recommends that high-risk patients (more than 40% risk of febrile neutropenia) receive G- or GM-CSF prophylactically. Similarly, during the onset of febrile neutropenia in patients not receiving a HGF, G-, or GM-CSF are suggested when the duration of neutropenia is predicted to be long. Although no data exist, patients who have had an episode of IFI in the past and become neutropenic again should be treated with a HGF. With regard to

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fungal Infections</th>
<th>Antifungal Therapy</th>
<th>Cytokine</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>143</td>
<td><em>Paecilomyces variotii</em> soft tissue infection on the right heel</td>
<td>Amphotericin B followed by itraconazole</td>
<td>IFN-γ</td>
<td>Complete response</td>
</tr>
<tr>
<td>144</td>
<td>Disseminated infection with <em>Pseudallescheria boydii</em></td>
<td>Amphotericin B followed by itraconazole</td>
<td>IFN-γ</td>
<td>Complete response</td>
</tr>
<tr>
<td>145</td>
<td><em>P. variotii</em> multifocal osteomyelitis</td>
<td>Amphotericin B followed by itraconazole</td>
<td>IFN-γ</td>
<td>Response</td>
</tr>
<tr>
<td>146</td>
<td><em>A. fumigatus</em> femoral osteomyelitis</td>
<td>Itraconazole</td>
<td>IFN-γ</td>
<td>Complete response</td>
</tr>
<tr>
<td>147</td>
<td><em>Aspergillus nidulans</em> invasive multifocal infection</td>
<td>Liposomal amphotericin B</td>
<td>G-CSF and G-CSF-elicited PMNs</td>
<td>Complete response</td>
</tr>
<tr>
<td>148</td>
<td><em>Chryosporium zonatum</em> lobar pneumonia and tibia osteomyelitis</td>
<td>Liposomal amphotericin B</td>
<td>IFN-γ</td>
<td>Complete response</td>
</tr>
<tr>
<td>149</td>
<td><em>A. fumigatus</em> brain abscesses</td>
<td>Various anti-fungal agents and surgery</td>
<td>IFN-γ</td>
<td>Complete response</td>
</tr>
<tr>
<td>150</td>
<td><em>A. fumigatus</em> tibia osteomyelitis</td>
<td>Amphotericin B</td>
<td>IFN-γ</td>
<td>Response</td>
</tr>
<tr>
<td>151</td>
<td><em>A. nidulans</em> femoral osteomyelitis</td>
<td>Liposomal amphotericin B</td>
<td>G-CSF</td>
<td>Complete response</td>
</tr>
<tr>
<td>152</td>
<td><em>A. nidulans</em> multifocal osteomyelitis</td>
<td>Amphotericin B followed by liposomal amphotericin B plus flucytosine</td>
<td>IFN-γ</td>
<td>Complete response</td>
</tr>
<tr>
<td>153</td>
<td><em>A. fumigatus</em> humeral osteomyelitis</td>
<td>Amphotericin B plus flucytosine followed by itraconazole</td>
<td>IFN-γ</td>
<td>Complete response</td>
</tr>
<tr>
<td>154</td>
<td><em>A. fumigatus</em> thoracic vertebral osteomyelitis</td>
<td>Amphotericin B plus itraconazole followed by lipid complex amphotericin B plus itraconazole</td>
<td>IFN-γ</td>
<td>Complete response</td>
</tr>
</tbody>
</table>
the management of documented IFIs in neutropenic patients, the 1997 Guidelines of Infectious Diseases Society of America state that these factors “may be indicated” (158) and have not been changed up to now. So far, potential direct applications of cytokines and HGFs against IFIs are limited to the following indications:

1. Use of GM-CSF or G-CSF in the prevention and treatment of IFIs in neutropenic patients, especially those with myelogenous leukemia or HSCT. The G-CSF may not be as effective as the macrophage-stimulating HGFs. In addition, patients with other types of neoplastic diseases including AIDS-related malignancies, which are associated with high probability of development of IFIs may benefit from HGF therapy. The M-CSF is used in Japan and since it has not been licensed in the United States and European Union it cannot be used clinically in these countries.

2. Prophylactic use of IFN-γ in patients with CGD.

3. Under certain conditions of defective host defenses without neutropenia (i.e., GVHD or therapy with immunosuppressive agents), IFN-γ with or without a HGF may be justified as adjunctive therapy for IFIs.

With completion of more clinical studies, indications also might include surgical and other non-neutropenic immunodeficient patients with IFIs, i.e., those with solid organ transplant or HIV-infection, neonates, and others. The role of administration of neutralizing antibodies or inhibitors of Th2 cytokines on prevention and outcome of IFIs needs further clinical study.

Two forms of G-CSF are commercially available. One is a recombinant non-glycosylated protein expressed in *Escherichia coli* (filgrastim). The other is a glycosylated form expressed in Chinese hamster ovarian cells in vitro (lenograstim). Both products have the same net effect, acceleration of myelopoiesis, and enhancement of functional responses. As an immediate effect, G-CSF causes an actual decrease of PMN count, which is followed by a sustained dose-dependent rise in PMN counts.

The G-CSF has been recommended at a dose of 5 μg/kg/day *sc* or *iv* for high-risk patients after cytotoxic cancer chemotherapy. Starting the day after the last chemotherapy dose it continues with subsequent individualized adjustment of dosage depending on the PMN count until this increases to 1000/μL for three consecutive days. A higher dosage of 10 μg/kg/day can be used in early phases of HSCT followed by a standard dose of 5 μg/kg/day or when G-CSF is administered as adjunctive therapy for a documented IFI.
The GM-CSF is a recombinant non-glycosylated protein expressed in *E. coli* (molgramostim) and glycosylated protein expressed in *Saccharomyces cerevisiae* (sargramostim) or in mammalian cells (regramostim). The GM-CSF transiently decreases leukocyte counts immediately after administration and causes sustained rises of PMN, eosinophil, and MNC counts afterwards. Various dosing regimens of GM-CSF have been used in different studies. The recommended dosage is 250 μg/m² daily during the period of profound neutropenia or when a documented IFI is treated. The dosage is individualized depending on response and development of adverse effects.

The IFN-γ has been administered at a dose of 50 μg/m² three times a week subcutaneously as prophylaxis in CGD patients (142). Doses up to 100 μg/m² three times a week have been subsequently suggested. Similar doses have been used as adjunctive therapy.

The GM-CSF has been described in some cases as inducing pleuritic pain, pulmonary edema, and a capillary leak syndrome. Another potential complication of its use stems from its activity in stimulating recovery of leukocyte function. Massive fatal hemoptysis has been reported to follow (159). Although bone pain is described in 20% of patients receiving G-CSF, the other adverse effects associated with GM-CSF are not commonly observed in G-CSF treated patients. The toxicity of GM-CSF appears to be related to the non-glycosylated preparations expressed in *E. coli*. By comparison, the glycosylated form of GM-CSF is not associated with these adverse effects. The toxicity profile of recombinant GM-CSF is consistent with priming of macrophages for increased formation and release of inflammatory cytokines, whereas G-CSF induces production of anti-inflammatory factors, such as IL-1 receptor antagonist and soluble TNF receptor, and is protective against endotoxin- and sepsis-induced organ injury. Although administration of G-CSF to patients with acute myeloid leukemia (AML) carries the theoretical risk of accelerating the leukemic blast cells, this has not been observed. Indeed, G-CSF has been safely used in patients with AML and myelodysplasia (160). The induction of Th2 response by G-CSF may not be deleterious; indeed it may be beneficial for the mediation of excessive inflammation (161). The adverse effect of GM-CSF accelerating HIV replication in MNCs may be offset by simultaneous administration of antiretroviral agents.

With regard to IFN-γ therapy the most common adverse effects are minor and consist of “flu-like” or constitutional symptoms such as fever, headache, chills, myalgia, or fatigue. These symptoms may decrease in severity as treatment continues.

VI. WHITE BLOOD CELL TRANSFUSIONS (WBCTx)

A potential application of combined cytokine-phagocyte therapy together with antifungal chemotherapy is the transfusion of cytokine-elicited PMNs to assist recovery from antifungal chemotherapy-refractory IFIs. In a review of 32 studies, the overall efficacy of WBCTx was 62% in 206 patients with bacterial infection (162). With regard to IFIs the data were less encouraging with a positive clinical outcome observed in only 29% of recipients. Bhatia et al. reported that there was no significant improvement in outcome of IFIs in 50 patients, despite showing the feasibility of administering WBCTx (163). However, subsequent reports have provided encouraging data with G-CSF elicited WBCTx as compared to studies that used conventional WBCTx stimulated with steroids, which necessitate follow-up studies.
Indeed, in non-G-CSF strategies the number of neutrophils collected ranged between $10^9$ and $10^{10}$, whereas in strategies where G-CSF was combined with a corticosteroid the range was $10^{11}$–$10^{12}$ (170).

A pilot study evaluated the safety and efficacy of G-CSF-elicited WBCTxs in 15 patients with neutropenia-related IFIs that were refractory to therapy with amphotericin B alone (165). There was a favorable response reported in 11 patients at the end of therapy. Although only three of the 11 patients were alive at three months after starting WBCTx, the IFI contributed to the death in six of eight patients in the setting of persistent or progressive immunosuppression (relapsed or refractory leukemia, or allogeneic BMT). The beneficial effect of the transfusions seemed to be enhanced by their administration to patients with good performance status, as well as administration early during neutropenia and soon after onset of the IFI.

A separate prospective multicenter phase I/II clinical trial evaluated the feasibility and tolerability of WBCTxs in neutropenic patients with infection, 13 of whom had IFIs (167). A favorable outcome was observed in five of nine patients with aspergillosis, and two of four patients with yeast infections. In addition to WBCTxs, treatment consisted of amphotericin B (partly liposomal preparation) and itraconazole, as well as surgical resection of the lesions in five patients following stabilization of the septic condition. Another multicenter trial evaluated the feasibility and tolerability of PMN transfusions in neutropenic patients with refractory infections. A favorable outcome was observed in a portion of patients with yeast infections (168).

A study of WBCTx in children with cancer evaluated 15 courses of WBCTx in 13 neutropenic children with refractory bacterial and fungal infections (171) and reported a 60% response rate and without serious adverse events.

Another study evaluated WBCTx in 22 patients with hematological malignancies who developed refractory neutropenia-related bacterial and fungal infections (172). Control of infection at day 30 after the first WBCTx could be achieved in 50% of patients. In this study, the ultimate recovery of the patient’s marrow was

<table>
<thead>
<tr>
<th>Reference</th>
<th>Underlying Condition</th>
<th>No. of Pts</th>
<th>Fungus</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>166</td>
<td>Aplastic anemia/BMT</td>
<td>1</td>
<td>Aspergillus</td>
<td>CR</td>
</tr>
<tr>
<td>164</td>
<td>Neutropenia</td>
<td>2</td>
<td>Candida tropicalis</td>
<td>2/2 CR</td>
</tr>
<tr>
<td>165</td>
<td>Neutropenia</td>
<td>15</td>
<td>11 molds, four yeasts</td>
<td>11/15 PR; (only 3/11 survived &gt;3 months post-WBCTx)</td>
</tr>
<tr>
<td>147</td>
<td>CGD</td>
<td>1</td>
<td>Aspergillus</td>
<td>CR</td>
</tr>
<tr>
<td>167</td>
<td>Neutropenia</td>
<td>13</td>
<td>Nine Aspergillus</td>
<td>5/9 CR</td>
</tr>
<tr>
<td>168</td>
<td>Stem cell transplant</td>
<td>15</td>
<td>Four Candida</td>
<td>2/4 CR</td>
</tr>
<tr>
<td>133</td>
<td>Hematologic cancer</td>
<td>7</td>
<td>Fusarium</td>
<td>3/7 CR</td>
</tr>
<tr>
<td>169</td>
<td>Neutropenia</td>
<td>12</td>
<td>Nine Aspergillus</td>
<td>4/9 CR and 1/9 PR</td>
</tr>
<tr>
<td>147</td>
<td>CGD</td>
<td>1</td>
<td>Aspergillus</td>
<td>CR</td>
</tr>
<tr>
<td>167</td>
<td>Neutropenia</td>
<td>13</td>
<td>Nine Aspergillus</td>
<td>5/9 CR</td>
</tr>
<tr>
<td>168</td>
<td>Stem cell transplant</td>
<td>15</td>
<td>Eight molds</td>
<td>0/8 R</td>
</tr>
<tr>
<td>133</td>
<td>Hematologic cancer</td>
<td>7</td>
<td>Fusarium</td>
<td>3/7 CR</td>
</tr>
<tr>
<td>169</td>
<td>Neutropenia</td>
<td>12</td>
<td>Nine Aspergillus</td>
<td>4/9 CR and 1/9 PR</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>66</td>
<td>46 molds</td>
<td>14/35 CR (40%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20 yeasts</td>
<td>10/16 CR (63%)</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete response; R, response; PR, partial response.
Source: Modified from Ref. 170.
the only parameter that significantly and independently correlated with a favorable response to WBCTx.

The prophylactic use of WBCTx was described in nine allogeneic stem cell transplant recipients with either previous invasive aspergillosis or considered to be at high risk for aspergillosis during their transplant. Compared to a control, untransfused group, these nine patients had a significant reduction in the incidence and duration of fevers and maximum C-reactive protein and fewer days of neutropenia ($p < 0.05$). Radiological improvement of pulmonary infiltrates was also noted in some patients (173).

In addition, a number of successful case reports using G-CSF-mobilized WBCTxs have been reported. For example, a CGD patient with invasive aspergillosis due to *A. nidulans*, who failed five months of treatment with liposomal amphotericin B and IFN-$\gamma$, was successfully treated with BMT, G-CSF-mobilized PMNs, and liposomal amphotericin B (147). In these studies, yeast infections tended to respond to WBCTx better than mould infections (Table 10). Thus, the rapid increase in PMNs coupled with the relatively low adverse effects, suggest that WBCTx in combination with cytokines may be a useful approach and deserves further study in the setting of refractory IFIs.

The major indication for WBCTx continues to be restricted to progressive, documented infection in the profoundly and persistently neutropenic host (162). Drug-refractory IFIs in patients with underlying phagocytic defect, namely CGD, are a particularly important indication for WBCTx adjunctive therapy. Prophylactic use of WBCTx therapy has not yet been incorporated into clinical practice, primarily because of cost and toxicity. It is still unclear that WBCTx should be widely embraced until further studies are completed, which should better define its niche in supportive care. Still, this is an approach that should be investigated in multi-institutional studies so that a satisfactory enrollment can be achieved.

Patients with evidence of alloimmunization (platelet refractoriness, antileukocyte antibodies, repeated febrile transfusion reactions, or post-transfusion pulmonary infiltrates) may not benefit from WBCTx (162). This happens because they usually have a low post-transfusion increment, more pulmonary reactions and transfused PMNs unable to migrate to the sites of infection.

The higher the number of cells transfused per square meter of body surface area, the better the clinical response to WBCTx (174–177). For mobilization of PMNs in healthy donors, most experts have favored G-CSF, which is indeed, the standard method in blood processing centers (178). Both forms of G-CSF commercially available have the same net effect, acceleration of myelopoiesis, and enhancement of functional responses (including bactericidal activity, chemotaxis, phagocytosis, respiratory burst, and surface expression of low affinity Fc receptors) (179–181). The G-CSF also delays apoptosis in PMNs, which is particularly important for harvested PMNs, because it appears that G-CSF-stimulated PMNs have a longer shelf life and perhaps, once transfused, persist longer in vivo (182,183).

The G-CSF increases the yield of PMNs by roughly five fold, which is greater than the 2–3-fold increase accomplished with prednisolone (50–100 mg given intravenously or orally once 2 hr before the donation) or dexamethasone (8 mg given orally 12 hr before the donation) (167,168). Four hundred fifty micrograms of G-CSF has been recommended as optimal safe dose required for mobilization of PMNs in adults (48). Only one dose is needed 12–24 hr prior to collection. Several recent studies have evaluated the role of the combination of G-CSF and corticosteroids, suggesting that the higher dose of the former with the latter results in the
highest yield (12-fold above premobilization) (184). The functional properties of G-CSF-mobilized PMNs are essentially unchanged. Specifically, the respiratory burst activity is normal or elevated, due to priming of PMNs. The half-life of transfused PMNs previously mobilized with G-CSF is at least twice as long (184). Single dose exposure to corticosteroids probably does not represent a major suppressant of PMN function in mobilized products. No G-CSF related long-term effects have been observed to the donors, even one year later, when all hematological measurements were comparable to pre-G-CSF levels (185,186). Storage at 10°C might lengthen the shelf life of mobilized PMNs and may preserve antifungal and PMN function better (187).

The leukapheresis technique used today is the continuous flow centrifugation with the addition of a rouleauxing agent such as hydroxyethyl, which produces a better quality PMN (186). This technique is very advantageous over previous techniques. It allows processing of larger volumes of blood, which has been translated into increased yields. Traditionally, related donors have been preferred to avoid toxicity related to incompatibility, but more recently, community donors have been effectively used to safely and rapidly mobilize PMNs, thus increasing the availability of WBCTxs to a larger number of individuals for whom the indication is sound (168).

The WBCTxs have been associated with a low incidence of complications. Mild reactions to the transfusion product are common, including fever and chills, which can be reduced if the infusion rate is reduced. Severe side effects, namely hypotension or respiratory distress, are estimated to occur in ~1% of recipients. In two reports, respiratory complications have been temporally linked to co-administration of deoxycholate amphotericin B (188,189). Transfusion-related acute lung injury is rare and probably not associated with G-CSF mobilization (190). The GVHD is prevented by irradiation of the product pre-infusion with 15–30 Gy, which does not appear to adversely affect the function of the transfused PMNs (191). Alloimmunization can be a formidable problem in CGD patients, but interestingly does not occur in cancer or transplant recipients, who have received immunosuppressive therapy. A recent study in HSCT reported that recipients who received G-CSF-mobilized PMNs from an incompatible donor had a delayed engraftment (192).

VII. OTHER MODES OF ADJUNCTIVE IMMUNOTHERAPY

Antibody-mediated host defense contributes to fighting against certain IFIs. Mice with experimental Candida infection treated with human IV immunoglobulin (IVIG) combined with amphotericin B had modest prolongation of survival, suggesting the potential efficacy of serum antibodies against fungi (193).

In humans, IVIG has been used in liver transplant recipients receiving anticytomegalovirus prophylaxis and in patients who had undergone BMT. In the first case, IVIG therapy was associated with a significant reduction in the incidence of IFIs (194), whereas in the second group, therapy was not associated with a significant reduction (195). This finding was despite a previous finding that oral administration of bovine anti-C. albicans antibodies to BMT recipients reduced Candida colonization in seven of 10 patients (196), which suggests that pathogen-specific antibodies can be effective in patients with immune defects.

For both C. albicans and C. neoformans, several protective monoclonal antibodies have been described not always with success (reviewed in Ref. 197).
Human serum antibodies and a mouse monoclonal antibody to fungal heat shock protein 90 (hsp90) were protective against candidiasis in mice as well as a human recombinant antibody to a hsp90 linear epitope mediated protection against invasive murine candidiasis (198). Mycograb, a human genetically recombinant antibody against fungal hsp90 synergized with amphotericin B for complete resolution of infection in models of murine candidiasis (199). Antibodies to *C. albicans* polysaccharides have also been shown to be protective in murine models of infection (200).

Another approach to antibody therapy has been to engineer antibodies with dual functions, or bispecific antibodies. A bispecific antibody that binds both the Fcε receptor (CD89) and *C. albicans* has been shown to enhance PMN-mediated antifungal activity in G-CSF-primed cells (201). However, although serum antibodies may promote natural resistance to infection, they may not necessarily ameliorate established or chronic infections. Hence, their efficacy against some fungi may be dependent on intact cellular immunity (202). Therefore, their clinical use has not been recommended in the management of IFIs.

The T-cell adoptive therapy and vaccination seems to be a promising strategy of prevention or even adjunctive therapy of IFIs in immunocompromised patients. Studies have begun to assess the ability of fungal antigens to induce Th1 type reactivity as potential candidates for fungal vaccines. Treatment of immunocompetent mice with *Aspergillus* crude culture filtrate antigens resulted in the development of local and peripheral protective Th1 memory responses. This finding suggests the existence of fungal antigens useful as a potential candidate vaccine against invasive pulmonary aspergillosis (203).

**VIII. FUTURE DIRECTIONS**

In view of the drug-resistant nature of many IFIs, the promise of certain immunotherapeutic agents underscores the need for interdisciplinary research to establish parameters for their use, and presents a major challenge to clinicians and scientists to translate preclinical data on immunotherapeutic agents into clinical benefit. In tandem with destroying fungi using potent antifungal agents, reconstitution and up-regulation of immune response by either exogenous administration of cytokines or transfusion of cytokine-elicited allogeneic phagocytes appear to be promising adjuncts to antifungal chemotherapy for these life-threatening infections. Further evaluation of the safety and efficacy of immunotherapeutic modalities is an urgent priority for research during the near future. Well-controlled studies in patients at very high risk of developing fungal infections, such as profoundly neutropenic, HSCT patients, should be the goal of future studies. The large number of immune defects that predispose to fungal infections, the biological differences among fungi, and the variable responses to immune modulators are likely to complicate the design of clinical studies, and large sample sizes will likely be required for valid conclusions. If such studies are proved to be impossible, physicians are left with great amount of positive preclinical data but with limited clinical proof of efficacy of the immunotherapy as adjunctive treatment of IFIs that are difficult to treat otherwise. Unfortunately, this is not the only management strategy in Medicine that is supported by theoretical and preclinical data but lacks statistically significant clinical proof, as we understand this proof in the beginning of the 21st century.
IX. CONCLUSIONS

The increasing incidence of IFIs and the emergence of previously rare opportunistic fungal pathogens is of major importance in the management of immunocompromised patients. The prevention and treatment of IFIs, which are still a great challenge for clinicians, may be greatly enhanced by strategies to normalize host defense mechanisms. This has been experimentally achieved by reconstitution of effector cells numerically and/or functionally with cytokines and/or WBCTx, or by manipulation of cytokine dysbalance. Undoubtedly, immunotherapy is a promising therapy and the interest in its use as adjuncts to antifungal agents has been extremely increased. Evaluation of the benefits of the HGF/cytokine prevention and adjunctive therapy with concomitant antifungal therapy are urgent priorities for clinical research. A better understanding of the synergy between cytokines and specific antifungal agents may provide additional powerful tools for managing these serious infections.

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Adjunctive Immunotherapy


I. RATIONALE

The outcomes of various invasive fungal infections have historically been suboptimal and for certain pathogens such as Aspergillus and Fusarium, frankly poor, with most infections ending in death. Although various pharmacologic agents have demonstrable in vitro antifungal activity, clinical responses have not been as good as one might anticipate. Several reasons have been posited. First, the early diagnosis of infection is often difficult to make, as noted in foregoing chapters. This means that treatment is often started late in the course of infection. Once the burden of organisms is high, with spread of the infection to multiple sites, and the physiological status of the patient is impaired, the prospect for successful resolution of the infection is compromised. Second, the host defenses of the infected patient are crucial for resolution of the infection, and patients who become infected have substantial compromise of such protective defenses, key to resolution of infection. Unless recovery of host defenses occurs concomitantly with antifungal therapy, prospects for successful treatment are limited. Third, substantial toxicities of several of the antifungal agents (e.g., amphotericin B) have limited the ability to administer the therapy at high enough doses or for long enough. An inadequate course of therapy seriously compromises the likelihood for treatment success.

To meet the challenge of poor treatment outcomes, prophylaxis is an alternative strategy to improve outcomes. There are a number of considerations one should weigh in determining if prophylaxis is appropriate for a given patient or patient population to address a specific infection (Table 1). First, the frequency of infection should be sufficient to warrant the cost, potential toxicities, and inconvenience imposed by administration of the prophylactic agent. Second, the infection of interest should be clinically important, with substantial morbidity or risk of death. Third, the option of waiting to treat the infection should be less attractive because of poor treatment results, toxicities of the therapy, or because substantial morbidity occurs even if the eventual outcome is salutary. Fourth,
suitable agents should be available for prophylaxis, with favorable antimicrobial activity and safety profiles and formulations that are convenient and inexpensive for administration of the regimen. Fifth, the strategy should be tested in controlled trials to demonstrate that it is effective in achieving the desired goal, and untoward or unanticipated adverse sequelae are not associated with it.

The earlier-mentioned considerations are important because prophylaxis can have potential undesirable consequences. The emergence of resistance is a constant threat for any antimicrobial agent. Overuse of an agent contributes to the risk for resistance. Shifts in microbial colonization may lead to changes in patterns of infections by other less-susceptible organisms. Toxicities of an agent may offset its antifungal efficacy. Unanticipated interactions with concomitant medications may occur and lead to altered effects of medications. For all of these reasons, prophylaxis should be used only when the benefits outweigh the risks.

II. TYPES OF PROPHYLAXIS

There are several types of prophylaxis strategies. Generally speaking, prophylaxis can be regarded as “primary” when prevention targets an individual or a group that has not been infected in the past, but who is vulnerable for infection. “Secondary” prophylaxis refers to the approach used in individuals who have been previously infected, but the infection has been brought under control; continued immunodeficiency or further immune-suppressing therapy places the patient at risk for loss of infection control, exacerbation, or reinfection. This is also sometimes referred to as chronic suppressive or maintenance therapy. An example of the latter would be a patient with acute leukemia who develops aspergillosis (which is then controlled with antifungal therapy), but postremission faces additional antileukemic consolidation therapy or hematopoietic cell transplantation (HCT). Another example is an HIV-infected patient who develops an acute opportunistic fungal infection, which is controlled but continues to have severely compromised cell-mediated immunity. Continued treatment would be given with the purpose to prevent a recurrence of the acute infection.

Another way to categorize prophylaxis is global vs. targeted. Global prophylaxis refers to administration to all patients deemed at risk for a given infection. This is attractive because it standardizes the approach for all patients, but is less desirable because of potential excessive exposure to patients. An alternative is to target those at greatest risk (“targeted” prophylaxis), using some parameter of host susceptibility to determine the targeted group at risk. An example is instead of offering all allogeneic HCT patients’ antimold prophylaxis, reserving it only for patients with...
graft versus host disease, the subgroup at greatest risk; a second example is offering prophylaxis only to HIV-infected patients with a CD4+ T-lymphocyte count below a certain threshold value instead of administration to all HIV-infected patients. In each case, certain risk factors or markers of immunodeficiency are used to define those who are offered prophylaxis. Various prophylaxis strategies have been evaluated in controlled trials, and several have been adopted in clinical practice as accepted good patient-care measures. It is important to note that prophylaxis against a specific fungal pathogen may be appropriate for one patient population, while not appropriate for another. Nearly half of all serious fungal infections occur in cancer, HIV, and transplant recipients (1). Not surprisingly, most of the studies that have evaluated prophylaxis have been conducted in these patient groups. Discussed in what follows are generally accepted practices for certain fungal pathogens and the studies that have formed their basis in various patient populations.

III. CANDIDA

Candida organisms are commensal organisms that cause superficial mucosal or cutaneous infections and invasive, systemic infections. Polyene and azole antifungals are highly active against most Candida species. Although nonabsorbable topical antifungals are available and several studies have demonstrated benefit, recent prophylaxis trials have emphasized absorbable azoles, especially fluconazole (or itraconazole) because of their systemic effect and excellent tolerability. The role of prophylaxis varies by patient population.

A. Hematopoietic Cell Transplantation

Candida has historically been the most common fungal pathogen in both allogeneic and autologous HCT recipients. Although mucosal infections occur, systemic infections are more problematic. Systemic Candida infections are frequent in HCT patients with infection rates of 15–20% during the first month after HCT. Prospective randomized trials have demonstrated the effectiveness of fluconazole prophylaxis when given from time of transplant until engraftment to reduce systemic infection and infection-related mortality (2,3). In one trial, prolonged administration (until day 75) after allogeneic HCT had a similar antifungal benefit but in addition was associated with a survival advantage as well (4), persisting even beyond cessation of fluconazole (5). Initial studies evaluated a dose of 400 mg/day, but one study demonstrated a dose of 200 mg/day was also efficacious (6). Emergence of resistance has not been reported to date. However, isolated reports of outbreaks of fluconazole prophylaxis when given from time of transplant until engraftment to reduce systemic infection and infection-related mortality (2,3). In one trial, prolonged administration (until day 75) after allogeneic HCT had a similar antifungal benefit but in addition was associated with a survival advantage as well (4), persisting even beyond cessation of fluconazole (5). Initial studies evaluated a dose of 400 mg/day, but one study demonstrated a dose of 200 mg/day was also efficacious (6). Emergence of resistance has not been reported to date. However, isolated reports of outbreaks of fluconazole, resistant organisms, such as C. krusei and C. glabrata, have been reported in several HCT centers (7–9). Fluconazole prophylaxis has been endorsed by consensus guidelines developed by the Center for Disease Control, the American Society of Blood and Marrow Transplantation, and the Infectious Disease Society of America (Table 2) (10) for all allogeneic HCT recipients and autologous HCT recipients transplanted for hematologic malignancies. An alternative to fluconazole is low-dose amphotericin B (dosed <0.5 mg/kg/day), which is also protective but more toxic (11,12).

B. Hematologic Malignancy

Both mucosal and systemic Candida infections occur because of neutropenia (especially in acute leukemia patients) or diminished cell-mediated immunity (in chronic
Table 2  Prophylaxis Against *Candida*

<table>
<thead>
<tr>
<th>Patient group references</th>
<th>Infection type</th>
<th>Antifungal agent</th>
<th>Alternative</th>
<th>Prophylaxis type</th>
<th>Concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allogeneic HCT</td>
<td>Systemic</td>
<td>Fluconazole 200–400 mg/day</td>
<td>Global</td>
<td>Drug interactions (cytochrome P450)</td>
<td></td>
</tr>
<tr>
<td>Autologous HCT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Systemic</td>
<td>Fluconazole 200–400 mg/day</td>
<td>Itraconazole, amphotericin B</td>
<td>Targeted</td>
<td>Variable infection risk</td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>Systemic</td>
<td>Fluconazole 400 mg/day</td>
<td>Amphotericin B</td>
<td>Global</td>
<td>Interaction with calcineurium inhibitors</td>
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<tr>
<td>Liver transplant</td>
<td>Systemic</td>
<td>Fluconazole 400 mg/day</td>
<td>Liposomal amphotericin B</td>
<td>Global</td>
<td>Interaction with calcineurium inhibitors</td>
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<td>Pancreas transplant</td>
<td>Systemic</td>
<td>Fluconazole 400 mg/day</td>
<td>Ketoconazole</td>
<td>Targeted</td>
<td>Increase in <em>C. glabrata</em>; definition of high risk</td>
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<td>Extremely low birth weight (&lt;1000 g)</td>
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<td>Neonatal ICU</td>
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<td>Fluconazole&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Itraconazole</td>
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<sup>a</sup>when performed for the treatment of hematologic malignancy.

<sup>b</sup>At a dose of 3 mg/kg every third day for the first 2 weeks, every other day during the third and fourth weeks, and every day during the fifth and sixth weeks.
leukemias, lymphomas, and those receiving corticosteroids). The rate of systemic infection is more variable in leukemic patients than with HCT and is highly dependent on the treatment regimen. Systemic infection rates of 8–16% have been reported during induction therapy for acute leukemia. Several antifungal agents have been evaluated as prophylaxis. The benefits of the nonabsorbable antifungal agents have been mixed in various trials; tolerability has limited their use. In a meta-analysis of antifungal prophylaxis and empirical therapy trials in neutropenic cancer patients using a variety of agents (mostly agents other than fluconazole), a reduction in infection and colonization was found, but no decrease in survival, except for amphotericin B (13). In contrast, a larger meta-analysis of trials testing azoles or IV amphotericin B products demonstrated reductions in rates of superficial and invasive fungal infections, decreased use of parenteral antifungal therapy, and lower fungal-related mortality, although no reduction in overall mortality (14). In a meta-analysis of fluconazole prophylaxis trials only (15), a reduction in systemic fungal infections was noted in patient populations in which the incidence of systemic fungal infection exceeded 15%. Such patient populations would typically include HCT patients and patients with hematologic malignancy receiving intensive chemotherapy regimens (3,16). Because of the heterogeneity of risk, clearly, prophylaxis must be tailored to the intensity of antileukemic treatment approach and the degree of risk posed by the particular treatment regimen. With respect to other patients with chemotherapy-induced myelosuppression, current data do not support the routine use of fluconazole prophylaxis. Of note, no increase in systemic infections by fluconazole-resistant fungi has been noted, although colonization by fluconazole-resistant organisms has been seen (15).

C. Solid Organ Transplantation

*Candida* infections are problematic in recipients of liver transplants. Rates of infection have been reported in the range of 5–42% (17). Risk factors include prolonged surgical time, elevated creatinine, retransplantation, reoperation, and cytomegalovirus infection (17). Randomized trials have demonstrated a reduction in *Candida* infections by the use of fluconazole (18,19). Alternatively, liposomal or lipid-complex amphotericin B is also effective (20,21). A similar benefit in pancreatic transplantation has also been suggested (22). Serious *Candida* infections are generally infrequent after renal transplantation, unless rejection episodes necessitate intensive or prolonged immunosuppression. Accordingly, routine prophylaxis is not appropriate after renal transplantation; however, recurring candiduria may signify upper urinary tract infection, should be fully investigated, and may be grounds for “preemptive” therapy to prevent obstructive uropathy from fungal balls at the ureterovesicle junction and pyelonephritis, especially in patients with impaired bladder emptying.

D. Critical Care

The risk of invasive *Candida* infection is low in unselected patients in medical or surgical intensive care units (ICUs), ~2% (23). Because of the low risk, several consensus guidelines developed by expert panels do not recommend routine prophylaxis (24–26). The risk is higher in certain subgroups, according to the complexity of critical care administered and the duration of its necessity. Risk factors identified include central venous catheters, parenteral nutrition, multiple antibiotics, extensive surgery (especially gastrointestinal surgery), burns, renal failure, hemodialysis,
mechanical ventilation, chronic intestinal perforation, poor physiological status of the patient, use of gut decontamination, prolonged ICU stay, surgery for liver or pancreatic transplantation, and colonization by *Candida* (27–29). Two small trials suggested ketoconazole or fluconazole could reduce *Candida* infections in critically ill surgical patients (30,31). In a recent prospective randomized placebo-controlled trial in patients who were expected to remain in the ICU for a minimum of ≥3 days, the frequency of invasive infections was reduced by 55% in patients given fluconazole at a dose of 400 mg/day (32). Clearly, these studies suggest there is a potential role for prophylaxis in carefully selected subgroups of ICU patients, but further study is needed to determine how to optimally select such individuals in current ICU practice environments (27).

E. Neonatal ICU

The risk of invasive *Candida* infection is high in critically ill preterm infants, accounting for 9% of late-onset sepsis in infants weighing <1500 g (33). Practices associated with the care of prematurity are relatively homogeneous in contrast to practices in the adult ICU patient population. In a randomized placebo-controlled trial conducted in infants weighing <1000 g at birth, IV fluconazole was given at a dose of 3 mg/kg every third day for the first 2 weeks, every other day during the third and fourth weeks, and every day during the fifth and sixth weeks. This regimen was effective in reducing the rate of invasive infection (from 20% to 0%) (34).

F. HIV Infection

*Candida* infections of the oral cavity, oropharynx, vagina, and esophagus are frequent and are perhaps the most common infection seen in HIV disease. Deep-seated candidiasis has been one of the infections included in the case definition of the acquired immunodeficiency syndrome. Most patients with advanced HIV infections develop mucocutaneous candidiasis and frequent recurrences occur (35). The risk of *Candida* infection is especially high when the CD4 lymphocyte count is low (e.g., <200 cells/μL) or the HIV burden is high. The advent of highly active antiretroviral therapy (HAART) has led to a dramatic reduction in the frequency of *Candida* infections (36,37). This appears to be independent of reconstitution of anti-*Candida* cell-mediated immune responses (36). Candidemia is infrequent except in late-stage HIV infection or in those with central venous catheters. Infections may be unresponsive to antifungal therapy when the CD4+ T-lymphocyte count is very low (e.g., <50 cells/μL). Fluconazole therapy of mucocutaneous candidiasis (38) and prolonged fluconazole prophylaxis (39,40) have also been associated with the emergence of resistance.

Multiple randomized trials have demonstrated the effectiveness of fluconazole in prevention of candidiasis in HIV-infected patients (41). Notwithstanding, consensus guidelines do not recommend routine prophylaxis in HIV-infected patients (42,24). There are several reasons. Patients with the first episode of oropharyngeal candidiasis generally respond promptly to therapy. The first episode has a low risk for serious morbidity or mortality. Chronic administration of azoles has been associated with a substantive risk for resistance, particularly in patients with CD4+ T-lymphocyte counts <200 cells/μL (43). Drug interactions and cost are other considerations. Accordingly, if recurrences of oropharyngeal or vulvovaginal candidiasis are frequent or severe, an azole such as fluconazole may be given (42) at a daily
dose of 100–200 mg. Similarly, multiple episodes of esophageal candidiasis are important considerations for chronic suppressive therapy or secondary prophylaxis. Fluconazole has been best studied, but other azoles have also been evaluated including itraconazole or ketoconazole as alternatives to fluconazole. Itraconazole (in solution) appears to be as effective, but there are fewer trials. Ketoconazole appears to be not as effective as fluconazole. Fluconazole is more effective than clotrimazole (44).

**IV. ASPERGILLUS**

**A. HCT and Hematologic Malignancy**

*Aspergillus* is a cause of life-threatening pneumonia and, less commonly, sinusitis. Prolonged neutropenia has long been known to be a major risk factor for aspergillosis (45). The intensive chemotherapy regimens typically used to treat acute leukemia are the most common scenarios in which such prolonged neutropenias are encountered. After allogeneic HCT, development of graft versus host disease and the use of corticosteroids also pose major risks with other factors such as use of T-cell depletion of the stem-cell graft, the occurrence of postengraftment neutropenia or lymphopenia, and infection by cytomegalovirus or respiratory viruses (46). With the increasing use of hematopoietic growth factors and stem-cell products containing large numbers of hematopoietic progenitors occasioned by peripheral blood stem-cell grafts, the interval to engraftment after HCT has shortened and there has been a concomitant reduction in the incidence of early aspergillosis. Most *Aspergillus* infections now occur after engraftment 2–4 months later. Because of graft versus host disease, the risk of aspergillosis after reduced intensity allogeneic HCT is similar to allogeneic HCT using standard intensity conditioning regimens (47,48). The rate of *Aspergillus* infections after HCT is 10–15% in various series and this has increased in recent years (49). The rate of *Aspergillus* infections during acute leukemia therapy is variable depending on the chemotherapy regimen and the response of the leukemia to therapy. After an initial episode of aspergillosis, the risk of later recurrence during subsequent antileukemic therapy (either additional cycles of chemotherapy or HCT) is practically 100%. Amphotericin B given at full treatment doses (1.0 mg/kg/day) (with or without flucytosine) started at the start of subsequent antileukemic treatments has found to be effective secondary prophylaxis (Table 3) (50,51). The evaluation of mold-active azoles (itraconazole and voriconazole) as secondary prophylaxis is more limited, but recent case series suggest that they are effective as well. Liposomal amphotericin B has been tested as primary prophylaxis in randomized trials after HCT, but because of small sample sizes, these trials were inconclusive (52,53). The need for prolonged prophylaxis has placed parenteral drugs at a substantial disadvantage. Oral itraconazole has anti-*Aspergillus* activity, and a number of randomized trials in patients with chemotherapy-induced neutropenia have tested its utility as prophylaxis (54–59). These studies have indicated itraconazole to be effective in reducing invasive fungal infections, but mostly the infections prevented were *Candida*, with the rate of aspergillosis in the control groups being too low to determine efficacy. A recent meta-analysis of itraconazole trials suggests that there is a reduction in *Aspergillus* infections but only if a certain threshold of bioavailable dosing is used (60). Two recent trials in allogeneic HCT patients tested prolonged courses of itraconazole (for 100 days) to cover the extended risk period for aspergillosis (61,62). In one trial (61), there was a reduction in the overall rate of invasive fungal infections, but there were too few *Aspergillus* infec-
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Patient group</th>
<th>Prophylaxis type</th>
<th>Antifungal agent</th>
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<td>HIV infection</td>
<td>Secondary</td>
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<td>Pentamidine, dapsone, atovaquone</td>
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<td>Autologous or allogeneic</td>
<td>Primary</td>
<td>TMP-SMZ</td>
<td>Pentamidine, dapsone, atovaquone</td>
<td>Pentamidine, dapsone, atovaquone, TMP-SMZ may be associated with toxicity</td>
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<td>Solid organ transplant</td>
<td>Primary</td>
<td>TMP-SMZ</td>
<td>Pentamidine, dapsone, atovaquone</td>
<td>Pentamidine, dapsone, atovaquone, TMP-SMZ may be associated with toxicity</td>
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<td>Other T-cell-deficient states</td>
<td>Primary targeted</td>
<td>TMP-SMZ</td>
<td>Pentamidine, dapsone, atovaquone</td>
<td>Approach must be individualized according to risk</td>
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tions to determine effectiveness for *Aspergillus* per se. Moreover, there were more deaths in the itraconazole arm. In the second trial (62), a higher dose regimen of itraconazole was used, and excessive toxicity in the itraconazole arm forced premature closure of the study. Although more than 200 patients were enrolled, there was no significant reduction in *Aspergillus* infections, but in a post hoc subset analysis, there was a trend toward reduction in patients who were able to remain on itraconazole. One concern that has been raised is the possibility that exposure to an azole (used in prophylaxis) may attenuate the effectiveness of a polyene given subsequently (63). Another approach is the use of nebulized amphoterin B. Uncontrolled trials suggested a benefit (64), but a randomized trial in patients with prolonged neutropenia showed no benefit (65).

**B. Solid Organ Transplantation**

Lung-transplant recipients are at substantial risk for invasive aspergillosis with a frequency of 8–18% (66,67) and a dissemination rate exceeding 25%. Inhaled amphotericin (either lipid-based or conventional) has been shown in preliminary studies to reduce invasive infection (68,69), but other studies have been inconclusive. Heart-transplant recipients have a risk of ~6% (67). Liver-transplant recipients have an *Aspergillus* risk that ranges between 1% and 6% (67). *Aspergillus* infections are less frequent in other transplant types: 1% kidney, 1% pancreas (67).

**C. HIV Infection**

*Aspergillus* infection is infrequent in patients with HIV infection except in the advanced stages in which CD4+ lymphocyte counts are <50 cells/μL. Clinical signs can be subtle, and aspergillosis is often discovered after death. Thus, there may be a need for targeted prophylaxis in advanced HIV infection, but this has not been tested as yet.

**V. CRYPTOCOCCUS**

**A. HIV Infection**

*Cryptococcus* can cause meningitis, fungemia, or pulmonary infection in advanced HIV disease. This is especially common in patients in developing countries. The risk increases as the CD4 count declines and in patients with high HIV burden. Other risk factors include IV drug use and tobacco use, and blacks seem to be at greater risk (70). Prospective controlled trials indicate that both fluconazole and itraconazole can reduce the occurrence of cryptococcal disease in advanced HIV infection (42). Similar to the reasoning for *Candida*, routine primary prophylaxis is not generally recommended (lack of survival benefit, possibility of drug interactions, risk for emergence of resistance, and cost). Moreover, in contrast to *Candida*, *Cryptococcus* is relatively infrequent. Doses of fluconazole at 100–200 mg/day are generally used for patients with CD4+ T-lymphocyte counts of <50 cells/μL (44). Once infection occurs and the acute infection is successfully controlled, maintenance is generally given for a prolonged course (at least 24 weeks) (Table 3). Continued secondary prophylaxis should be given indefinitely with a potential to stop it if the CD4+ count improves (to 100–200 cells/μL and this improvement is sustained) (71,72) and the HIV burden declines with effective antiretroviral therapy. Maintenance should be restarted if
the CD4+ cell count declines <100 cells/μL again. Fluconazole appears to be more effective than itraconazole for secondary prophylaxis against *Cryptococcus* (73).

**B. Solid Organ Transplant**

*Cryptococcus* infection is infrequent, generally occurring after the first 6 months, especially in patients receiving systemic corticosteroids. Heart-transplant recipients are at greater risk than liver-transplant recipients. No prophylaxis strategy has been tested for this group of patients.

**VI. HISTOPLASMOSIS**

**A. HIV Infection**

Histoplasmosis is a cause of pulmonary infection in certain geographic regions, such as the Mississippi River valley. Inhalation of the organisms can occur particularly after activities that lead to organisms in soil or detritus being stirred up, including working with soil, cleaning chicken coops contaminated with droppings, cleaning or renovating old houses, or exploring caves. These activities should be avoided by individuals with CD4+ T-lymphocyte counts <200 cells/μL (42). Disseminated infection typically occurs in patients with CD4+ T-lymphocyte counts <200 cells/μL. Older age is a risk factor (74). After treatment of acute infection, maintenance therapy with itraconazole should be administered at a dose of 200 mg twice daily, using similar guidelines as for *Cryptococcus* (75) (Table 3). Fluconazole at a dose of ≥200 mg/day can be used as an alternative for secondary prophylaxis in patients who cannot tolerate itraconazole (76). Itraconazole is effective as primary prophylaxis as well in endemic areas (77). In general, it is not recommended for primary prophylaxis except in individuals with CD4+ T-lymphocyte counts <100 cells/μL, who have occupational exposure or who live in a community with a very high rate of histoplasmosis (42).

**B. Other Patient Groups**

*Histoplasma* infections are infrequent occurrences in solid organ-transplant and HCT recipients. Prophylaxis in at-risk geographic areas has not been evaluated.

**VII. COCCIDIOIDES**

**A. HIV Infection**

*Coccidioides immitis* can cause pneumonia in certain geographic areas (especially the Southwest of the United States). Activities that stir up dust and soil in endemic areas lead to exposure and inhalation of organisms and should be avoided by at-risk individuals. The major risk factor is a low CD4+ T-lymphocyte count of <100 cells/μL. Other risk factors implicated to increase risk include black race, and history of oropharyngeal or esophageal candidiasis, and protease-inhibitor therapy lessens the risk (78). After the treatment of the acute infection, maintenance with either fluconazole (400 mg/day) or itraconazole (200 mg twice daily) should be given (79), using similar guidelines for duration as for *Cryptococcus* (42) (Table 3).
B. Other Patient Groups

*Coccidioides* infections are infrequent occurrences in solid organ-transplant and HCT recipients. Prophylaxis in endemic geographic areas has not been evaluated.

VIII. *PNEUMOCYSTIS JIROVECI*

*Pneumocystis jiroveci* (formerly *P. carinii*) can cause interstitial pneumonia in patients with impaired T-cell immunity. Although pneumonia is by far the most frequent manifestation, extrapulmonary manifestations can occasionally occur as well, especially in individuals receiving aerosolized pentamidine (see in what follows); extrapulmonary organs most frequently involved include lymph nodes, spleen, liver, and bone marrow. Patient groups in which *P. jiroveci* infections have been best described include HIV-infected patients, children with acute lymphoblastic leukemia, and HCT recipients. Consensus recommendations suggest that routine primary prophylaxis should be given to all adults with HIV-infected individuals with CD4⁺ T-lymphocyte count of <200 cells/µL or a history of oropharyngeal candidiasis (42,80–82) (Table 3). For HIV-infected children of age <6 years, the appropriateness for prophylaxis may vary with age (82). Secondary prophylaxis should be given indefinitely to HIV-infected individuals who have experienced an earlier episode of *P. jiroveci* pneumonia. HCT patients should receive prophylaxis for at least 6 months (the greatest risk period) and longer if the immunosuppressive regimen continues for treatment of chronic GVHD (10). Children with acute lymphoblastic leukemia should be given prophylaxis (83). For other patient groups, there are no national guidelines, and the appropriateness of primary prophylaxis must be individualized by patient risk group and individual immune status because the underlying immunodeficiency and the type of immunosuppressive regimen may vary considerably and the risk for infection may be difficult to judge. Solid organ-transplant recipients generally are given prophylaxis for 6 months. Other risk groups in which prophylaxis should be considered included immune deficiency conditions, severe protein malnutrition, illnesses that result in low CD4⁺ lymphocyte counts (<200 cells/µL), connective tissue diseases treated by immunosuppressive regimens, and various cancers, especially lymphoreticular cancers, in which treatment regimens result in suppression of T-lymphocyte immunity (e.g., corticosteroids, purine analogs, and anti-T-cell antibodies). Infection can be prevented by trimethoprim–sulfamethoxazole (TMP-SMZ) (84). TMP–SMZ is generally given as one double-strength tablet (160 mg TMP plus 800 mg SMZ) once or twice daily, but three times weekly also is effective. Aerosolized pentamidine given once monthly at a 300-mg dose is an alternative, avoiding some of TMP–SMZ’s toxicities, but is less effective (85,86). Dapsone and atovaquone are also alternatives.

IX. DURATION OF PROPHYLAXIS

For prophylaxis to be effective, the period of vulnerability must be known to determine the duration of prophylaxis. Correction of the underlying host defense that led to the susceptibility is the key determinant. For example, resolution of neutropenia for chemotherapy-induced myelosuppression and control of active HIV infection with recovery of CD4⁺T-lymphocyte counts to >200 cells/µL are two examples.
Notwithstanding, there is a relative paucity of data to clearly determine the optimal duration of prophylaxis. The best data are in HIV-infected patients in which improvement in the CD4+ T-lymphocyte count takes place. Several studies have clearly shown that once a threshold level of a CD4+ count of at least 200 cells/μL is achieved and maintained for at least 3 months, prophylaxis against *P. jiroveci* can be stopped with a low likelihood of recurrence (42). There is a small body of data suggesting that discontinuation of secondary prophylaxis for *Candida* and *Cryptococcus* can also be done when the CD4+ T-lymphocyte count rises to above 100 or 200 cells/μL and remains above this level (76,87–90). There is a paucity of published data for discontinuation strategies for other opportunistic fungal pathogens in advanced HIV disease, but expert opinion suggests that this can be considered when the CD4+ T-lymphocyte count rises to >100 cells/μL with HAART therapy (42).

For patients with hematologic malignancies, few studies have evaluated the duration of prophylaxis. Generally, cessation of prophylaxis in leukemia therapy takes place at the completion of the treatment course and recovery of myelosuppression. For HCT recipients, *Candida* prophylaxis generally is discontinued at engraftment, but some continue during the peak risk period of acute graft versus host disease (4). For solid organ transplantation, various studies have evaluated antifungal prophylaxis durations between 1 and 10 weeks in duration. The general view is that prophylaxis should continue during the peak risk period, which is early after transplant.

**X. INFECTION CONTROL**

As important as pharmacologic agents are in a prophylaxis strategy, even more important are the measures necessary to minimize exposure of susceptible patients to potential pathogens. For pathogens infecting or colonizing patients, measures should be taken to minimize patient to patient transmission.

*Candida* organisms are generally endogenous, and thus no measures are ordinarily needed to prevent acquisition. However, some studies have suggested patient to patient transmission in hospital environments, presumably by healthcare workers, in transplant recipients, leukemia patients, ICU patients, and surgical patients (91–101). Thus, hand washing is an important facet of infection control. Outbreaks of infections in which nosocomial transmission is a possibility should be investigated by the hospital infection-control team. Molecular testing for DNA polymorphisms can be quite useful to determine if one or more strains are present in multiple patients (91). Other point sources of infection that have been identified (other than healthcare workers) include IV solutions, medications, and plastic tubing.

Because *Aspergillus* conidia are exogenous organisms present in the environment and are primarily air-borne, transmission of organisms can occur in the hospital environment during construction, renovation, or other activities in which organisms can be spread and inhaled by susceptible immunocompromised patients. Accordingly, high-efficiency air filtration is important in hospital rooms in which highly susceptible patients reside to prevent outbreaks (102–107). These include HCT recipients, and the routine use of HEPA filters is recommended in consensus guidelines (10,49). The use of high-efficiency masks worn during transport when leaving their rooms may also be helpful (108). The CDC has promulgated environmental guidelines to prevent inadvertent exposures to *Aspergillus* (109).
Recently, patient-shower facilities have been implicated as potential sources of nosocomial *Aspergillus* acquisition (110,111). Avoidance or cleaning procedures have been proposed to reduce the risk to susceptible patients (112).

*Cryptococcus* is associated with IV drug use and tobacco use. Avoidance of invasive disease from this pathogen is yet another reason that such activities should be avoided. As noted earlier, histoplasmosis can be acquired by activities that result in dispersal of organisms from soil into the atmosphere, including working with soil, cleaning chicken coupes contaminated with droppings, cleaning or renovating old houses, or exploring caves. These activities should be avoided by individuals with CD4+T-lymphocyte counts <200 cells/μL in endemic areas (42). Similarly, in coccidiomycosis endemic areas, activities that stir up dust and soil should be avoided by at-risk individuals.

**XI. CONCLUSIONS**

Prophylaxis can be an important strategy to improve antifungal treatment outcomes. Toxicity, emergence of drug resistance, and financial costs may offset the benefits, however. Accordingly, careful selection of at-risk patients is important. A prerequisite for this is an understanding of the spectrum of infectious pathogens, time of vulnerability for infection, and the risk factors that identify susceptible patients. Moreover, monitoring of the strategy over time must be performed to ascertain that the strategy continues to be effective and benefits outweigh risks.

**REFERENCES**


Empirical Therapy of Suspected Infections

I. INTRODUCTION

Invasive fungal infections (IFI) have become a major cause of morbidity and mortality in immunocompromised patients (1–5). The incidence and the mortality rate varies among the different underlying diseases, with relapsed acute leukemia patients and those undergoing allogeneic hematopoietic stem cell transplantation having the highest risk, followed by solid organ transplant recipients, critically ill surgical patients, and premature neonates (6,7). Candida- and Aspergillus species still account for the vast majority of documented infections, but recent epidemiological surveys have revealed the emergence of previously uncommon pathogens that are often less susceptible to conventional antifungal agents (8,9). This shift will have an impact on the choice of appropriate agents for empirical antifungal therapy.

Hospital-acquired infections caused by Candida species—both superficial and deep-seated forms—have increased substantially over the past two decades. Prospective surveillance studies have shown an approximately 500% increase in the rate of nosocomial primary bloodstream infections by Candida species in large teaching hospitals, with Candida now representing the fourth most commonly isolated bloodstream pathogens in the United States (10). Today, the contribution of candidemia has placed this pathogen ahead of more traditional nosocomial pathogens, including Enterobacter spp., Escherichia coli, Pseudomonas aeruginosa, and Klebsiella. At-risk patients are encountered in various settings, but most frequently in intensive care, abdominal surgery, transplantation, and oncology units (11). However, more alarming is the high overall and attributable mortality rate. In a tightly matched historical control study, Wey et al. reported an attributable mortality rate of 38% and a significantly increased duration of hospitalization for survivors (12); these data are in accordance with European studies (13,14).

Although Candida albicans remains the most commonly encountered pathogenic yeast in humans, recent studies have demonstrated a shift towards infection with “non-albicans” Candida species (15–18). In the SCOPE National Surveillance System, 48% of Candida isolates from nosocomial bloodstream infections were non-albicans species, including C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, and C. lusitaniae (19). Although some of these species are considered less virulent
than *C. albicans*, they are also inherently less susceptible to commonly used antifungals, often resulting in a higher failure rate (2). In addition, recent data have revealed important differences between patient groups at-risk (e.g., oncology vs. non-oncology), between institutions, and between countries (2,19). The causes of this changing spectrum are multifactorial and have not yet been evaluated systematically, but the selective pressure resulting from the routine prophylactic use of fluconazole in immunocompromised patients has undoubtedly played a major role in the observed shift (20). However, institution-related differences in anti-infective protocols and treatment-specific factors may have an equally important impact on fungal colonization and subsequent infection.

In prolonged neutropenic cancer patients and allogeneic hematopoietic stem cell transplant recipients, *Aspergillus* species is the most common mold causing fungal disease (4,5). Estimating the incidence of invasive aspergillosis is more problematic given the large variations between institutions and the diagnostic difficulties, especially in patients who have a suppressed inflammatory response. However, the incidence of *Aspergillus* and other filamentous mold infections has been increasing from 18% to 29% following the routine prophylactic use of fluconazole (21).

Finally, a battery of previously uncommon species—both yeasts and filamentous fungi—is increasingly recognized as opportunistic pathogens. Of particular concern is the fact that many of these emerging pathogens display decreased in vitro and in vivo susceptibilities to current antifungal agents, including amphotericin B. These infections caused by *Trichosporon* spp. (22), *Fusarium* spp. (23), *Scedosporium* spp. (24), *Aspergillus terreus*, Zygomycetes (25), and dematiaceous or darkly pigmented fungi carry a high morbidity and mortality rate. These changing epidemiological trends reinforce the requirement of broad-spectrum antifungal agents for empirical treatment in order to prevent breakthrough infections.

II. RATIONALE AND GOAL OF EMPIRICAL THERAPY

The overall case fatality rate of an established invasive opportunistic fungal infection ranges between 40% and 90% with an average of about 60% (2,26). It is evident that the outcome of these infections depends upon the early institution of appropriate therapy (27). However, the major obstacle for the prompt institution of antifungal therapy lies in the difficulty and delay in establishing a definite diagnosis. In many instances, gold standard (invasive) diagnostic tests are not feasible due to cytopenia or due to the critical condition of the patient. In addition, withholding therapy while awaiting time-consuming laboratory tests or clinical confirmation will allow dissemination to occur and will result in a high failure rate. These problems are compounded by the fact that the clinical presentations are often non-specific and suggestive signs and symptoms are frequently absent in the early stages of disease. Also, attempts to make an accurate diagnosis may be thwarted by blunted host defense mechanisms or masked by the high incidence of concomitant clinical processes, especially in transplant recipients (28). By consequence, as evidenced by autopsy surveys, a large number of these infections are never diagnosed nor treated ante mortem (1,7).

Although primary chemoprophylaxis has been effective in the prevention of invasive *Candida* infections, especially in the setting of hematopoietic stem cell transplantation (29,30), it has thus far not been demonstrated to be protective against *Aspergillus* infections (31). Given these diagnostic and preventive shortcomings, a strategy of *empirical* initiation of antifungal therapy has been advocated, especially
in profound and prolonged neutropenic cancer patients. This implies the commence-
ment of antifungal therapy at the first suspicion (though without definite proof) of a
fungal infection. The aim of this approach is to ensure that all patients with a
possible systemic mycotic infection receive appropriate therapy early in the course
of the disease. In neutropenic patients, these infections are usually suspected solely
on the basis of a continuing or recurring fever despite a predefined period of therapy
(4–7 days) to cover likely bacterial pathogens. A similar approach had already been
well established during the early 1970s for the early treatment of bacterial infections
in neutropenia; patients experiencing a primary febrile episode received broad-
spectrum antibiotics empirically because withholding therapy while awaiting culture
confirmation resulted also in a too high mortality rate (32).

III. HISTORICAL PERSPECTIVE OF EMPIRICAL
ANTIFUNGAL THERAPY

A. To Treat Empirically

Although this scenario has become the standard practice of care in many cancer
centers worldwide, the concept has never been firmly validated. Historically, two
controlled trials in support of empirical antifungal therapy in neutropenic patients
were conducted in the late 1970s and the early 1980s. The first study by Pizzo et al.
at the National Institute of Health compared the outcomes of three strategies in 50
neutropenic cancer patients with persistent fever despite seven or more days of
broad-spectrum antibacterial therapy (cephalotin, gentamycin, and carbenicillin) in
the absence of any documentation of infection: discontinuation of antibacterial agents,
no change in therapy, or the addition of amphotericin B deoxycholate 0.5 mg/kg/day
(33). The primary endpoint was defervescence, which indicated that amphotericin B
controlled an occult fungal infection. The addition of amphotericin B decreased the
incidence of breakthrough fungal infections, but there was no improved overall survi-
val for those treated with amphotericin B. The apparent lack of any survival benefit
may be explained by the small number of patients, the insufficient dose of amphotericin
B, or the rather late initiation of antifungal therapy. A second, larger multicenter trial
conducted by The European Organization for Research and Treatment of Cancer
(EORTC) randomized 132 patients who remained febrile after 4 days of antibacterial
therapy to receive empirical amphotericin B (0.6 mg/kg/day) or to continue therapy
without modification (34). No statistically significant difference was seen between the
two groups in terms of defervescence (the primary endpoint of efficacy) and survival,
although fewer individuals died with invasive fungal disease in the amphotericin B
group. Again, the number of documented fungal infections was higher in the patient
not receiving amphotericin B. Interestingly, subgroup analysis revealed a better
clinical response to empirical therapy in specific subgroups: those who were not
receiving any antifungal prophylaxis; those who were severely granulocytopenic
(< 100/μL) at randomization; those with a clinically documented site of infection;
those who were more than 15-year old. These data suggested that early institution
of conventional amphotericin B may reduce the number of proven fungal infections
and that the benefit may depend upon patient characteristics and laboratory values.
B. Or not to Treat Empirically

However, despite these beneficial trends towards reduced fungal morbidity and mortality, it should be noted that none of these studies was blinded and that both trials suffered from inadequate statistical power, especially for subgroup analysis. Moreover, an approach that has been established at the beginning of the 1980s may currently no longer apply to standard medical practice, given drastic changes in anti-neoplastic therapy (e.g., peripheral blood stem cells have replaced bone marrow in hematopoietic transplantation), in prophylactic strategies (widespread use of triazoles), in supportive care measures (use of hematopoietic growth factors; preemptive anti-cytomegalovirus strategies), and in availability of more accurate diagnostic tools (antigen detection and advanced radiological tools). Also, not all neutropenic patients share the same risk of acquiring a pulmonary or disseminated mycotic infection. Risk-adapted strategies need to be developed and validated.

Starting antifungal therapy on the grounds of persistent fever alone will inevitably result in overtreatment, since the incidence of proven invasive fungal infections appears to be less than 10% of all febrile neutropenic episodes, whereas often as much as 40% of the patients receive antifungal therapy. This approach will inevitably induce or select for resistant pathogens and may jeopardize the quality of life of patients and/or the hospital budget. Finally, much controversy still surrounds the optimal timing, dosage, and duration of therapy.

Confirmatory evidence of efficacy of a particular antifungal agent cannot be obtained from empirical studies because of the low yield of proven fungal infections; in this setting, efficacy is often primarily (or even wholly) assessed by defervescence. However, resolution of fever is a highly imprecise and non-specific end point: it may be the result of concomitant successful therapy for a non-fungal infection, may be due to resolution of a drug-induced fever, the successful management of an underlying illness (graft-vs.-host disease and malignancy-induced fever), or following neutrophil recovery. Although the rate of breakthrough infection remains the optimal endpoint when evaluating the efficacy of empirical therapy, such a trial would require an unfeasibly large sample size. Therefore, in the mid 1990s, experts have introduced a composite score, combining defervescence as well as data on survival, breakthrough infections, and safety issues, for the alternative evaluation of “success” (Table 1).

IV. THE EVOLVING EMPIRICAL ARSENAL

Despite the small number of patients enrolled in the first two trials, as well as in other non-randomized studies, empirical institution of amphotericin B deoxycholate has become standard of care for neutropenic cancer patients with persistent fever unresponsive to 4–7 days of broad-spectrum antibacterial therapy. The clinical utility, however, is hampered by the suboptimal safety profile of conventional amphotericin B with dose-limiting renal toxicity and, to a lesser extent, infusion-related side effects.

More recently, new modes of administration (e.g., continuous infusion) (35) and commercially available delivery systems with significantly reduced nephrotoxicity [liposomal amphotericin B (AmBisome®), amphotericin B lipid complex (ABLC, Abelcet®), and amphotericin B colloidal dispersion (ABCD, Amphocil®, Amphotec®)] have resulted in an overall improvement of the therapeutic index.
compared with conventional amphotericin B (36). However, when evaluated in documented *Candida* and *Aspergillus* infections—the two predominant fungal pathogens in neutropenic patients—none of the lipid-associated formulations proved to be more efficacious than conventional amphotericin B (37,38). Their utility in the empirical setting has been challenged in four large, randomized, multicenter trials, comparing these formulations with the parent compound or with each other (39–42). In summary, none of these four studies shows differences in the primary efficacy end point, using a composite score of success (including defervescence, survival, breakthrough fungal infection, and major toxicity). Differences in secondary end points (such as numbers of documented fungal infections and mortality) have been reported in some though not all studies and have been explained by others by imbalances in patient characteristics (43) and/or diagnostic uncertainties (44).

With respect to toxicity, these studies report clear differences between the formulations: all three lipid formulations are considerably less nephrotoxic than is conventional amphotericin B, whereas only therapy with liposomal amphotericin B results in fewer infusion-related toxicity, both in adult and pediatric patients (39,41,42). However, whereas infusional toxicity remains mostly manageable and transient, this is not the case for renal toxicity. Several analyses now underline the increased morbidity, cost, and mortality associated with (the management of) nephrotoxicity (45,46). Unfortunately, at the high current acquisition cost, the routine first-line empirical use of lipid-based formulations is not cost-effective (only demonstrated for liposomal amphotericin B) (47). However, the risk for amphotericin B-associated nephrotoxicity varies considerably among different groups of patients and depends on factors such as sex, dose, and duration of therapy.

### Table 1 Composite Endpoints Used as Primary Endpoints in Recent, Large Trials on Empirical Antifungal Therapy

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Resolution of fever during neutropenia</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Resolution of fever at discontinuation of therapy</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful treatment of any baseline fungal infection</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Survival for 7 days beyond the end of therapy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>No death during study period (any cause)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No breakthrough fungal infection during drug administration or within 7 days of study completion</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>No premature discontinuation of study drug due to toxicity or lack of efficacy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>No withdrawal from study by patient/physician</td>
<td></td>
<td></td>
<td></td>
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<td>+</td>
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</tr>
</tbody>
</table>
pre-existing renal disease, and the concomitant use of other nephrotoxic drugs (in particular cyclosporine or amikacine) (48). For instance, most allogeneic bone marrow transplant recipients who receive immunosuppressants will not tolerate the nephrotoxicity of conventional amphotericin B and may benefit from a first-line empirical therapy with a lipid-based formulation. Alternatively, for low-risk patients, a strategy based on careful monitoring of their renal function while receiving conventional amphotericin B followed by a timely switch to a lipid formulation may be preferred.

Thus, the lack of cost-effectiveness associated with the indiscriminate empirical use of lipid-based formulations at their current acquisition cost and the fact that breakthrough fungal infections as well as toxicity (including renal toxicity) still occur while using these less toxic alternatives, has prompted clinicians and pharmaceutical companies to look for alternatives (Table 2).

The triazole fluconazole represented an attractive alternative by virtue of its favorable pharmacokinetics and excellent safety profile. The agent had been used successfully for the treatment of oropharyngeal and esophageal candidiasis, cryptococcal meningitis and hepatosplenic candidiasis and proved to be an equally effective (but less toxic) alternative to conventional amphotericin B in non-neutropenic candidemia (49). In addition, the prophylactic use of fluconazole prevented colonization and development of superficial Candida infections in patients with leukemia and reduced the incidence of both superficial and systemic fungal infections in bone marrow transplant recipients (29,30,50).

Three randomized, multicenter trials have compared fluconazole to conventional amphotericin B (51–53). Not unexpectedly, all three studies found that fluconazole at a dosage of 400 mg/day (oral or IV) was associated with less infusional toxicity, less nephrotoxicity, and less hypokalemia than was amphotericin B at a dosage of 0.5 mg/kg/day. Therapeutic response, defined by defervescence or by a composite endpoint, was similar for both therapy groups in all three studies and appeared to be adversely influenced by the presence of pneumonia and the persistence of neutropenia. However, the exclusion of patients at increased risk for azole-resistant Candida and Aspergillus infections makes it difficult to assess the efficacy of fluconazole for empirical therapy. In fact, given its widespread and often indiscriminate prophylactic use and considering the emergence of causative organisms with intrinsic or acquired resistance, fluconazole may no longer be a suitable candidate for empirical therapy in many cancer centers (2). An extended-spectrum antifungal agent will likely be needed for patients known to be colonized with Aspergillus species, C. glabrata, or C. krusei; for patients who develop new or progressive pulmonary infiltrates; and for those at high risk for mold infections (prolonged neutropenia, refractory disease, corticosteroids, etc.).

The broad-spectrum triazole itraconazole displays good activity against Aspergillus and Candida species, including fluconazole-resistant strains, but the erratic absorption of itraconazole capsules discouraged clinicians from using it for patients who were suffering from therapy-induced mucositis. The recent development of an oral cyclodextrin solution with increased oral bioavailability in a variety of at-risk patients and the availability of an IV solution has increased the options for the use of this drug (54). The empirical use of itraconazole has been assessed in an open-label randomized trial (55). IV itraconazole (400 mg/day for 2 days followed by 200 mg/day for 5–12 days), followed by oral solution (400 mg/day for 14 days) was compared with amphotericin B (0.7–1.0 mg/kg/day for up to 28 days) in 384 neutropenic patients. Based on a composite endpoint of success,
Itraconazole was as effective as amphotericin B. However, itraconazole was associated with significantly less severe adverse events and fewer patients were withdrawn prematurely from therapy due to adverse drug reactions. In addition, itraconazole offered the flexibility to switch to oral therapy in selected patients. Unfortunately, this study did not evaluate response to treatment in patients with a documented fungal infection and excluded patients undergoing allogeneic stem cell transplantation. Also, clinical experience with this new parenteral formulation is limited and additional data on efficacy in documented *Candida* and *Aspergillus* infections is needed.

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Trial Design</th>
<th>Sample Size</th>
<th>Primary end Point</th>
<th>Antifungal Agents</th>
<th>Response Rate</th>
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<tr>
<td>Viscoli (1996)</td>
<td>Open-label</td>
<td>112</td>
<td>Defervescence</td>
<td>Amphotericin B</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Deoxycholate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fluconazole</td>
<td>=</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>52%</td>
</tr>
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<td>Prentice (1997)</td>
<td>Open-label</td>
<td>338</td>
<td>Safety</td>
<td>Amphotericin B</td>
<td>46%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Deoxycholate</td>
<td>&lt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liposomal</td>
<td>64%</td>
</tr>
<tr>
<td>Malik (1998)</td>
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<td>106</td>
<td>Efficacy (composite)</td>
<td>Amphotericin B</td>
<td>46%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Deoxycholate</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fluconazole</td>
<td></td>
</tr>
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<td>White (1998)</td>
<td>Open-label</td>
<td>213</td>
<td>Efficacy (composite)</td>
<td>Amphotericin B</td>
<td>43%</td>
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<td></td>
<td></td>
<td>Deoxycholate</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Amphotericin B</td>
<td>50%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Colloidal dispersion</td>
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<td>Walsh (1999)</td>
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<td>687</td>
<td>Efficacy (composite)</td>
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<td>Deoxycholate</td>
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<td></td>
<td></td>
<td></td>
<td>Liposomal</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Amphotericin B</td>
<td></td>
</tr>
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<td>Winston (2000)</td>
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<td>Efficacy (composite)</td>
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<td>Wingard (2000)</td>
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<td>Safety</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liposomal</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Amphotericin B</td>
<td>33%</td>
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<td></td>
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<td>Liposomal complex</td>
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<td>Boogaerts (2001)</td>
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<td>Efficacy (composite)</td>
<td>Amphotericin B</td>
<td>38%</td>
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<tr>
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<td></td>
<td></td>
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<td>Walsh (2002)</td>
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<td>849</td>
<td>Efficacy (composite)</td>
<td>Itraconazole</td>
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<td></td>
<td>Liposomal</td>
<td>31%</td>
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<td>Walsh (2004)</td>
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<td>1095</td>
<td>Efficacy (composite)</td>
<td>Voriconazole</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>Casofungin</td>
<td>(33.9%)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liposomal</td>
<td>=</td>
</tr>
<tr>
<td>Walsh (2004)</td>
<td>Double-blind</td>
<td>1095</td>
<td>Efficacy (composite)</td>
<td>Amphotericin B</td>
<td>(33.7%)</td>
</tr>
</tbody>
</table>

*= equivalence >: superior to <: inferior to.

*Voriconazole failed to fulfill protocol-defined criteria for non-inferiority to liposomal amphotericin B.
The new generation triazole voriconazole is available in oral and IV formulation, demonstrates a broad-spectrum of activity, covering both yeasts as well as classic and emerging filamentous fungi (including *Fusarium* species but not the *Mucorales*), and displays a good pharmacokinetic profile (56). The empirical use of voriconazole has been assessed in an open-label, randomized trial that compared voriconazole with liposomal amphotericin B (57). Eight hundred and thirty-seven neutropenic patients, stratified according to their risk for fungal infection and by the use or nonuse of systemic antifungal prophylaxis, were randomized to receive either voriconazole (6 mg/kg bid on the 1st day, then 3 mg/kg twice daily) or IV liposomal amphotericin B (3 mg/kg/day). In that study, voriconazole failed to meet predefined criteria for non-inferiority on the basis of the same composite end point that was previously used by the same group. Exploratory analysis of the five individual components of the composite score favored liposomal amphotericin B except for the prevention of breakthrough infections. The better activity of voriconazole in preventing breakthrough infections—the primary goal of empirical therapy—was particularly evident among high-risk patients (allogeneic transplant recipients and relapsed leukemia). Not unexpectedly, significantly more patients in the voriconazole group suffered from visual disturbances while more patients on liposomal amphotericin B had infusional toxicity and hypokalemia. There was no difference in terms of hepatotoxicity, while more patients in the liposomal group developed mild nephrotoxicity. Interestingly, the incidence of severe renal impairment was similar in both groups. The interpretation of these results is challenging, since the overall success rate according to the composite score, the response according to each of its five components, and the subgroup analyses do not point into the same direction (58,59).

**V. TOWARDS PREEMPTIVE OR EARLY-THERAPY STRATEGIES**

In spite of the presumed advantages of empirical therapy, this strategy can easily be challenged: since not all neutropenic patients and transplant recipients have the same risk of fungal disease, starting therapy solely directed to the management of fever will inevitably result in overtreatment, induction or selection of resistance, increased toxicity, and higher medical costs. A more targeted therapy, directed towards the high-risk patients and based upon a battery of clinical, radiological, and microbiological data that suggest the presence of an invasive mycosis—though still without histopathological proof—would be welcome. However, the feasibility of such a targeted strategy depends upon the availability of rapid and accurate diagnostic tests. Progress may come from the incorporation of new diagnostic tests, such as the sandwich-enzyme-linked immunosorbert assay (ELISA) for the detection of galactomannan (60) or mannan (61) and/or the detection of (pan)fungal DNA by a polymerase chain reaction (62), especially in combination with modern imaging techniques (63). As evidenced in a French study, a simple CT-scan-based approach can already substantially improve the early diagnosis of invasive aspergillosis in neutropenic patients and may have a favorable impact on survival (64). Single- as well as multicenter studies comparing this preemptive or early-therapy approach (e.g., PCR-based) vs. empirical therapy are currently in progress; the impact on infection-related mortality still needs to be demonstrated in prospective studies (65). If such a strategy should prove to be sufficiently robust to withhold therapy in persistently febrile neutropenic patients, then we will steer away from an empirical approach towards a pre-emptive approach.
VI. FUTURE PERSPECTIVES

While such new treatment algorithms are being properly validated, the empirical approach continues to be the standard of care. Given the non-specific nature of “persistent neutropenic fever”, an empirically used antifungal drug should not only be efficacious, but above all safe (Table 3). If it were not for dose-limiting renal toxicity, amphotericin B deoxycholate would have been used at a higher dose and longer duration for empirical therapy. An excellent safety and toxicity profile is of the utmost importance since many of these patients are receiving concomitantly nephrotoxic agents or drugs that are metabolized through the cytochrome P450 enzyme system. The candins (such as caspofungin, anidulafungin, and micafungin), a class of antifungals that target the fungal cell wall instead of the plasma membrane, appear to be very attractive candidates (66). Confirmatory data of the efficacy in the treatment of Candida and Aspergillus infections is currently available for caspofungin some agents (67,68). In addition, the drugs are very well tolerated and prove to be safe in patients with a wide spectrum of diseases and many concomitant medications (69). Recently, a randomised, double-blind, multicenter trial involving more than 1100 patients compared the efficacy and safety of caspofungin (loading dose of 70 mg followed by 50 mg/d) with liposomal amphotericin B (AmBisome® 3 mg/kg/d) for empirical antifungal therapy of persistently febrile neutropenic patients. Eligible patients were randomised by risk factor (allogeneic transplants and relapsed leukaemia were considered high risk) and previous antifungal therapy. The primary efficacy endpoint was percentage of treated patients (modified intent-to-treat) with a successful outcome as defined by a classical composite endpoint. In this large study, caspofungin proved to be non-inferior to AmBisome. In addition, caspofungin was more successful in the treatment of baseline fungal infections and proved to be significantly better tolerated (70). Empirical studies with echinocandins are currently being conducted.

Concomitantly with antifungal therapy, one should also try to restore or enhance host defense mechanisms. For some patients this is easy to obtain, while it remains problematic in others. In haematological patients the depth and duration of neutropenia remain the major independent predictor of outcome.

Table 3 Profile of the Ideal Agent for Empirical Antifungal Therapy

| Fungicidal activity against the most common fungal pathogens, including non-albicans and azole-resistant Candida spp. and all pathogenic Aspergillus spp.
| Activity against emerging yeasts and filamentous fungal pathogens
| No antifungal resistance/no potential for cross-resistance with agents commonly used in prophylaxis
| Excellent safety and toxicity profile allowing prolonged therapy without dose reductions
| No potentially hazardous drug-drug interactions
| Linear and predictable pharmacokinetics
| Affordable at the recommended dose |
Increasing the number and function of circulating granulocytes and monocytes by colony-stimulating factors or cytokines has shown favorable results in vitro and in animal experiments, but confirmation from clinical studies is lacking (71). The infusion of donor elicited granulocytes remains investigational, especially in the empirical setting (72). Achieving remission of the underlying disease may be the best option for survival.

VII. CONCLUSION

In conclusion, although guidelines (including the Infectious Diseases Society of America) state that conventional amphotericin B remains the drug of choice for the empirical treatment of neutropenic fever not responding to a predefined period of antibacterial therapy (73), the lipid formulations of amphotericin B, as well as voriconazole and the new formulations of itraconazole, can be used as alternatives within certain clinical boundaries. However, only amphotericin B deoxycholate, liposomal amphotericin B, the IV and oral solutions of itraconazole, and caspofungin are currently approved by the U.S. Food and Drug Administration (Table 4). Policies of the timing and specific agents are most often made on an institutional basis and are frequently influenced by cost considerations. The implementation of validated, reliable diagnostic tests and the ongoing evaluation of new antifungal compounds may significantly impact on our future thinking about empirical or pre-emptive therapy in persistently febrile neutropenic patients. New therapeutic strategies should be based on risk-assessment with consideration of both efficacy and safety issues. At this moment empirical antifungal therapy in neutropenic patients is at least sub-optimal and improvement by novel strategies, safe and potent antifungal agents, and adjunctive immunotherapy seems hardly needed.

REFERENCES


Table 4  FDA-Approved Drugs for Empirical Therapy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosing regimen used in controlled trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B deoxycholate</td>
<td>0.6–1.0 mg/kg/day (IV)</td>
</tr>
<tr>
<td>Liposomal amphotericin B</td>
<td>3 mg/kg/day (IV)</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>400 mg/day for 2 days followed by 200 mg/day for 5–12 days (IV), followed by oral solution 400 mg/day for 14 days</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>70 mg loading dose, followed by 50 mg daily dose</td>
</tr>
</tbody>
</table>


19. Pfaller MA, Jones RN, Messer SA, Edmond MB, Wenzel RP. National surveillance of nosocomial blood stream infection due to species of Candida other than Candida albicans:


I. INTRODUCTION

Enormous strides have been made in the management of invasive fungal infections (IFIs). New drugs, randomized trials testing comparative efficacy and safety, and development of adjunctive measures to complement pharmacologic agents have combined to improve treatment outcomes. Sufficient studies have been done to allow evidence-based consensus guidelines for Candida, Aspergillus, and Cryptococcus to be developed. For other pathogens, case series with different treatment modalities permit guidance of treatment preferences. In this chapter, treatment strategies of the most common IFIs will be summarized.

II. CANDIDIASIS

Amphotericin B has been the gold standard of therapy for Candidiasis for decades. Until the past decade, however, there were few randomized trials. With the advent of new antifungals, comparative trials have allowed clinicians a better understanding of the relative merits of various treatment options. There are several excellent options for antifungal therapy supported by randomized trials (Table 1).

A. Amphotericin B Deoxycholate

Despite methodologic problems in interpretation of in vitro susceptibility testing for amphotericin B, several conclusions appear warranted from the data available (reviewed in Refs. 1 and 2). Resistance is infrequent in isolates of Candida albicans, Candida tropicalis, and Candida parapsilosis, while resistance is not uncommon in isolates of Candida lusitaniae. Some isolates of Candida glabrata and Candida krusei appear to be less susceptible and may require higher doses of amphotericin B (1 mg/kg/day).
<table>
<thead>
<tr>
<th>Study</th>
<th>Percent Success</th>
<th>Toxicity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rex (13)</td>
<td>70</td>
<td>79</td>
<td>Favors fluconazole (less nephrotoxicity)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gaps in fluconazole coverage</td>
</tr>
<tr>
<td>Anaissie (5)</td>
<td>68</td>
<td>63</td>
<td>Favors ABLC (less nephrotoxicity)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Never published; no survival difference</td>
</tr>
<tr>
<td>Phillips (12)</td>
<td>50</td>
<td>56</td>
<td>Favors fluconazole</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gaps in fluconazole coverage</td>
</tr>
<tr>
<td>Rex (31)</td>
<td>56(^b)</td>
<td>69</td>
<td>Favors fluconazole (less nephrotoxicity)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Shorter time to negative blood cultures with combination</td>
</tr>
<tr>
<td>Mora-Duarte (28)</td>
<td>62</td>
<td>73</td>
<td>Favors caspofungin (less nephrotoxicity)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Composite success criterion contained both efficacy and toxicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>parameters, most of difference in failure was due to toxicity</td>
</tr>
</tbody>
</table>

\(^a\)The combination of amphotericin B with fluconazole.

\(^b\)800 mg/day.
There is a considerable body of treatment experience of using amphotericin B for therapy of systemic Candidiasis in various patient populations, including both neutropenic and non-neutropenic patients (1–3).

B. Amphotericin B Lipid Formulations

Intolerance to amphotericin B deoxycholate due to infusional reactions or nephrotoxicity has limited its utility. It has been especially difficult to use in patients receiving calcineurin inhibitors (4). Amphotericin B lipid complex (ABLC) given at a dose of 5 mg/kg/day has been shown to be as effective as amphotericin B deoxycholate at a dose of 0.6–1.0 mg/kg/day (5) (Table 1). Response and survival rates were not different, but there was considerably less renal toxicity. There are no randomized trials for the other licensed lipid formulations for Candidiasis. However, there is sufficient experience with the other lipid formulations to permit a reasonable conclusion that all three lipid formulations are acceptable alternatives to amphotericin B deoxycholate.

Although the lipid amphotericin B formulations are considerably less toxic they are also much more expensive and they have not been shown to improve the rates of response or survival. Accordingly, there has been considerable debate as to when and in whom the lipid formulations should be used (6–8) and when used which one (9). Certainly, the lipid formulations are excellent substitutes for patients intolerant of amphotericin B deoxycholate. Additionally, the intolerance of the deoxycholate formulation in patients receiving concomitant nephrotoxins, those with antecedent renal impairment, and in those receiving prolonged courses of doses approximating 1 mg/kg/day make such individuals good prospects for the lipid formulations in preference to amphotericin B deoxycholate upfront.

C. Fluconazole and Other Azoles

In vitro susceptibility assays have been standardized for azole testing. Most Candida species are highly susceptible to fluconazole (defined as MIC ≤8 mg/L) (1,2,10). However, several species are not reliably inhibited by fluconazole. Candida krusei and Candida dubliniensis isolates are resistant to fluconazole. Isolates of C. glabrata are less susceptible to fluconazole and are characterized as “susceptible-dose dependent (S-DD),” because higher concentrations are required for in vitro inhibition (MIC 16–32 mg/L). Some C. glabrata strains are resistant (≥64 mg/L). Fortunately, C. krusei and C. dubliniensis infections are infrequent. However, C. glabrata infections account for 10–20% of invasive Candida infections in various series and these appear to be increasing over time. This increase was first noted coincident with the introduction of fluconazole into clinical practice, suggesting that its widespread use has led to selection of less susceptible fungal organisms. Fortunately, bloodstream isolates of C. albicans have remained largely susceptible to fluconazole now a decade later after its introduction into clinical practice (11).

The standard dose of fluconazole in the treatment of Candida mucosal infections is generally 100–200 mg/day. For candidemia caused by susceptible Candida spp., doses of 400 mg are recommended. For children, dosing for serious Candida infections is 6 mg/kg/day. For C. glabrata, doses of 12 mg/kg/day are necessary.

Randomized trials and case-controlled studies have shown fluconazole (400 mg/day) to be highly effective as therapy of systemic Candida (12,13) (Table 1) with response and survival rates comparable to amphotericin B (0.5–0.6 mg/day). Time to clearance of Candida bloodstream infections is similar although slightly slower than with
amphotericin B. Most treatment trials were conducted in non-neutropenic patients, and there is a paucity of data in neutropenic patients. Thus, many experts believe amphotericin B to be preferable to fluconazole for the neutropenic patient with systemic Candidiasis.

Resistance to fluconazole occurs through several mechanisms (reviewed in Refs. 14 and 15): alteration of the target enzyme (14-alpha-sterol-demethylase encoded by ERG11) by mutation or overexpression of ERG11 or up-regulation of efflux transporters (encoded by CDR1, CDR2, and MDR1 genes). Emergence of resistance to C. albicans has been seen largely in patients with advanced acquired immunodeficiency syndrome with low (and declining) CD4+ T lymphocyte counts, where low doses of fluconazole (50–200 mg/day) were given for many months to suppress recurrent oropharyngeal candidiasis. This experience has contrasted to the experience in leukemia and BMT patients where shorter courses of higher doses (400 mg/day) are generally given and restoration of host defenses (neutrophil recovery and/or recovery of cell-mediated immune responses) generally occurs. It seems likely that these different trajectories of host differences are important in understanding the reasons for these different experiences. Notwithstanding, several outbreaks of Candida bloodstream infections have been reported in BMT patients receiving fluconazole prophylaxis by fluconazole resistant organisms (16–18). Fortunately, these have been infrequent; moreover, unpublished data of investigations of these outbreaks suggest most infections were caused by only one or several strains, suggesting a common source. However, the resistance story in advanced HIV infection should serve as a cautionary note for potential similar concerns which may pertain to patients with poor T-cell immune reconstitution after transplantation or conditions associated with poor T-cell function. Several instances of fluconazole-resistant C. albicans fungemia in leukemia patients have also been described (19,20).

Several azoles, in addition to fluconazole, have excellent activity against Candida spp. These include clotrimazole, ketoconazole, itraconazole, and voriconazole. Controlled trials have shown these agents to be effective as therapy for oropharyngeal candidiasis. Because of lack of systemic effect clotrimazole is generally used only for mucosal infections. Ketoconazole has largely been replaced by fluconazole because of variable bioavailability and dependence on gastric acidity for maximal absorption. Voriconazole has been shown to be as effective as fluconazole for the treatment of Candida esophagitis in HIV patients (21) and is being evaluated in a randomized trial for systemic candidiasis.

D. Caspofungin

Caspofungin has excellent in vitro activity against Candida (including azole-resistant species) (22–26). In vitro susceptibility studies have raised a concern of lower activity against C. parapsilosis and Candida guilliermondii, but whether these in vitro findings are clinically important is at present unclear.

Two randomized trials, one in Candida esophagitis in HIV-infected patients (27) and the other in systemic Candidiasis in immunocompromised adults (28) have demonstrated excellent clinical activity, comparable to amphotericin B, with substantially less toxicity than amphotericin B. In both trials response and survival rates were comparable. Caspofungin was associated with a lower rate of toxicities.

Caspofungin clearly is an excellent choice for Candidiasis, but caution is necessary if the patient is on cyclosporine, due to a potential for hepatotoxicity noted in normal volunteers receiving both concomitantly. One option is to switch cyclosporine to tacrolimus if the clinician deems that acceptable. Cyclosporine appears to
increase caspofungin blood levels up to 30%. Caspofungin increases tacrolimus levels by 20% (but has no effect on cyclosporine levels). Dosing in children is still being worked out, although preliminary results indicate that a dose of at least 1 mg/kg is required and computer modeling suggests dosing based on body surface area achieves more predictable blood concentrations (Walsh, unpublished observations).

Two other echinocandins are in clinical trials. Micafungin appears to have a similar antifungal spectrum of activity and toxicity profile as caspofungin (29). No hepatic transaminase elevations have been noted in patients receiving micafungin concomitant with cyclosporine, unlike caspofungin. Anidulafungin is also in clinical trials (30).

E. Combination Therapy

Rex et al. (31) evaluated combination therapy for the treatment of candidemia in non-neutropenic adults comparing amphotericin B plus fluconazole vs. high doses (800 mg/day) of fluconazole alone. There was a non-significant trend to higher success rates with the combination therapy compared to monotherapy (69% vs. 56%, \( p = 0.08 \)) and better clearance of the bloodstream infection by the combination therapy \( (p = 0.02) \). Unfortunately, there was also more toxicity with the combination therapy mitigating the net benefit. An analysis examining the association of patient physiological status with response found that the extra benefit offered by dual therapy seemed to be most evident for patients in whom treatment factors may be most germane rather than the host status to outcomes and least in patients very ill (in which no therapy has a chance to help) and in patients least ill (in which any therapy will suffice) (31).

F. Adjunctive Measures

Most experts recommend removal of central venous catheter or any other foreign body in an infected patient wherever possible (1,2). Candida parapsilosis is frequently associated with vascular catheters and catheter removal is especially important for this infection. Penetration by antifungal agents of biofilms on catheters may be impeded, hindering clearance of pathogenic fungi. Several studies suggest more rapid clearance of organisms from the bloodstream with catheter removal, although a recent evidence review questioned how well founded this recommendation is in empirical data (32). Clearly, there is a role for clinical judgment necessary to ascertain when and in whom catheter removal should be done (33).

G. Practical Considerations

With several excellent therapeutic options, there are practical considerations that influence treatment decisions. With its oral formulation, favorable safety profile, relative low cost, and a proven track record in clinical trials, fluconazole has considerable advantages over the other options. However, the gaps in its activity spectrum pose a dilemma for clinicians making treatment decisions before the isolate is known to be fluconazole susceptible. Several days may pass from notification of a positive culture before the isolate is speciated. Susceptibility testing is not widely available at present and testing (even if available) adds even more time. Knowledge of what species the pathogen is provides a good estimate of susceptibility, with C. krusei, C. glabrata, and C. dublienis not reliably susceptible, and all others susceptible (1,2). Accordingly, one option for serious infections is to start with caspofungin or
one of the amphotericin B formulations to provide broad coverage. Once the organism is speciated and the patient stabilizes, one may either continue the initial therapy or can change to fluconazole for susceptible isolates to complete the course of therapy.

The duration of therapy is problematic with no clear guidance from published literature. Generally, one should continue treatment until resolution of signs and symptoms, clearance of cultures, improvement of radiologic manifestations, and improvement of the host defenses that contributed to the infection.

III. ASPERGILLOSIS

There are three general categories of Aspergillus infections: invasive, saprophytic, and allergic. This section will address treatment approaches of invasive infection only. Early detection and prompt initiation of antifungal therapy are key determinants of success. To date, there are few randomized treatment trials of aspergillosis (Table 2).

A. Amphotericin B Deoxycholate

Most Aspergillus isolates are susceptible to amphotericin B. However, there are notable exceptions. Most Aspergillus terreus isolates demonstrate in vitro resistance to amphotericin B and do not respond well to amphotericin B. Similarly, Aspergillus ustus and Aspergillus versicolor may be resistant to amphotericin B.

As with Candidiasis, amphotericin B deoxycholate has long been the gold standard for primary therapy of invasive aspergillosis and until recently was the only licensed therapy for primary therapy of invasive aspergillosis in the United States. Unfortunately, amphotericin B deoxycholate must be given in high doses and the prolonged treatment courses needed are poorly tolerated in many individuals (due to nephrotoxicity and other toxicities) and success rates are poor.

B. Lipid Formulations of Amphotericin B

The lipid amphotericin B products are also effective against Aspergillus, although higher doses must be administered compared to amphotericin B deoxycholate. Two lipid formulations (ABLC and ABCD) were first licensed because of their efficacy as salvage therapy in patients with progressive Aspergillus infection or intolerance for amphotericin B, with responses in approximately 40%. Two randomized trials have evaluated lipid amphotericin B formulations as primary therapy (Table 2).

In one study (34), liposomal amphotericin B was evaluated in a randomized trial of two doses (1 and 4 mg/kg/day). Patients were required to have probable or proven invasive aspergillosis. No differences in either response rate or survival were seen, but it is important to note that the sample size was very small and the statistical power to detect a difference if present was inadequate to reject the null hypothesis; moreover, the complete response rate in the 4 mg/kg/day group was higher than in the 1 mg/kg/day group. Many experts believe this trial is inconclusive in recommending a lower dose schedule for the therapy of invasive aspergillosis.

In a second trial, ABCD at a dose of 6 mg/kg/day was compared to amphotericin B deoxycholate in a dose of 1–1.5 mg/kg/day (35). There was no difference...
**Table 2** Randomized Comparative Trials for Therapy of Invasive *Aspergillus* Infections

<table>
<thead>
<tr>
<th>Author</th>
<th>Test agent</th>
<th>Comparator</th>
<th>Test agent</th>
<th>Comparator</th>
<th>Response rates</th>
<th>Survival rates</th>
<th>Toxicity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ellis (34)</td>
<td>L-amph 1 mg/kg</td>
<td>L-amp 4 mg/kg</td>
<td>L-amp 4 mg/kg</td>
<td>64%</td>
<td>48%</td>
<td>43% (at 6 mos)</td>
<td>37% (at 6 mos)</td>
<td>Favors liposomal amphotericin B</td>
</tr>
<tr>
<td>Bowden (35)</td>
<td>ABCD 6 mg/kg</td>
<td>Amph (1–1.5 mg/kg)</td>
<td>35%</td>
<td>35%</td>
<td>50%</td>
<td>45%</td>
<td>Favors ABCD (less nephrotoxicity but more infusion reactions)</td>
<td>Improved tolerance did not translate into response or survival advantage</td>
</tr>
<tr>
<td>Herbrecht (36)</td>
<td>Vori 6 mg/kg LD, then 4 mg/kg Q12H</td>
<td>Amph (1–1.5 kg)</td>
<td>53%*</td>
<td>32%*</td>
<td>71% (at 12 weeks)</td>
<td>58% (at 12 weeks)</td>
<td>Favors voriconazole</td>
<td>Amphotericin B was poorly tolerated and most received other lipid amphotericin B formulations or itraconazole in the comparator arm</td>
</tr>
</tbody>
</table>

*p < 0.05.

**Abbreviations**: L-amp, liposomal amphotericin B; ABCD, amphotericin B colloidal dispersion; Amph, amphotericin B deoxycholate; Vori, voriconazole.
in response or survival rates between the two arms. However, the ABCD therapy was associated with lower rates of nephrotoxicity.

C. Aerosolized Amphotericin B

Inhaled amphotericin B and lipid formulations of amphotericin B have been tested in small numbers of patients either as prophylaxis or as adjunctive drug delivery to infected tissue. This is an appealing idea which has the potential to spare the patient systemic toxicity and deliver higher drug concentrations. Because of limited data, this remains investigational at present.

D. Voriconazole

A randomized trial compared amphotericin B deoxycholate and voriconazole as first-line therapy of invasive aspergillosis in immunocompromised adults (36). Voriconazole was dosed 6 mg/kg at 12-hour intervals for the first two doses, then followed by 4 mg/kg twice daily for at least 1 week. After stabilization, the patient could be switched to the oral formulation at a dose of 200 mg/kg twice daily.

Amphotericin B was dosed at 1–1.5 mg/kg/day. Other licensed antifungal therapy was allowed in both arms for progression or intolerance. Voriconazole was found to be more effective than amphotericin B, with higher response rates (53% vs. 32% complete or partial response at 12 weeks) and better overall survival (71% vs. 58% at 12 weeks). In addition, voriconazole was associated with fewer adverse events and greater tolerance. Based on these data, voriconazole is now considered the drug of choice for first-line therapy of aspergillosis. An important consideration for practical use is knowledge of drug interactions with a multitude of concomitant medications that are metabolized by cytochrome P450, which may need to be monitored or doses adjusted.

E. Itraconazole

This azole has long been known to have anti *Aspergillus* activity. Various case series have shown it to be an effective therapy for invasive aspergillosis (37–39) for both initial and salvage therapy. Unfortunately, there are no controlled trials testing it in comparison with amphotericin B or other treatment options. Thus, today we remain uncertain as to its role relative to other drugs. Several caveats pertain. Many of the patients treated with itraconazole were relatively less immunocompromised. Use of either of the two oral formulations (capsules or solution) is plagued by erratic absorption. The oral solution is better absorbed than the capsule (especially in the fasted state) and is preferred. Several studies suggest a correlation between trough plasma levels and response. Initially, trough concentrations of ≥0.25 mcg/mL were targeted, but more recent data suggest that targeting of trough concentrations of ≥0.5 mcg/mL are better associated with response (40). With the heterogeneity of bioavailability, measurement of plasma drug levels is advisable to ensure therapeutic levels are being achieved. The IV formulation is well tolerated and gets around some of these concerns. However, its excipient is a cyclodextrin, which is renally cleared (in contrast to the hepatic clearance of itraconazole) and accumulation in renal failure occurs and this may pose a safety concern. Thus, the IV formulation should be avoided in patients with renal failure (creatinine clearance of <30 cc/min). A negative inotropic effect has been noted. This is a concern for patients with antecedent
cardiac compromise and those receiving chemotherapeutic agents which are cardio-
toxic in their own right. For the IV formulation, the dose is 200 mg twice daily for
2 days, then 200 mg daily (for up to 12 days). The oral dose is 2.5 mg/kg twice daily
for the oral solution. In addition to the drug interaction issue noted for voriconazole,
the issue of negative inotropic effects and variable bioavailability are impediments
for clinical use.

There are rare reports of itraconazole resistance of *Aspergillus fumigatus*
to itraconazole (41,42). An amphotericin B formulation or echinocandin should be
considered for such circumstances.

F. Other Azoles

Posaconazole and ravuconazole are in clinical trials. In vitro susceptibility studies
and anecdotal experience suggest roles for these agents.

G. Caspofungin

Caspofungin was first licensed for use in salvage therapy of invasive aspergillosis,
refractory to amphotericin B, or in patients intolerant to licensed therapy. Response
rates of 40–45% have been noted (43). To date, there are no data using caspofungin
as first-line therapy. Other echinocandins are in development, including micafungin
and anidulofungin.

H. Combination Therapy

A number of labs have demonstrated additive effects of a polyene plus an echinocan-
din (44–46) or voriconazole plus an echinocandin (47–51). There are some limited
clinical data with this approach (52,53,53A). Not all combinations are beneficial
and thus caution is urged until more definitive study is undertaken (54).

I. Adjunctive Measures

The use of hematopoietic growth factors (G-CSF or GM-CSF) have been used to
speed neutrophil recovery for infected neutropenic patients. Gamma interferon has
also been used in patients with chronic granulomatous disease to reverse the phago-
cytic defect. Although intuitively appealing, there is a paucity of data to clearly
define the use of growth factors or immune modulators in these settings. Many clin-
icians administer them in treatment of serious infections, especially in those poorly
responsive to antifungal therapy alone.

Similarly, the use of granulocyte transfusions is also attractive. Some pilot data
using transfusions of large numbers of granulocytes obtained from donors pretreated
with G-CSF have shown that circulating neutrophil counts in recipients can be greatly
increased for a day or two and there is some suggestion of improvement of refractory
infection (55). They have also been used in patients with chronic granulomatous disease.
However, to date the true benefit of this approach is not established (56).

Surgical excision should be considered in patients with pulmonary *Aspergillus*
infections, where cavitary or necrotic tissues are persistent or where lesions are
centrally located adjacent to great vessels or pericardium and catastrophic hemor-
rhage may occur due to invasion of pulmonary vasculature, a single lesion causing
hemoptysis, or lesions eroding into pleural space, ribs, or pericardium (57). If
possible, reduction in the immunosuppressive treatment regimen is desirable to enhance host defenses.

J. Approaches to Aspergillosis Involving Sites Other than the Lungs

Sinusitis is an occasional manifestation and may be isolated or associated with either pulmonary or cerebral involvement. Surgical debridement is a key component of the management. For cerebral aspergillosis, case reports show some efficacy of various anti-*Aspergillus* agents, but overall mortality rates remain high (generally 80% or greater). There may be a role for surgical resection of amenable cerebral lesions, but this has not been formally studied. *Aspergillus* endocarditis should be approached with early aggressive surgical resection where feasible, along with antifungal therapy.

K. Practical Considerations

Based on clinical trial data, voriconazole is the first-line treatment of choice. There are certain patients for whom voriconazole may not be an option; these include patients with hepatic dysfunction or those with renal impairment and cannot receive an oral medication (IV voriconazole is not recommended due to the cyclodextrin accumulation during renal failure); for those an amphotericin B formulation would be indicated, with safety considerations giving considerable weight to a lipid formulation. For infections caused by *A. terreus*, an azole is preferred over a polyene due to susceptibility considerations.

For infection progression or treatment intolerance, a change to one of the lipid formulations of amphotericin B or caspofungin would be an excellent choice. When used, a lipid amphotericin B it should be given in a dose of 4–6 mg/kg/day. Some would advocate combination therapy as salvage therapy (either addition of caspofungin or change to an amphotericin B formulation plus caspofungin) based on the strength of the in vitro data and pilot clinical data.

In addition to pharmacologic therapy, resection of localized infarcted tissue should be considered as noted above and reduction of immunosuppressive therapy should also be considered. The use of myeloid growth factors or granulocyte transfusions should be strongly considered in persistently neutropenia with progressive infection.

The duration of therapy is not well worked out. As with Candidiasis, treatment until resolution of attributable clinical signs and symptoms, resolution, or maximal improvement of radiographic signs, and to the extent possible, improvement of host defenses should be done. The use of galactomannan antigen testing may be useful in monitoring clinical response along with clinical and other laboratory monitoring, but this has as yet not been well studied. At this time, much more experience is needed to clarify whether this is both practical and useful.

Important to note is the high likelihood of recurrence of infection in the event further compromise of host defenses occurs, through relapse of the underlying disease, further immunosuppressive or myelosuppressive therapy, or use of more intensive immunosuppressive therapy.

IV. CRYPTOCOCCUS

Treatment approaches vary according to the underlying state of the patient’s immune status (normal or immunocompromised) and the form of infection (CNS
This allows more intensive therapy (which is also more toxic) for those with serious disease and permits more convenient and less toxic oral therapy for those at lower risk for serious sequelae. Treatment is generally broken down into three phases: induction, consolidation, and secondary prophylaxis (sometimes referred to as chronic maintenance).

A. Pulmonary and Non-CNS Disease Without CNS Involvement in Non-Immunocompromised Patients

Few studies have been performed but expert opinion has been codified in IDSA treatment guidelines (58). For the immunocompetent individual who is asymptomatic, one can consider either observation or 3–6 months of fluconazole at a dose of 200–400 mg/day (58). If mild to moderate symptoms are present, then 6–12 months of fluconazole is advised. Itraconazole (200–400 mg/day) (but not ketoconazole) is an acceptable alternative. Patients with severe disease manifestations or those who are immunocompromised should be managed like those with CNS disease.

B. Meningitis or Pulmonary Infection in Immunocompromised Patients

The CNS infection in the form of meningitis is most commonly encountered in patients with advanced HIV infection; thus, the most well-developed trials have been conducted in this patient population (Table 3). For pulmonary disease associated with mild or moderate symptoms, treatment by either fluconazole or itraconazole in doses of 200–400 mg day can be given (58). For patients with more severe disease (pulmonary disease with severe symptoms or meningitis), an initial “induction” course of the combination of amphotericin B at a dose of 0.7–1.0 mg/kg/day with fluconazole 100 mg/kg/day should be used (58–62). The duration of the initial “induction” therapy has varied in different studies (generally 2–3 weeks), but amphotericin B should be continued at least until symptoms are controlled. Liposomal amphotericin B at a dose of 6 mg/kg/day for 2–3 weeks induction followed by fluconazole 400 mg day is as effective as amphotericin B in two randomized trials (63,64). Generally, after the induction course, a “consolidation” course of fluconazole given in a dose of 400–800 mg/kg/day is given for 8–10 weeks (65). The role of newer azoles has not been defined. The echinocandins are not active.

There have been few trials to determine if adjunctive measures are useful. Elevations in intracranial pressure can be harmful and should be monitored. Acetazolamide given to reduce intracranial pressure was not helpful in a small trial and was associated with considerable toxicity (66). Steroids and mannitol also do not appear to be helpful. Drainage by repeated lumbar punctures may be necessary if pressures exceed 200 mm of water. In vitro studies suggest G-CSF and GM-CSF may be helpful in enhancing azole killing (67), but this has not been tested in clinical trials. Other proinflammatory cytokines, including IL12, TNF alpha, and gamma interferon, are important in host responses (68), but these observations have not been exploited in therapeutic interventions.

Because of a high likelihood of recurrence in HIV infection, once initial induction and consolidation therapy is completed, chronic suppressive therapy should be instituted after control of the acute infection (69). Either fluconazole (200–400 mg day) or itraconazole (200 mg once or twice daily) is acceptable for chronic suppressive therapy, but fluconazole is more effective than itraconazole (70) and once weekly
Table 3  Randomized Trials for Therapy of Cryptococcosis

<table>
<thead>
<tr>
<th>Author</th>
<th>Indication</th>
<th>Test agent</th>
<th>Comparator Test agent</th>
<th>Response rates</th>
<th>Survival rates</th>
<th>Time to CSF clearance (day)</th>
<th>Toxicities</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larsen (60)</td>
<td>Induction therapy</td>
<td>Amphotericin B plus fluconazole</td>
<td>Fluconazole</td>
<td>100%</td>
<td>43%</td>
<td>100%</td>
<td>33%</td>
<td>15.6</td>
</tr>
<tr>
<td>Bennett (61)</td>
<td>Induction therapy</td>
<td>Amphotericin B plus fluconazole</td>
<td>Amphotericin B</td>
<td>68%</td>
<td>47%</td>
<td>76%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1 week&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Saag (62)</td>
<td>Induction therapy</td>
<td>Amphotericin B</td>
<td>Fluconazole</td>
<td>40%</td>
<td>34%</td>
<td>86%</td>
<td>82%</td>
<td>42</td>
</tr>
<tr>
<td>Leenders (63)</td>
<td>Induction therapy</td>
<td>Liposomal amphotericin B</td>
<td>Amphotericin B</td>
<td>80% (at 3 weeks)</td>
<td>86% (at 3 weeks)</td>
<td>93% (at 10 weeks)</td>
<td>85% (at 10 weeks)</td>
<td>73% at 3 weeks</td>
</tr>
<tr>
<td>Study</td>
<td>Setting</td>
<td>Treatment</td>
<td>Response 1</td>
<td>Response 2</td>
<td>Response 3</td>
<td>Response 4</td>
<td>Response 5</td>
<td>Comparison</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------</td>
<td>-----------------------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>Hamil (64)</td>
<td>Induction</td>
<td>Liposomal amphotericin B</td>
<td>86%/94%</td>
<td>87%</td>
<td>86%/90%</td>
<td>88% (at 10 weeks)</td>
<td>63%/54%</td>
<td>54% at 2 weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(dosed at 3 mg/kg/day and 6 mg/kg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amphotericin B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>No decrease</td>
<td>No decrease</td>
<td>84%</td>
<td>100%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Newton (66)</td>
<td>Adjunct</td>
<td>Acetazolamide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>van der Horst (59)</td>
<td>Induction therapy</td>
<td>Amphotericin B plus fluconazole</td>
<td>78% at 2 weeks</td>
<td>83% at 2 weeks</td>
<td>94% at 2 weeks</td>
<td>95% at 2 weeks</td>
<td>60% at 2 weeks</td>
<td>51% at 2 weeks</td>
</tr>
<tr>
<td>van der Horst (60)</td>
<td>Consolidation</td>
<td>Itraconazole</td>
<td>47%</td>
<td>42%</td>
<td>97%</td>
<td>99%</td>
<td>60%</td>
<td>72%</td>
</tr>
<tr>
<td>Saag (70)</td>
<td>Maintenance</td>
<td>Fluconazole</td>
<td>47%</td>
<td>42%</td>
<td>97%</td>
<td>99%</td>
<td>60%</td>
<td>72%</td>
</tr>
<tr>
<td>Powderly (71)</td>
<td>Maintenance</td>
<td>Amphotericin B</td>
<td>23%</td>
<td>4%</td>
<td>90%</td>
<td>84%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluconazole</td>
<td>2%</td>
<td>18%</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
Table 3  Randomized Trials for Therapy of Cryptococcosis (Continued)

<table>
<thead>
<tr>
<th>Author</th>
<th>Indication</th>
<th>Test agent</th>
<th>Comparator</th>
<th>Response rates</th>
<th>Survival rates</th>
<th>Time to CSF clearance (day)</th>
<th>Toxicities</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saag (70)</td>
<td>Secondary prophylaxis</td>
<td>Itraconazole</td>
<td>Fluconazole</td>
<td>23% relapse</td>
<td>4% relapse</td>
<td>0% relapses at 48 weeks</td>
<td>N/A</td>
<td>This and other studies confirm that reconstitution of immunity can permit discontinuation of antifungal maintenance therapy</td>
</tr>
<tr>
<td>Vibhagool (78)</td>
<td>Withdrawal of secondary prophylaxis</td>
<td>Withdrawal of prophylaxis</td>
<td>Continued prophylaxis</td>
<td>0% relapses at 48 weeks</td>
<td>0% relapses at 48 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05.*
One concern is the observation of fluconazole heteroresistance in some Cryptococcal isolates (now <5%) (72). Whether this will emerge as a substantial clinical issue remains to be seen. The role of maintenance therapy in cancer patients or patients on corticosteroids is less certain. Voriconazole has activity as salvage therapy (73).

Until recently, treatment was recommended lifelong, but studies have indicated that with improvement of host immunity (as can be seen with HAART), discontinuation can take place if there is a sustained increase in the CD4+ T lymphocyte count to above 100–200 cells/µL (74–78).

V. HISTOPLASMOSIS

Histoplasmosis is endemic in certain geographic regions. Disease is usually self-limited in non-immunocompromised individuals. In immunocompromised patients, disease can be severe, progressive, or become disseminated.

A. HIV-Infected Patients

Therapy for the acute manifestations of infection during the first 12 weeks consists of an amphotericin B formulation (79). Clearance of Histoplasma organisms from blood by culture and antigen assay occurs quicker with liposomal amphotericin B than with itraconazole in patients with moderately severe or severe disease (80) etc. Change to itraconazole can be made for patients responding well to take advantage of an oral regimen (81). In a randomized comparison of amphotericin B deoxycholate at a dose of 0.7 mg/kg/day and liposomal amphotericin B at a dose of 3 mg/kg/day, there was a higher response rate, fewer deaths, and less toxicity with liposomal amphotericin B (82) (Table 4). For patients with mild to moderate manifestations (and without CNS involvement), itraconazole can be given at a dose of 300 mg BID for 3 days, then 200 mg BID for 12 weeks (83). Antigen monitoring can be used to monitor response to therapy (84–86).

After control of the acute infection, maintenance should be given with itraconazole at a dose of 200 mg once or twice daily (87). Maintenance should be continued indefinitely. If a sustained increase in the CD4+ T lymphocyte count to above 100–200 cells/µL occurs discontinuation can be considered (69).

For patients with CD4+ T lymphocyte counts <200 cells/µL in endemic areas, avoidance of exposure is important. Prophylaxis with itraconazole is effective (88) and can be considered in patients with CD4+ T lymphocyte counts <100 cells/µL (69) in endemic areas at high risk due to occupational exposure or high rates of infection. It should not be used routinely, however, because of an increase in resistance to both fluconazole and itraconazole in patients receiving itraconazole (88).

B. Other Immunocompromised Patients

The treatment approach is similar. For maintenance therapy, prolonged treatment in the range of 6–18 months is generally advised (69) but this has not been formally studied.
<table>
<thead>
<tr>
<th>Author</th>
<th>Pathogen</th>
<th>Indication</th>
<th>Test agent</th>
<th>Comparator</th>
<th>Success/Relapse rates</th>
<th>Toxicities</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson (82)</td>
<td>Histoplasmosis</td>
<td>Induction therapy</td>
<td>Liposomal amphotericin B</td>
<td>Amphotericin B</td>
<td>88% 64%</td>
<td>Favors</td>
<td>liposomal amphotericin B</td>
</tr>
<tr>
<td>Galgiani (94)</td>
<td>Coccidiomycosis</td>
<td>Inductiona</td>
<td>Itraconazole</td>
<td>Fluconazole</td>
<td>63% 50% at 8 months; 72% at 12 months; 50% at 8 months; 57% at 12 months</td>
<td>No difference</td>
<td>Relapse rates after discontinuation 28% vs. 18%</td>
</tr>
</tbody>
</table>

*a p = 0.05.
VI. COCCIDIODOMYCOSIS

As with Histoplasmosis, Coccidiodomycosis is endemic in certain geographic areas. Its clinical course can be quite variable.

There are a number of agents with activity against coccidioides, but treatment guidelines are difficult to formulate because of the lack of controlled trials (89). Generally, oral or parenteral therapy is chosen based on the degree of illness of the patient. An azole, either itraconazole or fluconazole, is effective for progressive non-meningeal infection; responses to itraconazole appear to be slightly better than fluconazole (90,91) (Table 4). If parenteral therapy is judged more appropriate, amphotericin B at a dose of 0.5–0.7 mg/kg/day or a lipid amphotericin B formulation is recommended (90). Posaconazole, voriconazole, and caspofungin have activity but a clinical role has not yet been defined.

Treatment of meningitis is quite problematic. For amphotericin B to be effective, it must be given intrathecally or by CSF shunt in a dose of 0.5–1.0 mg. Azoles are effective but relapses are frequent. In an animal model, liposomal amphotericin B was more effective than amphotericin B (92).

Surgical resection of localized pulmonary cavities can be useful in selected patients, where a cavity is persistent, progressive over time, or if subpleurally located where communication to the pleural cavity can occur. Immunomodulators offer promise as adjuncts: gamma interferon and IL12 promote protective Th1 responses and could be important adjuncts to antifungal therapy (93,94).

For patients with advanced HIV infection, maintenance with either fluconazole at 400 mg/day or itraconazole at 200 mg BID should be done indefinitely or until a sustained increase in the CD4+ T lymphocyte count above 100 cells/μL occurs (69). Relapses may occur less frequently with itraconazole than with fluconazole. Relapses appear to be infrequent in cancer patients and the role for maintenance is uncertain. One center has reported prophylaxis with fluconazole in liver transplant recipients in an endemic area is effective (95).

VII. OTHER FUNGI

A. Other Yeasts

1. Trichosporon

Amphotericin B has traditionally been used for therapy. However, persistence of fungemia during treatment coupled with in vitro observations of inhibition but not killing by amphotericin B suggest that amphotericin B is not optimal (96–98). Azoles, including fluconazole, voriconazole, and posaconazole, are active (96,99). The combination of fluconazole and amphotericin B showed greater activity in an animal model (99). GM-CSF may enhance therapeutic potential (100). There may be a role for granulocyte transfusions as well in the presence of persistent neutropenia, but this has not been studied.

2. Blastoschizomyces Captitatus (Formerly T. capitatum)

In vitro susceptibilities for this organism are similar to those of Trichosporon with the exception of fluconazole resistance noted in some isolates (101). The combination of fluconazole, amphotericin B, and GM-CSF has been used anecdotally (102).
3. **Malassezia furfur (Pityrosporum)**
In vitro susceptibility testing suggests amphotericin B and azoles are good treatment options (103,104). Antifungal therapy alone is inadequate, however (105). Removal of catheter and lipid nutritional supplements (upon which the organism is dependent for growth) are important components of management.

4. **Rhodotorula**
Amphotericin B is active in vitro and is generally used for treatment. The azoles have variable activity (106). Removal of central venous catheters is generally recommended, although this has not been systemically studied (107).

### B. Other Molds

1. **Zygomycetes (Mucormycosis)**
   Traditional treatment is with amphotericin B given in high doses (108,109). Surgical debridement of necrotic tissue is an important adjunct to antifungal therapy. Results have been poor. Case series with amphotericin B colloidal dispersion (110) and with amphotericin B lipid complex (111) as salvage therapy may be better options than amphotericin B. Posaconazole has in vivo activity in an animal model (112). Voriconazole and caspofungin do not have activity.

3. **Blastomycosis**
   Amphotericin B in a dose of 0.7–1.0 mg/kg/day is recommended (113). In the absence of CNS disease, a change to itraconazole may be made once the patient stabilizes. Frequent relapses in HIV infection occur. Accordingly, maintenance therapy with an azole is recommended, preferably with itraconazole (113).

3. **Scedosporium (Scedosporium apiospermum or Pneumocystis boydii and Scedosporium prolificans)**
   Susceptibility to amphotericin B is poor in vitro and clinically there have been few successes. The lipid formulations of amphotericin B, amphotericin B deoxycholate, and voriconazole act additively with neutrophils to exert injury to *Scedosporium* hyphae (114,115). The addition of G-CSF to liposomal amphotericin B in an animal model appeared to be more effective than liposomal amphotericin B alone (116). Voriconazole and posaconazole have inhibitory activity in vitro (117–120). Voriconazole and posaconazole have been used anecdotally with some success (73,121–125). Voriconazole may be the therapy of choice at present. The echinocandins have some in vitro activity but have not been tested clinically (119,126). Itraconazole and terbinafine have demonstrated poor activity in vitro as single agents but in combination demonstrated synergy (127); the clinical significance of this observation is unknown. Where possible, surgical debridement of necrotic tissue is important.

4. **Fusarium**
   Amphotericin B in high doses has traditionally been used, although in vitro susceptibility to amphotericin B is suboptimal and some isolates demonstrate resistance in vitro (128) and treatment results have been poor. The lipid formulations of amphotericin B have been used clinically, but treatment results have remained suboptimal. Fluconazole and itraconazole have little activity. Voriconazole and posaconazole
have both shown in vitro activity (118,120,129–132) and voriconazole has demonstrated clinical efficacy in the salvage setting (73,133). In vitro testing suggest synergy of caspofungin and amphotericin B (44), but this has not been evaluated clinically. Neutrophil recovery appears crucial for successful outcomes. Growth factors and granulocyte transfusions have been used anecdotally for persistently neutropenic patients with some efficacy. Removal of vascular catheters and debridement of necrotic tissue are other adjunctive measures which can be useful (65).

5. *Alternaria*

Antifungal agents have poor activity. Amphotericin B, itraconazole, and voriconazole have been used (134,135). Surgical debridement and efforts to effect restoration of host responses are key.

VIII. *Pneumocystis jiroveci (formerly Pneumocystis carinii)*

Trimethoprim-sulfamethoxazole (TMP-SMX) is the preferred treatment for *Pneumocystis carinii* pneumonia (PCP), found in various randomized trials to be more effective than a variety of alternative therapies for mild, moderate, or severe PCP. The TMP-SMX is administered either orally or intravenously in a dose of 15–20 mg/kg/day of trimethoprim and 75–100 mg/kg/day sulfamethoxazole in 3–4 divided doses. Although generally well tolerated, adverse reactions may occur, especially in HIV-infected patients, with rash, fever, cytopenias, gastrointestinal intolerance being the most frequent toxicities. Alternative therapies for mild to moderate PCP include dapsone 100 mg/day with TMP 15–20 mg/kg/day (136,137). This combination is less toxic, but adverse reactions including methemoglobinemia and hemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency can occur. A third option for mild to moderate PCP is the combination of clindamycin given at a dose of 600 mg IV every 6 hr followed by 300–450 mg orally every 6 hr plus primaquine 15–30 mg base per day orally (137,138). Hemolysis from primaquine can occur in patients with G6PD deficiency. Atovaquone at a dose of 750 mg twice daily has been found to be less effective than TMP-SMX for mild to moderate PCP but it is as effective as pentamidine (139,140); it is better tolerated than either comparator. Its bioavailability has been an issue but the oral suspension is much better absorbed than the oral tablet (47% bioavailability compared with 23%).

For moderate to severe PCP, alternatives to TMP-SMX include pentamidine isethionate at a dose of 4 mg/kg/day once daily intravenously (141–144) and trimetrexate at a dose of 45 mg/m² once daily (145). Response rates to IV pentamidine have generally been similar to TMP-SMX. Trimetrexate is less effective than TMP-SMX but better tolerated; notwithstanding, myelosuppression, can occur, which can be lessened by administration of folinic acid.

Since the inflammatory response to PCP can lead to worsening of respiratory symptoms and greater hypoxia, corticosteroids have been used in moderate to severe PCP and found to beneficial and improve survival (146–148). Typically prednisone is given at a dose of 40 mg twice daily for 5 days and then tapered to cease by day 20.

Several agents are useful as second-line therapies for individuals progressing, or as intolerant of TMP-SMX. In a meta-analysis of salvage therapies for PCP (149), efficacy rates for salvage therapies were clindamycin-primaquine (88–92%),...
atovaquone (80%), efloornithine hydrochloride (57%), TMP-SMX (53%), pentamidine (39%), and trimethrexate (30%).

IX. CONCLUSION

Treatment approaches have been well studied and rigorous trials have been conducted for Candida and Cryptococcus infections forming strong bases for management strategies. Steady progress has been made and treatment outcomes have improved. Unfortunately, for other fungal infections, there have been few randomized trials, progress has been slower, and management strategies are less certain. In large part, the small numbers of cases have impeded progress. For many years the lack of antifungals to test has also contributed to slow gains; that has changed now with the introduction of several new agents which offer advantages in efficacy and safety. Another factor that has slowed progress is the heterogeneity of host factors that must also be taken into consideration in clinical trial design and evaluation of treatment outcomes. Some effort is being made to consider new trial designs (150–153) to maximize what can be learned from small numbers of patients and, in many instances, the inability to conduct a randomized trial to definitively answer which agent or strategy may be “best.” Renewed commitment of clinicians to enter patients wherever possible to clinical trials is also key for progress to be made.

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