

Trends in the Epidemiology of Opportunistic Fungal Infections: Predisposing Factors and the Impact of Antimicrobial Use Practices

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In the past decade, the frequency of opportunistic fungal infections has increased, and the spectrum of fungal pathogens has changed. The increasing number of susceptible hosts, the introduction of newer modalities for hematopoietic stem cell transplantation, the evolution of organ transplantation practices, the use of novel immunosuppressive agents, and current antimicrobial prophylactic strategies have likely contributed to the changing epidemiology of invasive mycoses. The introduction of azoles more than a decade ago has had a profound impact on curtailing candidal infections. However, a dramatic increase in azole-resistant *Candida* species and mold infections has been documented. The trends in time of onset, spectrum, and frequency of infections due to invasive molds and opportunistic yeasts are unique for different fungi and vary between subsets of immunocompromised hosts. This review discusses the implications of these trends for guiding judicious use of antimicrobial prophylactics and for unraveling the pathophysiological basis of fungal infections.

Although invasive mycoses have long been recognized as significant pathogens, particularly in immunocompromised patients, the frequency of opportunistic fungi is increasing over time and the spectrum of invasive mycoses is changing. The vast majority of the invasive fungal infections are still due to *Aspergillus* and *Candida* species, but infections due to mycelial fungi other than *Aspergillus* and to non-*albicans* species if *Candida* are increasingly common. In the present review, I show that the trends in fungal infections are unique for different fungi and different subsets of immunocompromised hosts. I discuss the evolution in predisposing factors, the impact of current antimicrobial prescription practices, and the implications of these trends for further unraveling the pathogenesis of infections with opportunistic fungi and optimizing their management.

TRENDS IN THE EPIDEMIOLOGY OF INVASIVE MYCOSES

An increase in the frequency of invasive aspergillosis and other molds has been reported [1–4]. A study of 11,000 autopsies at a university hospital in Europe documented a significant increase in invasive fungal infections in 1978–1992 ($P \leq .001$) [2]. This increase was due largely to a rise in aspergillus infections ($P < .001$). The prevalence of candidal infections was stable and even showed a declining trend in the later years of the study. Although the incidence of invasive aspergillosis (defined as the number autopsies performed divided by the number of cases diagnosed) among transplant recipients increased from 6% in 1983–1987 to 11% in 1988–1992, the incidence of invasive candidiasis declined from 12% to 5% during those time periods [2].

Mycelial fungal infections. A study of hematopoietic stem cell transplant recipients has documented an increase in the incidence of invasive aspergillosis, from 7.3% in 1992 to 16.9% in 1998 [3]. Although the risk for aspergillosis after allogeneic transplantation was greater than the risk after autologous transplantation, an increase had occurred among recipients of both

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types of transplants [3]. Traditionally, most aspergillus infections have occurred during the period when bone marrow transplant recipients are neutropenic; that is, before engraftment. Aspergillus infections have now been shown to occur more frequently after engraftment [3]. An overall increase was noted in the number of non-*Aspergillus* mycelial infections and infections due to non-*fumigatus* species of *Aspergillus* [3]. In another study, species other than *Aspergillus fumigatus* accounted for 53% of invasive mycelial infections in allogeneic bone marrow transplant recipients [4].

Virtually identical trends have been noted among organ transplant recipients. Traditionally, invasive mycelial infections in these patients have been almost exclusively due to *Aspergillus* species [5–7]. A ongoing prospective study, however, has documented that fungi other than *Aspergillus* species now account for 37% of all mold infections and 43% of all deaths among organ transplant recipients with mold infections [8]. Mortality among patients with infections due to mycelial fungi other than *Aspergillus* species exceeded that associated with invasive aspergillosis [8]. Infections due to mycelial fungi are also occurring later in the posttransplantation period; 56% of these infections occurred >3 months after transplantation, and 30% occurred >12 months after transplantation [8].

Despite heightened awareness of the profiles of patients at risk for aspergillus infections, and despite the advent of liposomal formulations of amphotericin B, invasive aspergillosis continues to be associated with inexorably high mortality rates [9–11]. The precise role of newer antifungal agents, such as echinocandins, for the treatment of aspergillus infections in the clinical setting remains to be determined, but the emerging data appear promising [12]. The management of infection with mycelial fungi other than *Aspergillus* species also remains challenging, since these fungi are erratically susceptible and often resistant to amphotericin B. Newer triazole antifungal drugs offer hope, particularly for treatment of infection with hyaline molds (e.g., *Pseudallescheria boydii* and dematiaceous fungi), but they have little activity against the zygomycetes [13, 14].

Candidal infections. Changes in the frequency of invasive candidiasis are most notable in the following subgroups of patients: those hospitalized in critical care units, patients with hematologic malignancies, hematopoietic stem cell transplant recipients, and organ transplant recipients. *Candida* species are currently the fourth most commonly recovered isolates in cases of nosocomial bloodstream infection in the United States. Twenty-five percent to 50% of the nosocomial candidal infections now occur in patients in critical care units [15]. The National Nosocomial Infection Surveillance Program documented a doubling in the rate of nosocomial fungal infections during 1980–1990, with the greatest increase (124%) occurring among surgical patients [16]. A greater severity of illness in hospitalized patients, advances in supportive medical care, use

of invasive devices, and use of broad-spectrum antibiotics have likely contributed to an increased predisposition of critically ill patients to candidal infections [17].

The use of fluconazole in intensive care units (ICUs) has increased exponentially in the past decade. Non-*albicans* species of *Candida* are increasingly being documented as pathogens in critically ill patients in ICUs [17, 18]. Ironically, the vast proportion of fluconazole use has been for prophylaxis or empirical therapy rather than for treatment of documented infections [18]. However, such fluconazole use may not necessarily be beneficial [17]. Comparison of critically ill patients who did receive fluconazole with those who did not showed that patients treated with fluconazole had a higher mortality rate (40% vs. 20%, respectively), longer stays in the ICU, and longer hospital stays [17]. This group also had a higher frequency of bacterial resistance subsequent to fluconazole therapy and a higher rate of isolation of *Candida* species resistant to fluconazole [17].

Among hematopoietic stem cell transplant recipients, an overall decrease has been documented in the frequency of candidal infections, as well as a shift toward isolation of non-*albicans* species of *Candida* [19, 20]. It has been proposed that use of fluconazole as antifungal prophylaxis largely accounts for these trends [19, 21]. Antifungal prophylaxis with fluconazole during neutropenia and acute graft-versus-host disease (until day 75 after transplantation) was associated with a significant reduction in the incidence of invasive candidiasis and improved survival rates [20]. However, although *C. albicans* was the most common colonizing isolate before transplantation, resistant species such as *Candida krusei* and *Candida glabrata* were isolated after transplantation and exposure to fluconazole [20]. In another study, fluconazole prophylaxis was the most important determinant for the relative increase in isolation of *C. krusei* (OR, 27.07) and *C. glabrata* (OR, 5.08) [19]. A meta-analysis of 16 randomized controlled trials, however, showed that fluconazole prophylaxis in neutropenic non-bone marrow transplant patients did not decrease fungus-related mortality or systemic fungal infections [22].

Among organ transplant recipients, invasive candidal infections is most relevant for liver and pancreas transplant recipients. An overall decline in the incidence of invasive candidiasis has been noted in liver transplant recipients, even in the absence of systemic antifungal prophylaxis: many centers now report incidences of <10% [23].

EVOLUTION IN PREDISPOSING FACTORS

An increasing incidence of invasive fungal infections could merely reflect greater laboratory expertise in the detection and identification of fungi, particularly molds (table 1). Increasing use of aggressive and intensive cancer chemotherapeutic regimens, immunosuppressive therapy for autoimmune disorders,

Table 1. Variables that likely account for the current trends in the epidemiology of opportunistic fungal infections.

Increasing number of susceptible hosts
Greater laboratory expertise in the detection and identification of fungi
Use of new transplantation modalities for hematopoietic stem cell transplantation (e.g., CD34 ⁺ selected autografts and peripheral blood stem cell transplantation)
Evolution in organ transplantation practices
Advances in surgical technology
Use of corticosteroid-sparing regimens and an overall conservative approach to immunosuppression
Use of novel immunosuppressive agents
Use of antimicrobial prophylactic practices, e.g., use of fluconazole for antifungal prophylaxis, ganciclovir for cytomegalovirus prophylaxis, quinolones for gram-negative bacterial prophylaxis

and transplantation have led to an increase in the number of susceptible hosts in recent years. However, I believe that the most significant variables accounting for the emerging trends, particularly the increase in the incidence of invasive mycelial infections, are the changes in transplantation practices and current antimicrobial prophylactic practices, which involve not only antifungal but also antiviral and antibacterial agents.

New transplantation modalities for hematologic malignancies continue to be developed. Preliminary data suggest that CD34⁺-selected autologous peripheral-blood stem cell (PBSC) transplants might confer a higher rate of cytomegalovirus and fungal infections than do other types of transplants [24, 25]. The use of cytokine-mobilized PBSC transplantation has facilitated engraftment and has led to faster immune reconstitution. Surprisingly, PBSC transplantation has not been associated with a higher rate of acute graft-versus-host disease [26, 27]; however, the frequency of chronic graft-versus-host disease has increased (or remained unchanged) in comparison with its frequency in bone marrow transplant recipients [26]. This may account for the delayed occurrence of invasive aspergillosis after transplantation.

It remains to be determined how the incidence and spectrum of opportunistic infections will be affected by even newer hematopoietic transplantation modalities; for example, adoptive immunotherapy with donor leukocyte infusions to induce a direct graft-versus-leukemia effect [28], and transplantation of T cell-depleted grafts to prevent graft-versus-host disease [29].

The incidence of invasive fungal infections, particularly candidal infections after liver transplantation, is influenced strongly by surgical factors, including the technical complexity of the surgery [5, 6, 30]. In recent years, there have been significant technical advances in liver transplantation practices and an evolution toward the conservative yet effective use of immunosuppressive agents. Liver transplantation surgery can now be performed with transfusion of 10 or fewer units of blood, and

up to 30% of the operations require no blood transfusions [31, 32]. Biliary anastomosis requiring neither stents or T-tubes has led to a striking reduction in the rate of biliary complications [33]. Finally, the use of corticosteroid-sparing and low-dose corticosteroid regimens is increasing. In the past 10 years at our institution, there has been a significant decline in surgical time, blood transfusion requirements, cold ischemic time, the use of roux-en-Y biliary anastomosis, and the rate of biopsy-proven rejection episodes [34]. Over the same period, a significant decrease in the incidence of invasive candidiasis was noted, even in the absence of systemic antifungal prophylaxis [34]. Thus, a decrease in putative risk factors may have contributed to an overall decline in the rate of invasive candidiasis among liver transplant recipients.

Patterns of antimicrobial use, particularly prolonged administration of prophylaxis against not only fungi but also other opportunistic pathogens, such as cytomegalovirus, may also be contributing to the changing epidemiology of fungal infections. Although the occurrence of azole-resistant candidal infections in patients receiving fluconazole prophylaxis is not surprising, an intriguing and worrisome observation is the higher incidence of mold infections in these patients. At a bone marrow transplantation center, the incidence of aspergillus and other mycelial infections increased from 18% in the years before the use of fluconazole prophylaxis to 29% in the period after use of fluconazole prophylaxis became routine [35]. The use of fluconazole was an independently significant predictor of invasive mold infections ($P = .009$). Invasive aspergillosis was documented in 8 (8%) of 101 liver transplant recipients who received fluconazole prophylaxis, an incidence virtually unheard of in the absence of an outbreak [36]. It is possible that eradication of susceptible microorganisms facilitates colonization and subsequent infection with pathogens that are innately resistant to the antimicrobial agent used. Thus, overcoming "colonization resistance" is a plausible explanation for a higher incidence of mold infections in patients receiving fluconazole prophylaxis.

Antiviral prophylaxis with prolonged courses of ganciclovir is now widely used for prevention of cytomegalovirus disease in transplant recipients. A study of bone marrow transplant recipients documented that receipt of ganciclovir therapy for >4 weeks correlated independently with a significantly higher risk of invasive aspergillosis; each week of ganciclovir therapy beyond 4 weeks increased the risk of invasive aspergillosis by a factor of 1.4 [37]. Strategies that not only target prophylaxis toward high-risk patients but also limit the duration of prophylaxis may be more rational approaches to cytomegalovirus prophylaxis in the transplantation setting [38]. Likewise, antibacterial prophylaxis with quinolones was among the most significant predictors of breakthrough fungemia in patients with cancer who were receiving antifungal prophylaxis [39].

Use of novel immunosuppressive agents may also have a role

in the changing frequency, spectrum, and clinical presentation of opportunistic mycoses. Mycophenolate mofetil use has been shown to be associated with a lower incidence of *Pneumocystis carinii* infection [40–43]. With the declining incidence of *Cryptococcus neoformans* infection in HIV-infected patients, organ transplant recipients have emerged as one of the leading groups of immunocompromised patients at risk for cryptococcal infections. The neurotropism and predilection of *C. neoformans* to cause CNS infection is well recognized. Indeed, CNS has traditionally been the most frequently involved site of cryptococcal infections. However, we have previously noted that 67% of the liver transplant recipients with cryptococcosis who received tacrolimus for primary immunosuppression had cutaneous and/or osteoarticular lesions, and only 17% had meningitis [44].

A review of cryptococcal infections in transplant recipients documented that the type of primary immunosuppression influenced the predominant clinical manifestations of cryptococcosis [45]. Patients receiving tacrolimus were significantly less likely to have CNS involvement than were those receiving a different agent (cyclosporine or azathioprine), on the basis of immunosuppression (78% vs. 11%, respectively; $P = .013$) [45]. On the contrary, skin, soft-tissue, and/or osteoarticular involvement was significantly more likely in patients receiving tacrolimus (66%) than in patients receiving a different agent, on the basis of immunosuppressive regimens (21%; $P = .006$). Patients who received tacrolimus had a lower rate of CNS involvement than did patients who received cyclosporine (11% vs. 67%; $P = .01$), and skin, soft-tissue, and/or osteoarticular involvement was significantly higher in the tacrolimus group (67% vs. 22%; $P = .04$) [45].

There are a number of plausible biological explanations for this observation. Tacrolimus is a natural macrolide antifungal product [46]. Although its immunosuppressive effect outweighs its antifungal action in vivo, tacrolimus is toxic to *C. neoformans* in vitro because it inhibits calcineurin, which is essential for growth at 37°C and virulence in cryptococci [46, 47]. It is possible that, in patients receiving tacrolimus for immunosuppression, meningitis occurs proportionately less frequently because tacrolimus is protective against cryptococci in tissues at 37°C but not a cooler sites (e.g., skin) or merely because tacrolimus achieves higher levels in CSF than do other immunosuppressants.

CLINICAL RELEVANCE OF EMERGING TRENDS

The evolving trends in invasive mycoses have a number of relevant implications. Understandable concerns about the critically ill patients have led to a push for the use of antimicrobial prophylaxis for every pathogen that can possibly be prevented from causing infection. The beneficial effects of liberal empirical

and widespread use of prolonged antimicrobial prophylaxis for all patients are arguable. However, the harm resulting unwittingly from these practices is increasingly apparent.

Given the increasing incidence of infection with *Aspergillus* species and mycelial fungi that are innately resistant to amphotericin B, rapid and accurate identification of these molds can be pivotal for the selection of appropriate therapy. Unmet challenges with regard to antifungal resistance testing for mycelial fungi include development of standardized susceptibility testing methods and correlation of in vitro susceptibility results with clinical outcome [48]. Amid an increase in the frequency of opportunistic fungal infections, particularly aspergillosis and infections with drug-resistant molds, it is evident that there is a critical need for more effective antifungal drugs and studies to assess the efficacy of combinations of antifungal agents for the management of such infections. Finally, careful documentation of epidemiological trends may provide insights into the pathophysiological basis of infections (as discussed above with respect to *C. neoformans*) and, ultimately, insights into the development of more effective antifungal strategies.

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