Antifungal Prophylaxis in Solid-Organ Transplant Recipients: Considerations for Clinical Trial Design

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Background. There are several key issues regarding clinical trial design for antifungal prophylaxis in organ transplant recipients.

Methods. The existing and emerging data on the epidemiology and risk factors for invasive mycoses in organ transplant recipients formed the basis for discerning the subgroups that may be targeted for prophylaxis, the unique end points to be considered, and the attributes of currently available drugs that may be suitable candidates for antifungal prophylaxis.

Results. Epidemiological characteristics of invasive mycoses in organ transplant recipients have evolved sufficiently to warrant thoughtful reconsideration of the subsets of patients and the fungal pathogens to be targeted for antifungal prophylactic trials in the current era. Safety and tolerability of antifungal agents and outcome stratified by severity of illness are important end points given the potential for drug interactions with immunosuppressive agents and the fact that a beneficial effect of prophylaxis on outcome has been difficult to document in organ transplant recipients.

Conclusions. Clinical trial design for antifungal prophylaxis must carefully consider the unique issues pertaining to the selection of patients most likely to benefit, as well as the tolerability and drug interactions of antifungal agents in organ transplant recipients.

Invasive mycoses have long been recognized as significant opportunistic infections in organ transplant recipients. The incidence of fungal infections varies from relatively low rates in renal transplant recipients to those exceeding 50% in small bowel transplantation [1–4]. On average, invasive fungal infections have been documented in 5%–20% of solid-organ transplant recipients (table 1). A vast majority of these infections are due to either Candida (35%–91%) or Aspergillus (9%–52%) species, with other opportunistic infections accounting for 1%–2% of fungal infections in these patients. Mortality in transplant recipients, particularly in those with Aspergillus infections, ranges from 55% to 92%; an estimated 9.3% and 16.9% of all posttransplantation deaths in lung and liver transplant recipients, respectively, have been due to invasive aspergillosis [2].

Studies on antifungal prophylaxis in organ transplant recipients have had numerous limitations, including small sample size, flawed or imprecise definitions of end points, uncontrolled trial design, comparison with retrospective cohorts, and an insufficient number of cases with documented fungal infections [5]. Consequently, an optimal or a standardized approach to antifungal prophylaxis for these patients does not exist.

Design issues that warrant consideration for clinical trials for antifungal prophylaxis among solid-organ transplant recipients are the following: the types of transplant recipients in whom such studies are warranted, whether prophylaxis should be used for all patients or targeted toward high-risk subgroups only, the unique end points to consider among organ transplant recipients, the attributes of an optimal antifungal agent, and currently available drugs that may be potential candidates for clinical trials.

DEFINING THE HIGH-RISK SUBGROUPS AND STRATEGIES FOR PROPHYLAXIS

Invasive aspergillosis has been reported in 1%–8% of liver transplant recipients [2, 8, 9]. These patients have unique predisposition to dissemination of Aspergillus infections beyond the lungs and have mortality rates that traditionally have exceeded 90%. In lung transplant...
recipients, the incidence of *Aspergillus* infections ranges from 3% to 15% [6, 9, 10]. Mortality in patients with invasive pulmonary or disseminated infections after lung transplantation ranges from 60% to 74%, with an estimated 9% of the deaths considered to be attributable to invasive aspergillosis. Thus, on the basis of the number of transplantations done, the rates of fungal infections, and their impact on overall morbidity and outcome, prophylactic strategies aimed at the prevention of *Aspergillus* infections are warranted for liver and lung transplant recipients.

Liver transplant recipients have also been shown to be at high risk for invasive candidiasis. Indeed, >90% of invasive fungal infections after liver transplantation have been due to candidiasis. Risk factors for invasive candidiasis in these patients have been largely reflective of the technical complexity of the surgery. In recent years, significant technical developments, advances in surgical practices, and notable changes in the characteristics of patients undergoing liver transplantation have occurred. A recent study has shown that the duration of operation time (*P* = .03), transfusion requirements (*P* = .0001), cold ischemic time (*P* < .0001), and use of Roux-en-Y biliary anastomosis (*P* = .0015) have decreased significantly over the last decade [11]. Over the same time period, a significant decrease in the incidence of invasive candidiasis (*P* = .015) was documented, even in the absence of any form of systemic antifungal prophylaxis.

Variables predictive of invasive candidiasis in liver transplant recipients in the current era are retransplantation (OR, 16.4), posttransplantation dialysis (OR, 7.6), and antibiotic prophylaxis for spontaneous bacterial peritonitis (OR, 8.3) [12]. These risk factors overlap considerably with those for invasive aspergillosis. Prophylactic trials specific to invasive candidiasis in liver transplant recipients, therefore, may not be warranted. Fluconazole has been shown to be effective for the prevention of superficial and invasive fungal infections in liver transplant recipients in a randomized trial [13].

Pancreas and small-bowel transplant recipients also have notably high rates of invasive fungal infections; a vast proportion of these are invasive *Candida* infections. Intra-abdominal abscesses and deep wound or surgical site infections due to *Candida* occurred in 7%–14% of pancreas transplant recipients in earlier studies and adversely affected allograft and patient survival [14, 15]. However, the overall risk of infections has been reduced dramatically with advances in immunosuppression and refinements in surgical techniques [16–18]. Given the small numbers of bowel transplantations done, it may not be feasible to conduct randomized, clinical trials among these patients at present.

**DESIGN ISSUES CONCERNING INVASIVE ASPERGILLOSIS IN LIVER TRANSPLANT RECIPIENTS**

Prophylaxis for invasive aspergillosis in liver transplant recipients should not be administered to all patients, that is, used universally, but instead should be targeted toward high-risk patients, on the basis of identifiable risk factors. Arguments against universal prophylaxis are the relatively low incidence of invasive aspergillosis and the expense and potential toxicity of antifungal agents if administered to all patients. A strategy of targeted prophylaxis is particularly well suited for liver transplant recipients, in whom the risk factors for invasive aspergillosis are rather well defined and the period of vulnerability is generally known. Retransplantation (OR, 29.9) and requirement of dialysis (OR, 24.5) have emerged as independently significant predictors of invasive aspergillosis in these patients [8, 9]. Prolonged intensive care unit stay and intubation are often used as surrogate markers to identify patients at high risk for invasive aspergillosis. However, up to 80% of the intubated patients who have developed invasive mycelial infections also had other risk factors, such as retransplantation and dialysis, that predisposed them to these infections [9]. Thus, retransplantation and requirement of renal replacement therapy are objective criteria that may be used to identify patients who should receive antifungal prophylaxis.

Colonization with *Aspergillus* is rare in liver transplant recipients. Detection of *Aspergillus* by culture of samples from these patients’ airways almost always indicates invasive infec-

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**Table 1. Incidence of fungal infection and predominant fungal pathogens in solid-organ transplant recipients.**

<table>
<thead>
<tr>
<th>Type of transplant</th>
<th>Incidence of invasive fungal infections</th>
<th>Usual etiologic agent(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal</td>
<td>1.4%–14%</td>
<td><em>Candida</em> (mainly urinary tract infections)</td>
</tr>
<tr>
<td>Heart</td>
<td>5%–21%</td>
<td>70%–90% <em>Aspergillus</em></td>
</tr>
<tr>
<td>Liver</td>
<td>7%–42%</td>
<td>35%–91% <em>Candida</em>, 9%–34% <em>Aspergillus</em></td>
</tr>
<tr>
<td>Lung and heart-lung</td>
<td>15%–44%</td>
<td>43%–72% <em>Candida</em>, 25%–50% <em>Aspergillus</em></td>
</tr>
<tr>
<td>Small bowel</td>
<td>40%–59%</td>
<td>90% <em>Candida</em></td>
</tr>
<tr>
<td>Pancreas</td>
<td>18%–38%</td>
<td>Virtually all <em>Candida</em></td>
</tr>
</tbody>
</table>

**NOTE.** Data are from [1–3, 5–7].
tion [2]. Therefore, liver transplant recipients with airway samples positive for *Aspergillus* should not be considered candidates for prophylaxis but, instead, should be considered for treatment of invasive aspergillosis.

*Aspergillus* infections in liver transplant recipients have traditionally occurred very early in the postoperative course [19]. Indeed, 81%–100% of liver transplant recipients who developed invasive aspergillosis were still in the intensive care unit after transplantation surgery. More recently, however, invasive aspergillosis has been shown to occur later in the posttransplantation period [9]. Nevertheless, the risk factors and characteristics of patients do not differ whether invasive aspergillosis develops in the late or early posttransplantation period [9]. Therefore, the aforementioned risk factors may be used to identify high-risk subgroups, regardless of the time elapsed since transplantation.

**End points and other considerations for clinical trials for invasive aspergillosis in liver transplant recipients.** Objective and standardized criteria for the diagnosis of invasive fungal infections, as proposed by the European Organization for Research on Treatment of Cancer and the Multicenter Study Group of the National Institute of Allergy and Infectious Diseases, represent a significant advance in overcoming the variability and imprecision in defining these infections [20]. Although the criteria for defining proven and probable aspergillosis are acceptable, the category of possible invasive aspergillosis may not reliably diagnose invasive aspergillosis in liver transplant recipients.

Safety and tolerability of the antifungal agents is an important end point, given the interactions of a number of these drugs with the inhibitors of the calcineurin/target of rapamycin (TOR) pathways. Azole antifungal agents are known to inhibit the metabolism of cyclosporine, tacrolimus, and sirolimus. The rank order of potency of the azoles for the inhibition of the P-450 isoenzymes is ketoconazole > voriconazole > itraconazole > fluconazole [21]. Coadministration of itraconazole with tacrolimus in serum [22, 23]. In human liver microsomes, voriconazole at a concentration of 4 μg/mL inhibited the metabolism of tacrolimus by 50% [21]. Given the extent of the interaction, the use of voriconazole is not recommended for patients receiving sirolimus. The azoles also have significant interaction with the statins. Concurrent use of cyclosporine, simvastatin, and itraconazole was associated with rhabdomyolysis in a transplant recipient [24].

Studies of antifungal prophylaxis for invasive aspergillosis in liver transplant recipients have not shown a beneficial effect on overall outcome [25–28]. Preemptive therapy with a lipid formulation of amphotericin B targeted toward liver transplant recipients who require renal replacement therapy in one study [25] and toward high-risk patients in another [28] significantly reduced the incidence of invasive fungal infections in this setting. However, a beneficial effect on survival has been difficult to document. Although antifungal prophylaxis was protective against invasive aspergillosis, these critically ill patients eventually died either of multisystem organ failure or antimicrobial-resistant bacterial infections [25]. Thus, clinical trials should include objective criteria for the assessment of severity of illness of the patients, such as the APACHE score [29], so as to identify those who are likely to have a poor response despite prevention of fungal infections.

**Antifungal agents for clinical trials of prophylaxis for invasive aspergillosis among liver transplant recipients.** Although an optimal antifungal agent for prophylaxis against invasive aspergillosis has not been defined in liver transplant recipients, ample data suggest that certain regimens are unlikely to be effective. Low-dose amphotericin B not only was ineffective but, in fact, increased the risk of invasive aspergillosis after liver transplantation [30]. Likewise, low doses of lipid formulations of amphotericin B (1 mg/kg/day) have not been shown to be effective [31, 32]. Concerns regarding poor bioavailability and absorption in the immediate posttransplantation period dissuade from the use of itraconazole as a potential candidate for prophylactic studies among transplant recipients.

Antifungal agents that may be considered for invasive aspergillosis trials are lipid formulations of amphotericin B deoxycholate, caspofungin, and newer azoles, such as voriconazole. Each one of these drugs has potential advantages but also has limitations (table 2). Lipid formulations of amphotericin B have a wide antifungal spectrum, including activity against non-*Aspergillus* molds, which may be potentially advantageous. Nearly 30% of the mold infections in these patients have been shown to be due to species other than *Aspergillus*; the time of onset for many of these infections is similar to that for *Aspergillus* infections [33]. An antifungal agent active against *Aspergillus* molds could theoretically be protective against most non-*Aspergillus* molds as well. Lipid formulations of amphotericin B, however, are expensive, require parenteral administration, and, in at least one study, had no significant impact on mortality.

The echinocandins (of which caspofungin is currently available) inhibit fungal (1,3)-β-d glucan, a polymer that is vital to the structural integrity of the fungal cell wall. There is evidence to suggest that caspofungin’s activity against *Aspergillus* species may be enhanced by the inhibitors of the calcineurin pathway [34]. Caspofungin, however, requires parenteral administration and has a relatively narrow antifungal spectrum with regard to molds other than *Aspergillus*.

The mold-active triazoles, of which voriconazole is available at present, are potentially promising drugs. Voriconazole has an extended spectrum of activity against a number of pathogenic molds. Its availability in an intravenous as well as an oral
Newer triazoles (e.g., voriconazole) have a wide range of activity, intravenous and oral formulations. Caspofungin has a good safety profile, potential synergism with immunosuppressive agents against Aspergillus, and may not be deemed ethical, given concerns regarding voriconazole's renally excreted carrier, sulfobutyl ether β cyclodextrin sodium, the use of intravenous voriconazole is contraindicated for patients with significant renal dysfunction. Posaconazole has a broader spectrum of activity than does voriconazole (e.g., against the zygomycetes) and potentially fewer drug interactions than does voriconazole.

Other design issues that remain unresolved and that warrant consideration are the duration of prophylaxis and the comparator for the study drug. If antifungal prophylaxis is targeted toward high-risk patients, should prophylaxis be used for a defined period or continued as long as the risk factor persists? With regard to the comparator, a trial design involving the drug versus placebo would require a smaller sample size to document the efficacy of the drug but may not be deemed ethical, given a high risk of invasive aspergillosis among the patients targeted. Comparison of an anti-Aspergillus drug against another would require a larger sample size but may be feasible in a multicenter study. Even if such a trial is designed to show comparable efficacy, it stands to address drug tolerability and safety concerns, which are often the key determinants in the selection of an antifungal agent for prophylaxis.

### CLINICAL TRIALS FOR INVASIVE ASPERGILLOSIS IN LUNG TRANSPLANT RECIPIENTS

Design issues that should be considered for clinical trials of prophylaxis of invasive aspergillosis in lung transplant recipients are the following: the unique characteristics of Aspergillus infections in lung transplant recipients, frequent occurrence of airway colonization with Aspergillus and its potential role in targeting patients for prophylaxis, other high-risk subgroups, definitions of infection and end points in lung transplant recipients, and universal versus targeted prophylactic strategies.

**Aspergillus infections in lung transplant recipients.** Aspergillus infections in lung transplant recipients demonstrate several unique features. Direct communication of the transplanted lung with the environment and impaired local host defenses, including mucociliary clearance, render airway colonization a common occurrence in these patients. During surgery, the bronchial arteries are disrupted at the site of anastomosis. Until collaterals from bronchial circulation develop, the anastomotic healing is dependent on blood supply from pulmonary circulation of the transplanted lung. The transiently devascularized anastomotic site, therefore, remains susceptible to ischemic injury, necrosis, and, potentially, infection with Aspergillus. Ulcerative tracheobronchitis and anastomotic infections are locally invasive aspergillosis with the potential to progress to disseminated infection. Lesions in the vicinity of or involving the anastomotic site can result in fatal bronchopleural fistulas.

Invasive aspergillosis occurs in an average of 6% (range, 3%–15%) of lung transplant recipients [6, 7, 35]. Of these infections, 58% are tracheobronchitis or bronchial anastomotic infections, 32% are invasive pulmonary aspergillosis, and 22% are systemic or extrapulmonary infections [35]. Aspergillus infections occur a median of 3.2 months after lung transplantation; 26% of all infections have been shown to occur within 1 month, 51% within 3 months, and 72% within 6 months of lung transplantation [35]. In addition, 16% of the infections occurred 6 months after transplantation, and 12% were documented after 12 months [35].

Time to onset of infections varies for different types of lung transplant recipients and generally spans several months. The median time to onset after transplantation was 0.7 months for heart-lung, 3.9 months for bilateral lung, and 5 months for single-lung transplant recipients (P = .046) [35]. Single-lung transplant recipients have been shown to develop Aspergillus infections significantly later after transplantation than do all other patients (median, 4.9 months vs. 2.1 months; P = .019)

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### Agent Advantages Limitations

| Lipid formulations of amphotericin B | Wide range of antifungal activity (30% of mold infections in these patients are due to non-Aspergillus species) | Expense, requirement for parenteral administration, no impact on mortality [25] |
| Caspofungin | Good safety profile, potential synergism with immunosuppressive agents against Aspergillus [34] | Parenteral administration, narrow spectrum against other molds |
| Newer triazoles (e.g., voriconazole) | Wide range of activity, intravenous and oral formulations | Inability to use intravenous formulation for patients undergoing dialysis, drug interactions (sirolimus) |
Up to 54% of patients with invasive aspergillosis have had prior cytomegalovirus infection [35]. However, these data are largely derived from studies done before the routine use of effective antiviral prophylaxis for cytomegalovirus. The frequency with which cytomegalovirus infection precedes invasive aspergillosis in the ganciclovir era has not been fully discerned. Rejection episodes have been documented in 63% of lung transplant recipients with invasive aspergillosis in earlier studies [35]. The role of rejection in predisposing patients to invasive aspergillosis, in the current immunosuppressive era, needs to be better defined before such a variable can be used as a preemptive therapy tool.

**Other risk factors.** The incidence of invasive aspergillosis appears to be higher for single-lung than for bilateral lung transplant recipients [9, 37]. Single-lung transplant recipients with invasive aspergillosis also have a significantly higher mortality rate than do bilateral lung or heart-lung transplant recipients [9]. Invasive aspergillosis in single-lung transplant recipients characteristically involves the native lung, and infections occur later, the median onset being 5 months after transplantation. Limiting prophylaxis to single-lung transplant recipients only, however, may not be desirable. The risk of *Aspergillus* infections, although relatively lower, is still significant for all other lung transplant recipients.

![Flow diagram](image)

**Figure 1.** Flow diagram representing the clinical course of lung transplant recipients with detection of *Aspergillus* in respiratory tract cultures. Data are based on [35].
by culture from airway specimens in the absence of invasive aspergillosis or tracheobronchitis should be considered to have colonization with *Aspergillus*.

**FUTURE DIRECTIONS**

One of the most significant advances in the field of transplantation has been the effective prevention of cytomegalovirus infection, considered to be the most pernicious opportunistic pathogen in the early years of transplantation. Two factors have largely accounted for a successful approach to prophylaxis for cytomegalovirus. First, a highly effective antiviral agent (ganciclovir) became available. Second, rapid and reliable diagnostic assays have been developed on the basis of which prophylaxis can be targeted toward high-risk patients before the onset of cytomegalovirus disease. These concepts may be extrapolated to fungal infections as well. Development of non–culture-based diagnostic assays that can be used to identify patients for whom early empirical therapy may be used would clearly be a significant advance in this field.

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**References**


