Fungal infections in solid organ transplantation

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Fungal infections in solid organ transplant recipients continue to be a significant cause of morbidity and mortality. Candida spp. and Aspergillus spp. account for most invasive fungal infections. The incidence of fungal infection varies with type of solid organ transplant. Liver transplant recipients have highest reported incidence of candida infections while lung transplant recipients have highest rate of Aspergillus infections. Recent epidemiological studies suggest the emergence of resistant strains of candida as well as mycelial fungi other than Aspergillus in these patients. The current review incorporates the recent changes in the epidemiology of fungal infections in solid organ transplant recipients and highlights the newer data on the diagnosis, prophylaxis and treatment of fungal infections in these patients.

Keywords fungal infection, solid organ transplant, treatment, prophylaxis

Introduction
Infections in solid organ transplant recipients remain a moving target. Use of various induction regimens, administration of novel immunosuppressive agents, and incorporation of newer prophylactic strategies continue to change the face of infections in solid organ transplant recipients. Amongst the infections, fungal infections still remain a major cause of mortality in these patients. The current review incorporates the recent changes in the epidemiology of fungal infections in solid organ transplant recipients and highlights the newer data on the prophylaxis and treatment of fungal infections in these patients.

Candida infections
Epidemiology and risk factors
Candida spp. are the most common cause of fungal infections in solid organ transplant (SOT) recipients. Infections are primarily noted in the early (0–30 days) post-transplant period. Risk factors include length of antibiotic therapy, use of broad-spectrum antibiotics, central venous catheters, requirement of dialysis and retransplantation [1,2].

Candida albicans remains the most common species causing infection, but a shift towards non-albicans species has been observed. C. glabrata is the second most common etiologic agent and currently, 1/3 of the candidiasis in liver transplant recipients are caused by non-albicans species [2]. The increased frequency of non-albicans species, particularly C. glabrata, is concerning due to decreased susceptibility to fluconazole and the possibility of cross-resistance with the newer azoles. In a study with 46 C. glabrata fluconazole-resistant isolates, only 13% were susceptible to voriconazole, 4% to posaconazole and 8.7% to ravuconazole [3].

Liver transplant recipients have the highest reported rate of Candida infections; 62–91% of the fungal infections occurring in these patients are caused by Candida, with an associated mortality ranging from 11–81% [2].

Pancreas transplant recipients are also at higher risk of candidiasis, with rates ranging from 6–38% [1,4,5]. The high risk is due to underlying diabetes and the often already present immunosuppression from a previous kidney graft [6]. The mortality rate in this group ranges from 20–27%, and the rates of graft loss...
are particularly high. Candidiasis occurs less often in kidney, lung and heart-lung recipients. While no good data exist in small bowel transplant recipients, small case series reported an incidence of candidiasis ranging from 28–55% [7,8]. Table 1 enumerates the general risk factors for the development of invasive fungal infections in organ transplant recipients. Table 2 lists the risk factors for candidiasis and antifungal prophylaxis in pancreas and small bowel recipients; the recipients who require specific antifungal prophylaxis against Candida.

Clinical presentation

The most common clinical presentation of candidiasis in organ transplant recipients is mucosal infection, especially oral candidiasis. Candida esophagitis may also be present. It is characterized by dysphagia, odynophagia and chest pain. Peritonitis is more commonly seen after liver and pancreas transplantation, when manipulation of the intestines and biliary tree may lead to dissemination of intraluminal candidiasis. Candidemia is often associated with a central venous catheter. Candida spp. can hematogenously disseminate to virtually any organ. Candiduria often represents colonization, but may also be a sign of disseminated infection to the kidneys. It occurs commonly in renal transplant recipients and is associated with reduced survival rates [9]. Although treatment of asymptomatic candiduria in renal transplant recipients does not appear to improve patient survival, suggesting that it is a marker of severity of illness, the impact on allograft survival is not clear [10,11].

Prophylaxis

Prophylaxis of candidiasis in SOT recipients is controversial. Only a few well-designed studies have been conducted and all of the controlled trials were performed in liver transplant recipients. Oral prophylaxis with non-absorbable antifungal agents (nystatin, clotrimazole, amphotericin B) has shown inconsistent results [12–15]. Two randomized controlled trials have shown the efficacy of fluconazole in the prophylaxis of invasive candidiasis. In one study, comparing fluconazole 100 mg/day for the first 4 weeks following liver transplantation to oral nystatin, fluconazole was associated with a reduction in Candida colonization and superficial infections, as well as a trend toward reduction of invasive infections [16]. In a randomized, placebo-controlled study, fluconazole 400 mg/day for 10 weeks after liver transplantation prevented most types of Candida infection, except those caused by C. glabrata and C. krusei. An increase of fluconazole-resistant Candida organisms was not observed [17]. In another double blind randomized control trial itraconazole decreased the rate of fungal infection from 24% to 4% in liver transplant recipients [18]. A recently published meta-analysis showed that antifungal prophylaxis in liver transplant recipients significantly reduced the total episodes of superficial and invasive fungal infection, as well as mortality attributable to

Table 1 General risk factors for the development of invasive fungal infections in solid organ transplant recipients

<table>
<thead>
<tr>
<th>Technical/anatomical abnormalities</th>
<th>Skill in operative/perioperative management</th>
<th>Vascular access devices</th>
<th>Drainage catheters/endotracheal tubes</th>
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<tbody>
<tr>
<td>Intensity of environmental exposures</td>
<td>Community</td>
<td>Nosocomial</td>
<td></td>
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<tr>
<td>Net state of immunosuppression</td>
<td>CMV and other herpesviruses</td>
<td>Treatment of rejection with steroids or monoclonal antibodies</td>
<td>Renal failure</td>
</tr>
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Table 2 Risk factors for infections caused by Candida spp., recommended agent for prophylaxis and duration

<table>
<thead>
<tr>
<th>Organ</th>
<th>Risk factors</th>
<th>Antifungal prophylaxis</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>Enteric drainage</td>
<td>Fluconazole 400 mg/day</td>
<td>(\geq4) weeks</td>
</tr>
<tr>
<td></td>
<td>Vascular graft thrombosis</td>
<td>Or</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-reperfusion pancreatitis</td>
<td>Lipid formulation of amphotericin B 3–5 mg/kg/day*</td>
<td></td>
</tr>
<tr>
<td>Small bowel</td>
<td>Graft rejection/dysfunction</td>
<td>Fluconazole 400 mg/day</td>
<td>At least 4 weeks; until healing of anastomosis and absence of rejection</td>
</tr>
<tr>
<td></td>
<td>Enhanced immunosuppression</td>
<td>Or</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anastomotic disruption</td>
<td>Lipid formulation of amphotericin B 3–5 mg/kg/day*</td>
<td></td>
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<tr>
<td></td>
<td>Multi-visceral transplant</td>
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</table>

*In centers with a high incidence of non-albicans species.
fungal infections; however it did not affect overall mortality or the need for empirical antifungal treatment [19]. Compared to controls, patients receiving prophylaxis experienced a higher proportion of episodes of Candida non-albicans infections.

**Aspergillus infections**

*Incidence and risk factors*

The incidence of invasive aspergillosis (IA) varies according to the transplanted organ. Kidney/pancreas recipients have the lowest rate, ranging from 0.4–5% [20,21]. Liver transplant recipients have a moderate risk, with incidence rates ranging from 1–8%, followed by heart recipients at 1–14% [21–23]. The highest incidence of aspergillosis is observed among lung transplant recipients, ranging from 6–16% [24–30]. The cumulative incidence of aspergillosis at 12 months following solid organ transplantation was found to be 2.4% after lung transplantation, 0.8% after heart transplantation, 0.3% after liver transplantation and 0.1% after kidney transplantation in a prospective multicenter surveillance program which included 4110 solid organ transplants in 19 centers in the US. In this study, the mortality at 3 months following the diagnosis of aspergillosis ranged from 20% for lung transplantation to 66.7% for heart and kidney transplantation [31].

Risk factors for IA that are common to all solid organ recipients include environmental exposure and the net state of immunosuppression, which is affected by the use of high dose steroids, anti-lymphocyte therapy and viral infections, particularly those caused by cytomegalovirus (CMV) [32–35].

Risk factors for IA specific to liver transplantation include: pretransplant fulminant hepatic failure, primary allograft failure or severe dysfunction, retransplantation, dialysis requirement after transplant and high transfusion requirement [36,37]. In another prospective study, patients were considered low risk if they had only one of the following conditions: choledochojejunostomy anastomosis; retransplantation; intra-operative administration of 40 or more units of blood products or return to the operating room for intra-abdominal bleeding; return to the operating room for anastomotic leak or vascular insufficiency; preoperative serum creatinine of greater than 2 mg/dl; and perioperative Candida colonization [38].

Lung transplant recipients are especially vulnerable to IA due to the direct exposure of the transplanted lungs to the environment, along with impaired defenses due to decreased cough and mucociliary clearance [39]. This risk is further enhanced by the relative ischemia at the anastomosis [40], receipt of single lung transplant, development of bronchiolitis obliterans, hypogammaglobulinemia and bronchial stent placement [30,41–43]. Pre and post-transplant colonization of the airways is also associated with the development of IA following lung transplantation. Cystic fibrosis patients with pretransplant airway colonization have a higher risk of post-transplant *Aspergillus* tracheobronchitis [44]. One study has shown that patients with airway colonization in the first 6 months post-lung transplantation are 11 times more likely to develop IA than those without colonization [29].

Table 3 lists the risk factors for *Aspergillus* infection in organ transplant recipients in liver and lung transplantation.

*Clinical presentation*

There are four clinical presentations of interest for discussion: *Aspergillus* airway colonization, tracheobronchitis, pulmonary aspergillosis and disseminated disease.

*Aspergillus* colonization is of particular interest among lung transplant recipients, in whom it is a risk factor for subsequent invasive disease. It is detected during surveillance bronchoscopy of asymptomatic patients, without any evidence of tissue invasion. Colonization occurs in as many as 46% of lung recipients [29].

Tracheobronchitis is found almost exclusively in lung transplant recipients, at the anastomotic site. One case of native lung tracheobronchitis has been reported in the literature in a heart transplant recipient [45]. It is characterized by involvement of the airways and bronchi without extension to the lungs (Fig. 1). It usually occurs in the first 3 months post-transplantation. Routine surveillance bronchoscopy is essential for the early diagnosis of this condition, which if not treated early may extend to the lung parenchyma and potentially disseminate. The pathologic features include necrosis, ulceration and pseudomembrane formation [46]. *Aspergillus* tracheobronchitis is considered a manifestation of invasive aspergillosis, and is the most common presentation of invasive aspergillosis following lung transplantation [47].

Pulmonary aspergillosis in solid organ transplant recipients lacks a distinguishing radiographic finding, in contrast to what is observed in bone marrow transplant recipients [48,49]. The halo sign, important early radiological finding in the diagnosis of pulmonary aspergillosis in neutropenic and stem cell transplant patients has very low sensitivity in solid organ transplant recipients and is often absent [47]. Patients
usually present with a dry cough and dyspnea. Low grade fever and hemoptysis may occur (Fig. 2).

*Aspergillus* have a predilection for the central nervous system but may disseminate to almost any organ, including the eye, liver, spleen, heart, kidneys and bone. A decrease in the incidence of disseminated and central nervous system infection has been observed in liver transplant recipients [50]. The currently used immunosuppressive agents, calcineurin and target of rapamycin inhibitors, which have in vitro activity against *Aspergillus*, have been implicated as a possible explanation of this observation [51]. It has been observed that late liver retransplant recipients are significantly more likely to have central nervous system infection compared to early retransplant recipients [52].

Early diagnosis and prompt initiation of therapy is necessary in the management of aspergillosis in organ transplant recipients. However, the diagnosis is challenging, and often involves a combination of clinical and radiological signs, galactomannan antigen detection, culture results and histopathological findings.

### Table 3 Risk factors for infections caused by *Aspergillus* spp., recommended agent for prophylaxis and duration

<table>
<thead>
<tr>
<th>Organ</th>
<th>Risk factors</th>
<th>Antifungal prophylaxis</th>
<th>Duration</th>
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<tbody>
<tr>
<td>Liver*</td>
<td>Pre-transplant fulminant hepatic failure</td>
<td>Lipid formulation of amphotericin B 2.5–5 mg/kg/day</td>
<td>4 weeks or until resolution of risk factors</td>
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<tr>
<td></td>
<td>Primary allograft failure</td>
<td>Or Voriconazole 400 mg/day</td>
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<td></td>
<td>Retransplantation</td>
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<tr>
<td></td>
<td>Requirement of renal replacement therapy</td>
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<td></td>
<td>High transfusion requirements</td>
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<td></td>
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<td></td>
<td>Use of monoclonal antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>Airway ischemia</td>
<td>Inhaled amphotericin B 6–30 mg/day</td>
<td>2 weeks to lifelong</td>
</tr>
<tr>
<td></td>
<td>Reperfusion injury</td>
<td>Or Voriconazole 400 mg/day</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Receipt of single lung transplant</td>
<td>Or Itraconazole 400 mg/day</td>
<td></td>
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<tr>
<td></td>
<td>Presence of bronchial stents</td>
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<tr>
<td></td>
<td>Acquired hypogammaglobulinemia</td>
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<td></td>
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<tr>
<td></td>
<td><em>Aspergillus</em> colonization</td>
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*These are risk factors for *Candida* as well, however, prophylaxis with an agent without anti-*Aspergillus* activity is not appropriate. Antifungal prophylaxis may be warranted whenever more than one risk factor is present.

Fig. 1 *Aspergillus* tracheobronchitis with pseudomembrane formation at the anastomotic site.

Fig. 2 *Aspergillus* pneumonia with cavitating lesion.
Chest tomography is recommended whenever aspergillosis is suspected, since its sensitivity is much greater than that of a chest radiograph. Common radiographic findings include focal areas of consolidation or infiltrate and nodular lesions with or without cavitation. The halo sign is rarely observed in organ transplant recipients.

**Diagnosis**

Since *Aspergillus* is ubiquitous in the air, culture results need to be interpreted in the context of the clinical picture, always keeping in mind that the unusual manifestations of disease may occur in this patient population. A study with liver and kidney recipients demonstrated that isolation of two or more colonies, or identification of more than one site of infection, particularly if the species were *A. fumigatus* or *A. flavus*, was associated with a greater than 70% likelihood of invasive disease [53]. Among organ transplant recipients, the positive predictive value of a respiratory tract culture for *Aspergillus* ranges from 17–72% [54–56]. Only a few studies have evaluated the utility of galactomannan assay in the diagnosis of IA in SOT recipients. The results showed the sensitivity, ranging from 30–55.6% and specificity from 87–95% [25,57,58]. A galactomannan cut-off value of 0.5, which is the manufacturer’s currently recommended cutoff value in the USA, was utilized in the studies by Husain and Kwak, while a value of 1.0 was used by Fortun. At least for lung transplant recipients, raising the cut-off value to 0.66 may be more clinically relevant. This slightly higher cut-off was associated with an increase in specificity and positive likelihood ratio, without a decrease in sensitivity. In a meta-analysis including 27 studies and approximately 4,000 patients, the test was shown to be more useful in patients with hematological malignancies or who underwent hematopoietic stem cell transplantation than in solid-organ transplant recipients. The overall sensitivity and specificity were 71% and 89%, respectively, for proven cases of IA. For solid-organ transplant recipients the overall sensitivity was 22% and the specificity was 84% [59].

More recently, the clinical utility of galactomannan antigen detection in BAL for diagnosis of IA in lung transplant recipients was prospectively evaluated. The incidence of IA was 5.2% (6/116 patients). Using an index cut-off value of ≥1.0 the sensitivity was 60%, specificity was 98% and positive and negative likelihood ratios were 28 and 0.40 respectively. Colonization with *Penicillium* species and use of piperacillin/tazobactam were associated with false-positive results. These data suggest that detection of galactomannan in the BAL of a lung transplant recipient with a compatible clinical illness, using a cut-off of ≥1.0, is highly suggestive of IA [60].

The usefulness of the (1→3)-β-D-glucan assay has not been assessed in the setting of organ transplantation. The major limitation of this assay is that it not only reacts with *Aspergillus* but also with *Candida*, *Trichosporon*, *Fusarium*, *Penicillium*, *Saccharomyces*, *Acremonium* and even *Pneumocystis jiroveci* [61]. *Aspergillus* PCR is an evolving technique, not yet available for clinical use. It has the potential to being quite useful; however, different methodologies are being utilized, not all with promising results.

The definitive diagnosis of aspergillosis is based on the demonstration of hyphal invasion in tissue and growth of *Aspergillus* in culture of the same tissue.

**Therapy**

The availability of echinocandins and new azoles has changed the management of IA and gave rise to interest in the use of combination antifungal therapy. Voriconazole became the drug of choice for the treatment of IA, after its superior efficacy and survival benefit was demonstrated in a randomized trial comparing it to amphotericin [62]. Its availability as an oral formulation facilitates the management when the patient becomes clinically stable. Voriconazole significantly interacts with tacrolimus, cyclosporine and sirolimus. In fact, co-administration with the later is contraindicated and close follow-up and adjustment of the immunosuppressant doses is necessary.

Itraconazole has been substituted by the new azoles with anti-*Aspergillus* activity, due to its unpredictable bioavailability. Posaconazole has potent activity against *Aspergillus* but is currently not approved for treatment of invasive aspergillosis in the United States; and in the European Union is approved for use only in refractory infection or in patients intolerant of standard therapy. Ravuconazole has potent in vitro activity against *Aspergillus*, but is not yet clinically available.

Echinocandins (anidulafungin, caspofungin, mica-fungin) have very low potential for drug interactions, although tacrolimus levels should be monitored as it can be lowered. Its different mode of action, blocking the synthesis of (1→3)-β-D-glucan cell wall have made it particularly interesting to be used in combination therapy.

Inhaled amphotericin B can be used as prophylaxis and for therapy of *Aspergillus* tracheobronchitis [63]. The use of aerosolized lipid formulations of amphotericin is usually better tolerated than conventional amphotericin [64].
The benefit of combination antifungal therapy with caspofungin and voriconazole in the management of IA was recently evaluated in 87 organ transplant recipients with IA in a prospective, multicenter, observational study, and was compared to a group of transplant patients who received a lipid formulation of amphotericin as single therapy. In multivariate analysis, combination therapy was independently associated with an improved 90-day survival in patients with renal failure and infection caused by *A. fumigatus* [65]. At the moment, however, there is no strong evidence to support the use of combination therapy. Interpretation of antifungal susceptibility testing for *Aspergillus* is difficult and it is not currently recommended.

The routine use of HEPA filters is not recommended in SOT, not even in the immediate post-operative period. Exposure to potential sources of molds should be minimized and special attention should be given during construction and renovation.

**Prophylaxis**

Antifungal prophylaxis in lung transplant recipients has been widely practiced with inhaled amphotericin. Although preemptive therapy based on the isolation of *Aspergillus* in the BAL has been reported [66], the majority of centers has used universal prophylaxis with nebulized amphotericin [64,67,68]. Voriconazole can be substituted for nebulized amphotericin especially during the first four months after lung transplantation. In one study, universal voriconazole prophylaxis was reported to decrease the rate of invasive aspergillosis as compared to targeted prophylaxis in lung transplant recipients [69].

In liver transplant recipients, lipid formulations of amphotericin at a dose of 5 mg/kg/day have been shown to be efficacious in reducing invasive fungal infections in high-risk liver transplant recipients on renal replacement therapy [70], although there was no reduction in mortality. A study of universal prophylaxis with cumulative doses of 1–1.5 grams of liposomal or lipid complex amphotericin showed a reduction in the incidence of IA, which was most significant among patients receiving renal replacement therapy (0% vs 32% on controls) [71]. However, low doses of lipid formulation of amphotericin failed to prevent IA [72].

The recommendations for prophylaxis against *Aspergillus* in SOT recipients are shown in Table 3.

**Cryptococcus infections**

**Epidemiology and risk factors**

Cryptococcosis is a significant opportunistic infection in solid organ transplant recipients, with a prevalence rate ranging from 0.26% to 5% and overall mortality of 42% [73–77]. However, the unique characteristics, optimal management and risk factors for acquisition of cryptococcosis in solid organ transplant recipients have not been defined.

The immunosuppression used to prevent rejection of the allograft, especially the use of steroids, is presumably the primary risk factor for its occurrence [75]. However, with new developments in transplantation, it is possible that new risk factors will be recognized. Recently, an increase in the cumulative incidence of cryptococcosis was associated with the use of two doses of antilymphocyte globulin or alemtuzumab, agents that cause profound and prolonged lymphocyte depletion [78].

**Clinical presentation**

Cryptococcosis most often presents as symptomatic disease and occurs late after transplantation, usually more than 6 months after transplantation [75,76].

The type of primary immunosuppression used seems to impact the clinical presentation of cryptococcosis in solid organ transplant recipients. Tacrolimus, cyclosporine and sirolimus have potent in vitro antifungal activity against *Cryptococcus*. Among 127 organ transplant recipients with cryptococcosis retrospectively evaluated, those receiving tacrolimus were significantly less likely to have central nervous system involvement and more likely to have skin, soft tissue and osteoarticular involvement than patients receiving non-tacrolimus based immunosuppression [73,79].

Headache, fever and mental status changes are the most frequent clinical presentations of cryptococcal meningitis. Meningismus is found in less than a 1/3 of patients [76]. Altered mental status, absence of headache and liver failure are associated with increased mortality in patients with cryptococcal meningitis [74].

Isolated pulmonary cryptococcosis usually manifests with non-specific symptoms and can be asymptomatic. One common presentation is the finding of nodular pulmonary infiltrates, often thought to be malignant in nature [73]. Acute respiratory failure requiring mechanical ventilation, pleural effusion and bilateral pulmonary infiltrates have been identified as predictors of mortality in cryptococcal pneumonia [80].
Cutaneous presentations vary from papular or nodular lesions to cellulitis resembling bacterial infections. Cutaneous infections are often a manifestation of disseminated disease [73], however primary cutaneous cryptococcosis may also occur [81].

An immune reconstitution syndrome-like entity has been identified as occurring in 4.8% of solid organ transplant recipients being treated for cryptococcal infection. Patients present with worsening of the clinical manifestations but cultures are negative for *C. neoformans* [82]. It can be misinterpreted as failure of therapy and it is thought to be secondary to a shift from a predominantly Th2 response to a Th1 proinflammatory response as a result of appropriate antifungal therapy and reduction in immunosuppression. This immune reconstitution syndrome has been associated to allograft loss in renal transplant recipients [83].

**Diagnosis**

The diagnosis can be made by culture, direct microscopic examination or detection of polysaccharide antigen in body fluid or tissues. It is important to keep in mind that serum cryptococcal antigen is not reliable to exclude isolated pulmonary disease; and false negative results may occur in the setting of cryptococcal meningitis and even disseminated disease. Therefore infection can not be ruled out solely based on a negative serum antigen [78,80,84].

**Treatment**

The principles for treatment of cryptococcal infections in solid organ transplant recipients are derived from the experience with HIV positive patients. An amphotericin B product together with 5-flucytosine for a 2-week induction is the treatment of choice for patients with cryptococcal meningitis or disseminated disease. Amphotericin B deoxycholate can be substituted with lipid preparation of amphotericin with dosing ranging from 3–6 mg/kg/day. Since baseline renal insufficiency is common in transplant recipients and they are often receiving other nephrotoxic drugs, lipid formulations of amphotericin B are generally better tolerated. If at the end of 2 weeks clinical and microbiological response is observed, therapy can be continued with fluconazole for a minimum of 10 weeks [85]. Selected patients with isolated pulmonary cryptococcosis and mild to moderate symptoms can be treated with fluconazole monotherapy.

Chronic suppressive therapy with fluconazole for a period of 6–12 months is recommended [85], however the exact duration of the secondary prophylaxis is not known. In a prospective cohort of 83 organ transplant recipients with cryptococcosis, suppressive therapy for 6 months was associated with a low risk (1.3%) of relapse [86]. We suggest lifelong secondary prophylaxis with fluconazole 200 mg/day.

Antifungal prophylaxis against *Cryptococcus* is not recommended. Organ transplant recipients should avoid birds and areas contaminated by bird droppings, which contain increased concentrations of *Cryptococcus* [87]. Zoonotic transmission of *Cryptococcus* has been demonstrated [88].

**Other molds**

Infections caused by non-*Aspergillus* molds have become increasingly common in the past decade among solid organ transplant recipients [89]. The reasons for this trend are not exactly known, but it may be a consequence of the currently used antifungal prophylaxis and changes in type and potency of immunosuppression [89,90]. Nonetheless, these changes are worrisome, as many of these fungi are resistant to conventional antifungal therapy.

**Fusarium**

Reports of fusariosis in solid organ transplant recipients remain rare and are found in the literature as isolated cases [91–98]. Neutropenia and impaired macrophage function are important risk factors for disseminated fusariosis [99].

Fusarium infections in solid organ transplant recipients tend to be localized and occur late after transplantation, at a median of 9 months, with an overall mortality of 33% [98].

Skin and soft tissue involvement is common. Lesions may be erythematous nodules, ecthyma gangrenosum-like lesions, target lesions, subcutaneous nodules or onychomycosis with periungual cellulitis [100].

Therapy of fusariosis is difficult. Amphotericin B is the primary therapy for fusariosis, however its efficacy is limited. Voriconazole is indicated in the treatment of patients intolerant of or refractory to primary therapy. Posaconazole has shown promising clinical efficacy in the management of fusariosis [96,101]. Decrease in immunosuppression is essential to reduce morbidity and mortality.

**Scedosporium**

*S. apiospermum* and *S. prolificans* are increasingly recognized as significant pathogens. They now account for approximately 25% of the non-*Aspergillus* molds causing infection in solid organ transplant recipients.
A combination of underlying immunosuppression, frequent occurrence of disseminated disease and lack of effective antifungal therapy makes scedosporiosis one of the most difficult fungal infections to treat.

Infections by *S. prolificans* are not homogenously distributed. Disseminated infections are reported mostly in Spain and Australia, while in the United States very few cases occur, even in immunocompromised patients [102].

The incidence of *Scedosporium* infection in organ transplant recipients is in the order of 1/1,000 patients, with a trend of higher incidence in patients receiving lung transplants compared with other organs [103]. Most of the infections are caused by *S. apiospermum* and about 50% are disseminated [104], with central nervous system involvement being observed in about 1/3 of the patients. Other manifestations include pulmonary involvement, cutaneous involvement, endophthalmitis, peritonitis and mycotic aneurysm. Mortality rate is 54%. Presence of disseminated disease has been identified as a predictor of lower survival, while the use of voriconazole and receipt of adjunctive therapy have been associated with a better survival [104].

Both *Scedosporium* species are inherently resistant to amphotericin. Voriconazole and posaconazole are active in vitro against *S. apiospermum*. *S. prolificans* is resistant to most currently available antifungal agent, including posaconazole, and the optimal treatment of this infection is not known. Duration of therapy remains uncertain, but certainly should not be less than 6 months.

**Dematiaceous molds**

The dematiaceous molds include *Exophiala*, *Alternaria*, *Dactylaria*, *Cladophialophora*, *Curvularia* and others. These fungi are increasingly recognized as pathogens in immunocompromised hosts, including organ transplant recipients, however most of the data in the literature is in the form of isolated case reports or small case series.

Most often, dematiaceous molds cause skin and subcutaneous infections [105–111], but invasive infections may occur [112–118], usually as brain abscesses. The most common presentation is a painless nodular lesion which often ulcerates. Organ transplant recipients, however, are more vulnerable to developing severe and disseminated infection.

In a review of 34 cases of infections due to dematiaceous fungi in organ transplant recipients, 76% of the patients had skin and/or soft tissue infections and 21% had systemic invasive infection. The median time to onset was 22 months following transplantation; only 21% of cases occurred within 6 months of transplantation. The prognosis was benign in the setting of cutaneous disease, without any deaths attributable to the fungal infection; however, the mortality rate was 57% among those with systemic invasive disease [119].

*Alternaria* cutaneous infections occur most frequently in the lower extremities. The clinical manifestations can be quite variable: papules, plaques, inflammatory nodules and recurrent cellulitis with secondary ulceration[120,121] (Fig. 3). It usually occurs in the first year following transplantation, and lesions are often multiple. Skin trauma, diabetes mellitus and frequent contact with soil are predisposing factors.

The cutaneous lesions caused by dematiaceous fungi should be excised whenever feasible, and that should be combined with systemic antifungal therapy, especially in the setting of invasive or disseminated disease. The agents of choice include itraconazole, amphotericin B and more recently voriconazole has been used [108,122,123].

**Zygomycetes**

These fungi are associated with a great potential for invasion and dissemination and as a consequence, with high mortality. This is a direct effect of its characteristic vascular tropism. They are primarily opportunistic pathogens, causing infection in immunocompromised hosts.

Recent reports have shown that zygomycetes are an emerging non-*Aspergillus* mold of major importance [124,125]. The increase in the incidence of zygomycosis was linked to the use of voriconazole prophylaxis in bone marrow transplant recipients in one study.
[126,127]. However, data from other large transplant centers showed an apparent increase in the cases of zygomycosis even before the availability of voriconazole [128]. In a prospective study of 57 organ transplant recipients with mold infections from 1998–2002, 5.7% were caused by zygomycetes [129]. None of these patients had used voriconazole.

Rhinocerebral infection is the most common presentation of zygomycosis [130] and the most frequent etiologic agent is *Rhizopus arrhizus* [131]. The infection originates in the paranasal sinuses and extends into the brain. Early signs and symptoms include fever, nasal congestion, serosanguinous nasal discharge, facial pain or swelling and headache. Later on, orbital cellulitis, ophthalmoplegia, proptosis and necrosis of the eyelid may occur. The presence of a black necrotic eschar on the palate or nasal mucosa is a classic sign, but it only appears late in infection. Computerized tomography and MRI (magnetic resonance imaging) show opacification of the sinuses, abnormal soft tissue and bone destruction.

Other presentations include pulmonary, cutaneous, gastrointestinal and disseminated disease. Pulmonary zygomycosis may be clinically and radiologically indistinguishable from pulmonary aspergillosis. Cutaneous infection may result from direct inoculation of traumatic wounds or burns. Gastrointestinal zygomycosis is a rare presentation of the disease and occurs as a result of ingestion of the pathogen. It may present as gastric or bowel perforation [130]. Disseminated disease is associated with the worst outcome, being almost uniformly fatal.

Therapy of zygomycosis involves extensive debridement of necrotic tissue, antifungal therapy and cessation or reduction of immunosuppression. The antifungal susceptibility profile of zygomycetes leaves few therapeutic options. The standard therapy consists of higher than usual doses of lipid formulations of amphotericin B (10 mg/kg/day) [130,132]. Echinocandins, fluconazole, voriconazole and fluocytosine are ineffective. Posaconazole has activity against the zygomycetes, but [133,134] it is only available as an oral formulation. It is approved by the US Food and Drug Administration and by the European Union for: (1) prophylaxis of invasive *Aspergillus* and *Candida* infections in hematopoietic stem cell transplant recipients, in graft-versus-host disease and in those with hematologic malignancies with prolonged neutropenia from chemotherapy; (2) and for treatment of esophageal candidiasis, but is likely to be used off-label for the treatment of Zygomycosis. Appropriate duration of therapy is unclear and should be individualized according to clinical and radiological response and it shouldn’t be shorter than 6–8 weeks.

### Endemic mycosis: coccidioidomycosis, histoplasmosis and blastomycosis

#### Coccidioidomycosis

Coccidioidomycosis is an endemic fungal infection of the southwestern United States and northern Mexico. Coccidioidomycosis in organ transplant recipients may occur from primary infection with *Coccidioides immitis* or *Coccidioides posadasii* after environmental exposure, from reactivation of latent infection or from primary infection from the donor organ. Donor-related coccidioidomycosis is rare, but is a severe complication of organ transplantation. Donor-related infections usually present early, are often disseminated and associated with high mortality [135–137].

In endemic areas, the incidence of coccidioidomycosis following solid organ transplantation is 1–8% [138–140]. Approximately half of the cases results from reactivation of previously acquired infection and occurs during the first year of transplantation [141]. Mortality can be as high as 70%, especially if the infection is disseminated [142].

A history of prior coccidioidomycosis, positive coccidioidal serologic tests at transplantation and clinical evidence of active infection at transplantation [142] have been identified as risk factors for symptomatic coccidioidomycosis after organ transplantation. The treatment of acute rejection is another risk factor for coccidioidomycosis after organ transplantation [142], even in the absence of prior infection. The use of corticosteroids has not been shown to increase the risk of infection in organ transplant recipients. Evidence of small calcified lung nodules or lymph nodes, suggestive of old granulomatous infections, in radiological studies is not a known risk factor for coccidioidomycosis [138].

The risk of infection among organ transplant recipients from nonendemic areas who relocate to or visit endemic areas is not known. In a study of liver transplant recipients who relocated to an endemic area, the incidence of new infection was 2.7% among those followed-up for at least 1 year [143].

Most cases will manifest with pulmonary symptoms [141], usually as an acute illness with fever, productive cough and shortness of breath. Fulminant respiratory failure with shock may occur, as well as an insidious presentation with fatigue, anorexia and weight loss. Dissemination to virtually any organ may occur, but is more common to skin, bone, joints and meninges [142].
The isolation of *Coccidioides* spp. from any clinical specimen is diagnostic, as well as the identification of spherules from tissue specimens. The microbiology laboratory should be notified whenever coccidioidomycosis is suspected, since the infectious mycelial form grows on artificial media, posing a significant laboratory hazard. The most commonly used serologic tests used for coccidioidomycosis are immunodiffusion, complement fixation and enzyme immunoassay. The first 2 are often performed in reference laboratories. Organ transplant recipients may have a decreased serological response, but if different serological methods are used, at least one should be positive [139]. The choice of serological method will depend on test availability, but combining results will improve sensitivity [144].

Management of coccidioidomycosis varies according to the clinical manifestations. Amphotericin B (deoxycholate or lipid formulation) is used by many authors as the drug of choice for rapidly progressing or severe disease. Other antifungal options include fluconazole at a dose of 400–800 mg/day and itraconazole (200 mg twice a day) [145]. The azoles are usually reserved as primary therapy for the transplant recipients with asymptomatic pulmonary nodules or mild disease. Voriconazole, posaconazole and echinocandins are potential alternatives in those intolerant or failing therapy with fluconazole or amphotericin B, however clinical reports are lacking. Depending on the extent of disease and response to antifungal therapy, immunosuppression may need to be decreased or withdrawn.

Patients living in endemic areas or with a prior history of coccidioidomycosis should undergo serologic testing prior to transplantation. The tests most commonly used are enzyme immunoassay, immunodiffusion and complement fixation.

Although no clear recommendations exist, targeted fluconazole prophylaxis given to patients with a history of coccidioidomycosis and/or positive serologies at the time of transplantation is well tolerated and efficacious in preventing reactivation of disease and should be given [146,147]. Duration of prophylaxis is unknown, with some authors defending its indefinite use when serologies are positive [139]. Patients who acquired coccidioidomycosis after transplantation should be on secondary prophylaxis with fluconazole for life, as relapse often occurs when prophylaxis is discontinued [148,149].

**Histoplasmosis**

Histoplasmosis is endemic in the Mississippi and Ohio River valleys, in many areas throughout Central and South America and in focal areas of Africa and Southeastern Asia.

It may occur after primary infection or after reactivation of latent infection. The exact incidence of histoplasmosis following organ transplantation among patients living in endemic areas is not exactly known, but it has been reported to range from 0% to as high as 2.1% in the setting of an outbreak [150–154].

A rare mode of acquisition of histoplasmosis in transplant recipients is transmission through an infected allograft from a donor with unrecognized infection [155–158].

The clinical manifestations of histoplasmosis in organ transplant recipients are protean. Prolonged fever is the predominant clinical finding [151] and pulmonary symptoms are usually subacute. In contrast to what is observed in immunocompetent hosts, dissemination is common [151]. Histoplasmosis usually occurs late after transplantation [152–154]; about 2/3 of cases occur within the first 18 months of surgery [151].

Cultures, fungal stains of affected tissues, antigen detection and serologic tests are useful for the diagnosis of histoplasmosis [159]. Cultures are most useful in disseminated or chronic pulmonary disease, but it may take several weeks to become positive.

Detection of antigen in serum, urine, cerebrospinal fluid and bronchoalveolar lavage is a rapid diagnostic method. Antigen can be detected in the urine of 90% of patients with disseminated disease [160] and in 75% of patients with diffuse acute pulmonary histoplasmosis. The use of antithymocyte globulin has been reported as a cause of false-positive antigenemia [161]. Detection of antigen in the urine is more sensitivity than in the serum. Cross-reaction may occur with penicilliosis, paracoccidioidomycosis and blastomycosis but not with aspergillosis, candidiasis, cryptococcosis or coccidioidomycosis [162].

Serologic testing is of limited value in the diagnosis of acute infection, especially in transplant recipients, who may have lower titers. Furthermore, antibody production is often delayed and evidence of antibody response does not differentiate between acute and prior infection.

Therapy of histoplasmosis in transplant recipients is initiated with amphotericin B. Most transplant centers favor the use of lipid formulations of amphotericin to lessen the risks of nephrotoxicity. Most patients respond rapidly to therapy, with defervescence observed in 1–2 weeks. When patients are afebrile and no longer critically ill, therapy can be switched to an oral azole. The preferred azole for the treatment of histoplasmosis is itraconazole, which can be also used intravenously in
patients intolerant to amphotericin. Fluconazole is a second-line agent and should be reserved to those patients who cannot tolerate itraconazole. Total duration of therapy should be 6–18 months [147,163]. Antigen testing may be used to monitor therapy and relapse in patients with disseminated disease.

It is not well established if organ transplant recipients should be maintained in long-term suppressive therapy with itraconazole. The decision should be individualized and depends on the overall state of immunosuppression.

The presence of calcified hilar and mediastinal lymphnodes and splenic calcifications in someone who has lived in an endemic area and without evidence of latent tuberculosis, is often indicative of past histoplasmosis. The risk of reactivation of histoplasmosis among patients who have lived in an endemic area has been deemed to be very low and pre-transplant serologies for *H. capsulatum* are not predictive of risk of disease [153]. Thus, a history of remote histoplasmosis is not a contra-indication to transplantation, and current evidence does not support the use of antifungal prophylaxis for persons from endemic areas or for those with radiographic or serologic evidence of past infection [164]. However, histoplasmosis should be considered in the differential diagnosis if the patient develops a febrile illness.

*Blastomyces*

Blastomycosis is endemic in the southeastern and south central United States, especially in the states that border the Mississippi and Ohio Rivers; in the midwestern states and Canadian provinces bordering the Great Lakes; and in a small area of Canada and New York adjacent to the St. Lawrence River [165]. Outside North America, cases are more often reported in Africa.

Blastomycosis is rare following solid organ transplantation and donor-transmitted infection has not been reported until now.

It is primarily a pulmonary infection, caused by inhalation of the conidia from the environment. Dissemination may occur, and immunocompromised patients are more likely to develop severe respiratory infections and disseminated disease, associated with a higher mortality, than the immunocompetent host [166]. The most frequent sites of dissemination are the skin, bones and genitourinary system. Involvement of the central nervous system with either meningitis or mass lesions is rare, but more often observed in immunocompromised patients [147]. Mortality rates of 30–40% have been reported in immunocompromised hosts [167].

Definitive diagnosis requires isolation of the fungus from a clinical specimen. The visualization of the characteristic yeast in histopathologic examination of a tissue or cytologic examination of sputum or bronchoalveolar lavage is much quicker, and in the appropriate clinical setting is enough to prompt initiation of antifungal therapy. Serologic tests by complement fixation, immunodiffusion and enzyme-linked immunosorbent assay are available, but have varying sensitivity and specificity and can not be used alone to exclude or confirm a diagnosis or support initiation of antifungal therapy, therefore are not widely used for diagnosis [166]. A *Blastomyces* antigen assay is commercially available [168]. Antigen can be detected in serum and several body fluids, including cerebrospinal fluid, but sensitivity is greatest in the urine. Cross-reaction can be observed in patients with histoplasmosis, paracoccidioidomycosis and penicilliosis [162].

Treatment options include amphotericin B, itraconazole, ketoconazole and fluconazole. Amphotericin B is the drug of choice for the initial treatment of blastomycosis in transplant patients [167]. The lipid preparations of amphotericin B have not been adequately evaluated in the management of blastomycosis but clinical experience indicates it should be effective, although dose and duration have not been established. Ketoconazole was the first azole to become an alternative to amphotericin in the management of blastomycosis, but it has been replaced by itraconazole, which is more readily absorbed, better tolerated and has enhanced antifungal activity. Fluconazole has a limited role in the treatment of blastomycosis. It is not as efficacious as itraconazole, but because it has good penetration into the central nervous system, it may have a role in the treatment of CNS infections [147,166,167]. Voriconazole and posaconazole may eventually become an option to the patients intolerant to or refractory to amphotericin B and itraconazole.

Frequent relapses have been noted in immunosuppressed patients and long-term suppressive therapy with an azole should be considered [147,166].

**Conclusion**

Fungal infections in solid organ transplant recipients continue to cause significant morbidity and mortality. The use of newer more potent immunosuppressive regimens as well as widespread use of antifungal drugs has changed the landscape of fungal infections. *Non-albicans* species and non-*Aspergillus* molds infections have become more prevalent. Moreover, significant
percentages of fungal infections are occurring late in the course of transplantation. Although significant strides have been made in the diagnosis of fungal infections, the diagnosis of mold and non-mold infections still remains elusive. PCR diagnosis of fungal infections appears to be promising but remains in its infancy.

Targeted prophylaxis against Candida and Aspergillus species is recommended in all solid organ transplant recipients, with the exception of lung transplant recipients, where universal prophylaxis against Aspergillus species is recommended owing to the lack of prospective data on the risk factor analysis. Fluconazole should be used for prophylaxis against Candida species unless the institution has a high rate of non-albicans infections. In this case, amphotericin B preparations, echinocandins or newer azoles should be used. Voriconazole can be used for prophylaxis against Aspergillus species; alternatives include posaconazole, echinocandins and amphotericin B preparations. Inhaled amphotericin B preparation can also be used in lung transplant recipients. The duration of prophylaxis however, still remains ill defined owing to the lack of randomized clinical trials.

Antifungal therapy is usually prolonged and interactions with the immunosuppressant drugs add to the complexity in management. Voriconazole has emerged as a drug of choice for the treatment of invasive aspergillosis and preliminary data suggests that the use of combination therapy with an echinocandin may be beneficial.

Future directions should include better definition of risk factors for fungal infection in solid organ transplant recipients in the current era by utilizing large collaborative prospective studies; and randomized studies of antifungal prophylaxis in solid organ transplant recipients.

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