Fungal Infection in Lung Transplantation

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Abstract

Invasive fungal infections threaten lung transplant outcomes with high associated morbidity and mortality. Pharmacologic prophylaxis may be key to prevent posttransplant invasive fungal infections, but cost, adverse effects, and absorption issues are barriers to effective prophylaxis. Trends in fungal infection diagnostic strategies utilize molecular diagnostic methodologies to complement traditional histopathology and culture techniques. While lung transplant recipients are susceptible to a variety of fungal pathogens, *Candida* spp. and *Aspergillus* spp. infections remain the most common. With emerging resistant organisms and multiple novel antifungal agents in the research pipeline, it is likely that treatment strategies will continue to evolve.

Keywords
- lung transplant
- fungal infection
- immunocompromised
- antifungal prophylaxis

Fungal infections lead to significant morbidity in lung transplant recipients. In addition, they have been directly and indirectly associated with increased mortality.[1] There are multiple reasons that account for the increased risk of fungal infections after lung transplantation. First, lung transplant patients receive high levels of pharmacologic immunosuppression to prevent or treat allograft rejection. This results in a functionally impaired immune system that predisposes to the occurrence of opportunistic fungal infections. Second, the lung is constantly exposed to the environment, allowing the direct access of fungal pathogens into the allograft. The hospital environment has occasionally been a source of fungal pathogens, with sporadic outbreaks of fungal infections in lung transplant units. Third, lung transplantation surgery impairs local physical and physiologic host defenses such as mucociliary clearance and cough mechanisms, respectively, impairing the transplant recipients' mechanisms of microbial clearance. Fourth, many lung transplant candidates have structural abnormalities that predispose to colonization with microbial pathogens, including fungi. Such colonization of sinuses and lung parenchyma with fungal pathogens has been associated with increased incidence of invasive fungal infections (IFIs) after lung transplantation.[2]

There is ongoing work to clarify and standardize the definitions of IFI. A consensus statement published in 2019 defined breakthrough fungal infections as any IFI that occurs during an exposure to antifungal therapy, including fungi outside of the spectrum of activity of the antifungal.[3] The IFI should occur within one dosing interval of the antifungal medication to be considered a breakthrough IFI.[3] The group also offered definitions for persistent, refractory, and relapsed infections in this document.

Epidemiology

The 1-year cumulative incidence of fungal infections in lung transplant recipients is estimated to be 10%, although the rates vary widely depending on multiple factors.[4,5] The occurrence and type of fungal infection depends on the intensity of physical and functional immunosuppression, the timing since transplantation, the type of antimicrobial prophylaxis, and the presence of colonization prior to transplantation. Overall, the median time to onset is roughly 11 months after lung transplantation.[4,5]

The majority of IFIs in lung transplant recipients are due to *Aspergillus* and *Candida* species. *Candida* sp. infections generally occur early after lung transplantation, usually as a complication of hospitalization and transplant surgery, in the form of mediastinitis, pleuritis, empyema, or surgical site infection. Bloodstream infection secondary to *Candida* species also occurs early, usually as a complication of indwelling

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vascular catheters or urinary catheters. Catheter-associated urinary tract infection with *Candida* species may also occur during the initial transplant hospitalization.

The 12-month cumulative incidence of invasive mold infection is 5.5%. *Aspergillus* species is the most common invasive mold infection, accounting for 73% of cases reported in a surveillance network of 15 lung transplant centers.[4,5] Most of the cases of aspergillosis occur during the first 6 months, and a median of 3.2 months, after lung transplantation. The vast majority of *Aspergillus* infections present as pneumonia or tracheobronchitis, with a risk of systemic and multiorgan dissemination in highly immune compromised patients.

Non-*Aspergillus* mold infections such as agents of mucormycosis, *Alternaria*, *Fusarium*, *Scedosporium*, among others occur at a much lower rate, at 27%, and at a much later time point since transplant surgery.[4,5] Certain groups of lung transplant patients are at increased risk of infections due to endemic fungi such as *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides immitis*.

However, clinicians should be aware that geographic boundaries of endemic mycoses are changing.[6] Cases of histoplasmosis have been reported to occur outside of the expected geographic endemic location, even in individuals without associated travel to an endemic area. *C. immitis* has been noted in soil environment, with local acquisition of patients in Washington State. *Blastomyces percursus* and *Blastomyces emzantsi* spp. have been better described as causes of human blastomycosis in South Africa.[6–8]

Geography seems to also play a role in the resistance patterns of certain IFIs. Clinicians should be aware of the potential for increased baseline azole resistance of *Aspergillus* sp. reported in Europe, particularly the Netherlands where 11 to 18% of isolates may be resistant.[9] Half of the environmental samples of *Aspergillus fumigatus* in azole-fungicide–containing plant waste in the Netherlands were azole resistant and carried resistance genes.[10] However, azole resistance has not been common in the United States. Only 1 of 181 *A. fumigatus* clinical isolates in the United States had azole resistance.[11]

Prevention

Prevention of mold infections typically begins with avoidance of high-risk exposures (e.g., avoiding compost and areas of construction and demolition) and utilizing respiratory protection whenever necessary. The use of antimicrobial prophylaxis is the most common approach—whether this is universal or a targeted approach. Universal antimicrobial prophylaxis to prevent *Pneumocystis jirovecii* is standard of care in lung transplant recipients, typically with sulfamethoxazole-trimethoprim.[12] The use of antifungal prophylaxis against mold infection is increasingly utilized following lung transplantation, although the details of this practice are highly variable. Surveys to define clinical practice regarding antifungal prevention strategies in lung transplant recipients indicate a trend toward increased utilization of antifungal prophylaxis protocols over time. Although approximately 59% of lung transplant programs utilized a universal prophylaxis strategy in a worldwide survey published in 2011,[13] by 2019 the rate was 90% in a survey of U.S. lung transplant centers.[14]

Antifungal prophylaxis against mold infection is commonly employed in the form of one of three strategies: (1) use of a topical inhaled antifungal agent (e.g., Amphotericin B nebulizer) to prevent postoperative tracheobronchitis; (2) use of a systemic antimold medication to prevent IFIs; or (3) use of both types of agents.[14,15]

The antimicrobial agents most commonly used for systemic antifungal prophylaxis are voriconazole and itraconazole. Barriers to prophylaxis are side effects, cost, and malabsorption.[16] A large retrospective study ($N = 662$) utilizing OptumLabs Data Warehouse claims data demonstrated that approximately 60% of lung transplant recipients filled antifungal prophylaxis following the index transplant hospitalization between 2005 and 2018.[17] This study was the first to demonstrate a significant survival benefit for lung transplant recipients obtaining prophylaxis in the first year following lung transplantation, compared with propensity-weighted controls who did not (8.4 events per 100 person-years compared with 19.5 in those without, $p = 0.003$).[17] The duration of antifungal prophylaxis, utility of pretransplant respiratory culture data (to assess fungal colonization), and specific medication regimen remains controversial. Although some centers use antifungal prophylaxis in all lung transplant recipients (the so-called universal prophylaxis), other centers limit antifungal prophylaxis to those with high-risk diagnoses or to those patients with evidence of colonization by fungal organisms on pre- or post-transplant respiratory cultures.[18,19]

Fungal Diagnostic Tools

Definitive diagnosis of IFI after lung transplantation relies on obtaining tissue for direct, histopathologic visualization of tissue invasion or fungal organisms in culture of specimens from sterile sites. Bronchoscopic and computed tomography (CT)-guided percutaneous lung biopsies are the two major ways to obtain tissue for histopathology and cultures for suspected pulmonary fungal disease. Fungal cultures and antifungal susceptibility tests generally guide the definitive management.

Molecular methods directed at detecting fungal antigens and deoxyribonucleic acid (DNA) allow for more rapid diagnosis when applied and interpreted in the appropriate clinical context. A recent study analyzed samples where hyphae were present on histopathology from immunosuppressed patients, and reported that broad-range fungal polymerase chain reaction (PCR) had sensitivity of 90% and a specificity of 86%.[20] Pathogen-specific *Aspergillus* PCR on hyphae-positive tissue had a sensitivity of 89%, but specificity was only 58%.[20] ►**Table 1** lists the characteristics, limitations, and controversies related to modern diagnostic tools. In an immunosuppressed population, antigen and DNA detection is favored over antibody testing, as antibody response can be unpredictable.

Galactomannan: Detection of galactomannan using enzyme immunoassay (EIA) has been utilized for nearly two decades.[21] Galactomannan is a major cell wall constituent of *Aspergillus* spp. and is released during fungal replication.[22]

Table 1 Serologic tests for detection of fungal antigens and DNA for common fungal pathogens in lung transplant recipients

Diagnostic test	Clinical use	Caveats
Galactomannan	*Serum*: - Diagnose invasive *Aspergillus* spp. or related molds in neutropenic patients *BAL*: - Diagnose pulmonary *Aspergillus* spp. or related molds in neutropenic and nonneutropenic patients	- Serum galactomannan has low sensitivity in nonneutropenic patients - Can cross-react with other mold species; culture data are still necessary - False positive can occur from dietary ingestion, penicillin antibiotics - Effect of antifungal medications on sensitivity is unknown
β-1,3-D glucan	- Used in conjunction with other diagnostic tools to screen for invasive fungal infection - May allow for safe discontinuation of antifungal medications in high-risk populations	- *Cryptococcus* spp. and *Mucorales* will not cause elevation - Should not be used as a standalone diagnostic test for invasive fungal disease - False positive can occur in patient receiving blood products, those on hemodialysis or cardiopulmonary bypass - Effect of antifungal medications on sensitivity is unknown
Aspergillus PCR	*Serum*: - Single test can help rule out invasive *Aspergillus* - Two positive serum tests can help rule in invasive *Aspergillus* *BAL*: - Can identify the presence of *Aspergillus* spp.	- Efforts to standardize PCR methods are ongoing - Most studies are in hematologic malignancy population - BAL cannot distinguish between colonization and infection - Antifungal medications significantly reduce sensitivity
Mucorales PCR	- Not clinically available	- Antifungal medications significantly reduce sensitivity
T2Candida nanodiagnostic panel	*Whole blood*: - Rapid detection of candidemia in high-risk populations	- Not evaluated for detection of deep-seated infection in the absence of candidemia - Effect of antifungal medications on sensitivity is unknown

Abbreviations: BAL, bronchoalveolar lavage; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction.

Serum galactomannan has the greatest sensitivity for the diagnosis of IFI in patients with severe neutropenia (<500 neutrophils per mm^3). In nonneutropenic patients, however, circulating galactomannan is consumed by the intact immune system. As such, the sensitivity of serum galactomannan for the diagnosis of IFI is estimated to be 71% in patients with hematologic malignancy and hematopoietic stem cell transplant and only 25% in nonneutropenic solid-organ transplant patients.[23] Galactomannan EIA sensitivity is improved to 88% in solid-organ transplant patients when utilizing bronchoalveolar lavage (BAL) specimens rather than serum or plasma.[23] The sensitivity of BAL galactomannan is reported between 57 and 100% in all-comers.[24–29] The specificity of galactomannan is estimated to be around 90% for serum and BAL specimens.[23–29]

Galactomannan: EIA can cross-react with mold species that are closely related to *Aspergillus* spp. such as *Fusarium* spp. and *Penicillium* spp.[30] In the case of pathologic molds such as *Fusarium* spp., this can be a helpful diagnostic adjunct. In the case of nonpathogenic *Penicillium* spp., this cross-reaction can lead to false-positive results with *Penicillium* spp. colonization or exposure to penicillin antibiotics[31]; however, the purification process for penicillin antibiotics has improved in recent years; so, false positives for this reason may no longer be a concern. Dietary ingestion of galactomannan in patients with mucositis may also be a cause for false-positive serum galactomannan. The impact of dietary galactomannan ingestion on BAL galactomannan is unknown. In the modern era where antifungal prophylaxis is commonplace for lung transplant recipients, it is important to note that the impact of cell-wall targeted antifungal medications, such as triazoles, on the sensitivity of serum or BAL galactomannan assays is unknown.

β-1,3-D glucan: Quantitative detection of β-1,3-D glucan has been utilized to aid in the diagnosis of IFI since the 1990s.[32] β-1,3-D glucan is contained in the cell wall of most fungal organisms, with the notable exceptions of *Blastomyces* sp., *Cryptococcus* spp., and *Mucorales*. While β-1,3-D glucan is an abundant constituent of *Candida* spp. cell-wall and elevated assays have traditionally been associated with invasive candidiasis, β-1,3-D glucan assays do not differentiate *Candida* spp. infection from other fungi. The sensitivity and specificity of β-1,3-D glucan for invasive candidiasis is around 80 and 60%, respectively.[33] An elevated β-1,3-D glucan level must be considered in appropriate clinical context. While low β-1,3-D glucan levels in high-risk populations suspected of invasive candidiasis may allow for the safe discontinuation of antifungal medications,[34,35] no evidence exists to support the initiation of antifungal treatment based solely on elevated (>80 pg/mL) β-1,3-D glucan levels.[33]

β-1,3-D glucan can be falsely elevated in patients receiving blood transfusions or intravenous immunoglobulin, and those on hemodialysis or cardiopulmonary bypass.[36] Notably, β-1,3-D glucan levels are characteristically high (>300 pg/mL) among patients with *Pneumocystis* pneumonia.[37] Similar to galactomannan, the effect of cell-wall–targeted antifungal prophylaxis or therapy on assay sensitivity is unknown.

Aspergillus PCR: While efforts are ongoing to standardize *Aspergillus* PCR methods, no universally accepted method for the detection of *Aspergillus* spp. currently exists. Methods vary by DNA extraction protocol, amplification technique, positivity threshold, and specimen type. A meta-analysis of serum *Aspergillus* PCR-based studies found a sensitivity of 80% and specificity of 79% for a single positive test, and sensitivity of 58% and specificity of 96% for two positive tests.[38] This indicates that single serum PCR may be helpful to rule out disease and two positive serum PCR tests may be helpful to rule in disease. However, many of the studies on *Aspergillus* PCR are on the detection or early diagnosis of invasive aspergillosis in patients with hematologic malignancy; so, applicability to lung transplant populations is unknown. Additionally, antifungal prophylaxis or therapy appears to reduce the positive predictive value of serum *Aspergillus* PCR from around 63% to a mere 5%,[39] indicating that the test may be best applied to antifungal naive patients.

Aspergillus: PCR can be used on BAL specimens. While the sensitivity for BAL *Aspergillus* PCR is greater than 90%,[40] it cannot distinguish between colonization and infection. Secondary to different BAL and PCR techniques, a threshold level to separate colonization and infection has not been established. A negative PCR on BAL may be helpful to exclude invasive aspergillosis,[27] but similar to serum PCR antifungal medications prior to testing greatly reduce the sensitivity.[40]

T2Candida nanodiagnostic panel: T2Candida panel is the only FDA-approved test to detect *Candida* spp. on whole blood samples. Using a combination of nuclear magnetic resonance and PCR, the platform allows for rapid (<3 hours) detection of *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*.[41,42] A multicenter trial of hospitalized patients with candidemia found the sensitivity of the T2Candida panel to be 89% with a positive predictive value of 84% in a setting of 10% candidemia prevalence.[43] The clinical utility of the T2Candida panel for suspected candidemia has not been fully evaluated. In patients at risk for candidemia, empiric antifungal therapy may be more clinically impactful than using the T2Candida panel.[44] The T2Candida panel has not been evaluated for the detection of deep-seated *Candida* spp. infections.

Endemic fungi antigen and antibody detection: B. dermatitidis, H. capsulatum, and *Coccidioides* spp. are the endemic fungi that cause disease in the United States. Aside from culture and direct visualization, diagnostic tests include antibody testing and antigen detection on urine, BAL, and serum samples.

B. dermatitidis and *H. capsulatum:* antigen testing detects capsule proteins. Secondary to their similarities in the capsule protein structure, there is a significant degree of cross-reactivity between these two fungi, lowering the specificity of these tests. Urine *Blastomyces* antigen testing has an overall sensitivity of 76 to 93% and a specificity of 80%.[45–47] Serum and BAL *Blastomyces* antigen testings have sensitivities less than 70%.[47] Conversely, *Histoplasma* serum and urine antigen testings have similar sensitivities (around 80% for disseminated disease and 65% for pulmonary disease).[48,49] Antigen testing is not routinely used to diagnose *Coccidioides* spp. infection.

The role for antibody testing in diagnosing active *B. dermatitidis* and *H. capsulatum* is limited. Traditional antibody testing for *Blastomyces* targets antibodies to a surface A antigen and has a sensitivity of 77% when using EIA techniques.[50] EIA techniques targeting antibodies to the surface protein, BAD-1, improved sensitivity to 88%[51] but is not commercially available. Compared with immunodiffusion and complement fixation, EIA techniques likewise appear to have the best sensitivity and specificity for diagnosing *H. capsulatum* infection. The sensitivity and specificity is 89 and 92%, respectively, when combining immunoglobulin M and immunoglobulin G antibodies.[49] Like other antibody methods, the host immune response influences the sensitivity of the test. The sensitivity is likely lower in lung transplant patients.

Antibody testing is the most commonly used method for diagnosing *Coccidioides* spp. infection. EIA has high sensitivity of 90%, and it is used to screen for disease.[52] Immunodiffusion and complement fixation techniques are used for confirmatory testing. As with other antibody tests, the sensitivity is likely lower in lung transplant patients, potentially due to the impaired antibody production in this population.

Important Pathogens

Fungal infection in lung transplant recipient can manifest as tracheobronchitis, fungal pneumonia, mediastinal infection, or disseminated fungal infection. Common fungal pathogens in lung transplant recipients and resultant disease manifestations are reviewed below.

Candida

Candida spp. can cause a spectrum of disease. Invasive candidiasis is the presence of *Candida* spp. in the blood stream, deep-seated tissue, or both. Candidemia is one of the most common nosocomial blood stream infections in critically ill patients and is associated with high mortality.[53] Invasive *Candida* spp. infections occur most commonly within the first 3 months posttransplant and are often the result of indwelling vascular catheters, surgical site infections, or airway anastomotic dehiscence resulting in pleural space contamination.[53] Risk factors for invasive candidiasis in lung transplant recipients include prolonged critical illness, neutropenia, and prolonged antibacterial therapy. The most common presentation in lung transplant recipients is line-related candidemia, candida empyema, or surgical site infection. Source control and prompt initiation of antifungal therapy (►**Table 2**) are associated with improved survival.[54]

Table 2 General treatment choices of selected fungal infections in lung transplant recipients

Fungal infection	Recommended antifungal treatment
Aspergillus sp.	Invasive aspergillosis - Voriconazole is treatment of choice Alternative therapies: - Liposomal amphotericin B product; inhalation may be given for tracheobronchitis in addition to systemic therapy - Other triazoles: posaconazole, isavuconazole - Echinocandins: caspofungin, anidulafungin, micafungin - Combination therapy may be considered
Candida sp.	Candidemia and invasive candidiasis - Echinocandins as initial empiric therapy: anidulafungin, micafungin, caspofungin Alternatives: - Fluconazole as alternative empiric therapy if azole-resistance is not a concern - Liposomal amphotericin B Targeted therapy based on susceptibility testing
Cryptococcus neoformans	Cryptococcal meningitis - Induction therapy: liposomal amphotericin B with flucytosine for 2–4 wk - Consolidation phase: fluconazole 400–800 mg daily for 8 wk - Maintenance phase: fluconazole 200–400 mg daily for at least 1 y Extra–central nervous system cryptococcal infections - Severe disease: similar treatment as meningitis - Nonsevere disease: fluconazole
Fusarium	Invasive fusariosis - Optimal antifungal therapy unclear - Choices: amphotericin B, voriconazole, posaconazole, isavuconazole
Mucormycosis	Invasive mucormycosis - Liposomal amphotericin B, high dose - Alternatives: posaconazole and isavuconazole
Pneumocystis jirovecii	*Pneumocystis* pneumonia - Trimethoprim-sulfamethoxazole; role of steroids not proven
Scedosporium and *Lomentospora*	Invasive scedosporiosis apiospermum - Voriconazole is the treatment of choice Invasive lomentosporiosis prolificans - Combination therapy (e.g., voriconazole plus terbinafine, others); multidrug resistant pathogen

Molds

Aspergillus

Aspergillus spp. are ubiquitous in the environment. The spectrum of clinical syndromes caused by *Aspergillus* spp. is largely dependent on host risk factors and includes respiratory tract colonization, mycetoma, and invasive aspergillosis. Invasive aspergillosis is the most serious and occurs in patients with significant immune compromise, critical illness, or underlying structural lung disease.[55] Severe neutropenia and lung transplantation are the greatest risk factors for the development of invasive aspergillosis. Lung transplant recipients are at greatest risk within the first 12 months following transplantation.

Aspergilloma tends to occur in the setting of structural lung disease and presents with a cavitary lung lesion with internal debris and surrounding ground-glass opacities or consolidation on CT of the chest. The surrounding areas of ground glass and consolidation represent local invasion into the surrounding lung tissue. ►**Fig. 1** demonstrates a right upper lobe aspergilloma that developed in a 65-year-old double-lung transplant recipient with chronic lung allograft dysfunction–bronchiolitis obliterans phenotype. He was 8 years posttransplant and treated with pulse dose steroids for potential acute cellular rejection episode 3 months prior. BAL cultures grew *A. fumigatus*, and BAL galactomannan was significantly elevated. Despite targeted antifungal therapy, this patient required a right upper lobectomy, as is often required for the management of mycetoma.

Aspergillosis can have disseminated involvement in lung transplant patients with higher-intensity T-cell–targeted immunosuppression. A 63-year-old patient presented 9 months after double-lung transplant with lower extremity ulcers and declining pulmonary function. She was treated with plasma exchange, steroid bolus, and antithymocyte globulin for antibody-mediated rejection in the months prior to presentation. ►**Fig. 2** demonstrates a diffuse nodular infiltrate with areas of ground-glass opacity and consolidation. BAL cultures grew *A. fumigatus*. Transbronchial biopsy and skin biopsy demonstrated fungal elements consistent with *Aspergillus* spp. The patient was treated with voriconazole and reduction in immune suppression but had continued disease progression. This required salvage combination therapy with posaconazole and caspofungin.

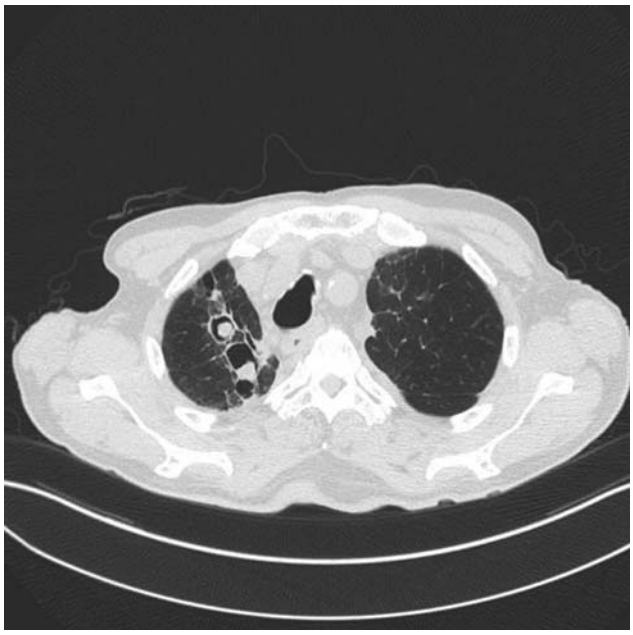

Fig. 1 Computed tomography of the chest demonstrating a right upper lobe cavity with internal debris and surrounding ground glass and consolidation consistent with a mycetoma.

Fusarium

Fusarium spp. are filamentous fungi that are found ubiquitously worldwide. They are an uncommon but important pathogen in lung transplant recipients, and are associated with high mortality. *Fusarium* causes infection in lung transplant recipients through inhalation and cutaneous and mucosal invasion. The respiratory system has been the portal of entry for the majority of *Fusarium* infections in lung transplant recipients, leading to sinusitis and pneumonia, while local cutaneous and mucosal invasion may lead to keratitis and skin and soft-tissue infections. The potential for hematogenous spread with subsequent multiorgan involvement is facilitated by severe and prolonged neutropenia, and severe T-cell immunodeficiency.[56]

Fusarium can cause a wide range of clinical manifestations, from superficial, locally invasive, and disseminated infections. The lungs are involved in the majority of cases of disseminated fusariosis. Radiographic studies show pneumonia, nodules, cavitary lesions, and parenchymal infiltrates. Patients usually present with cough, chest pain, and shortness of breath. Diagnosis is confirmed by the isolation of the fungi in culture. Biopsy may be performed to document invasive infection. Blood cultures may be positive. Disseminated fusariosis has poor prognosis. There is a high mortality rate, as high as 65%, particularly for disseminated infections, and those with severe neutropenia and markedly impaired immune status.[56]

Mucormycosis

Among IFIs with highest morbidity and mortality in the lung transplant population are those in the order of *Mucorales*. Mucormycosis is the third most common cause of IFI among lung transplant recipients with an incidence of 2% 1 year after transplant.[4] The genera most commonly implicated in human infections are *Rhizopus*, *Mucor*, and *Rhizomucor*.[57] These are classically described as broad, irregularly branched, pauci-septate ribbon-like hyphae on hematoxylin and eosin staining, and this distinct appearance allows for preliminary identification from histopathologic specimens to guide therapeutic management. Though not classical or exclusive to this infection, radiologic studies may show the reverse halo sign, describing a focal area of ground-glass attenuation surrounded by rim of consolidation.[58] Rapidly developing cavitary lesions on imaging (chest radiograph [CXR] or CT scan) can be seen (►**Fig. 3**).

Mucormycosis most commonly presents as pulmonary disease, although rhinocerebral, disseminated, anastomotic, gastrointestinal, and cutaneous manifestations have all been described.[59] The diagnosis is challenging, as noninvasive studies such as galactomannan and β-1,3-D-glucan and fungal blood cultures are classically negative for these organisms even in the presence of invasive disseminated disease. The possibility of mucormycosis should be considered in clinical scenarios suspicion of fungal infections especially when the host is receiving antifungal prophylaxis that is not active against this group of organisms (such as prophylaxis with fluconazole, itraconazole, or voriconazole). Transplant centers with recent cases of IFI due to mucormycosis should be aware of the implications of hospital-acquired infection and investigate such cases for causes to prevent further cases.[60–62]

Management relies on rapid diagnosis, aggressive and prompt surgical resection, and initiation of active antifungal therapy (►**Table 2**). The diffuse nature of pulmonary disease due to mucormycosis that is seen after lung transplantation may limit the options for surgical resection.[59] Poor outcomes in lung transplant recipients due to mucormycosis are attributed to rapid vascular invasion, necrosis, and destruction of tissues. Mortality due to mucormycosis is upward of 96% when disseminated but lower in localized pulmonary disease (76%).[57]

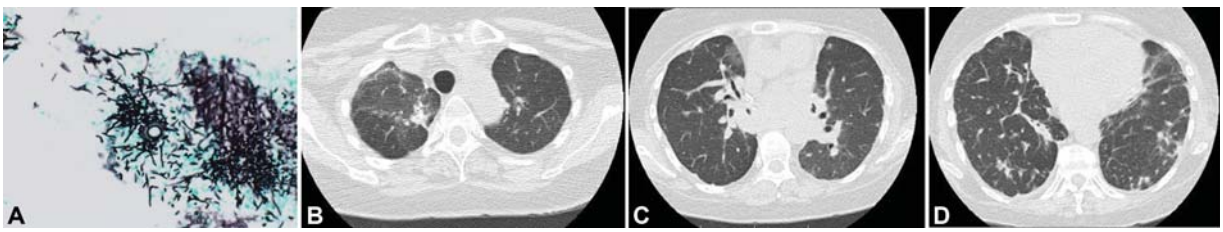

Fig. 2 Computed tomography (CT) of the chest demonstrating diffuse nodular and ground-glass infiltrates. In isolation, these CT findings are nonspecific but concerning for an evolving atypical infection. Transbronchial biopsy confirmed invasive aspergillosis. Histopathology showed acute branching hyphae with septae consistent with *Aspergillus* spp. *Image courtesy*: Dr. Audrey N. Schuetz, MD, Mayo Clinic, Rochester.

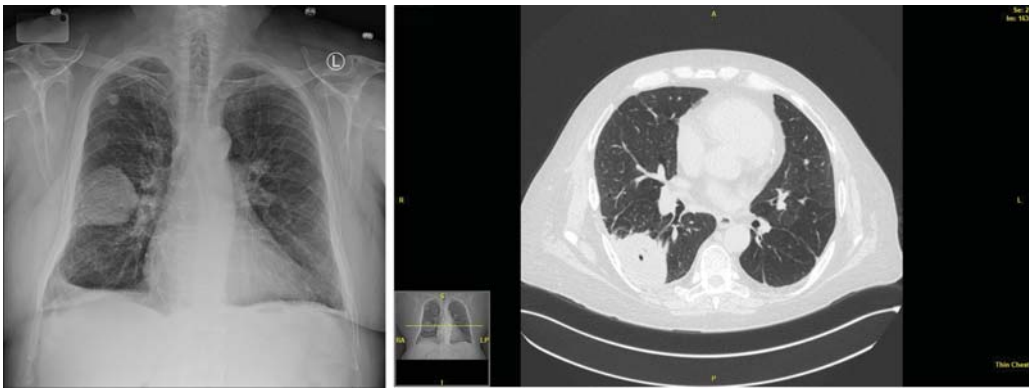

Fig. 3 (A) Chest X-ray demonstrating a cavitary mass in the right lower lobe, biopsy proven to be an infection due to *Rhizopus* sp. (B) Partially cavitary mass in right lower lobe with adjacent pleural thickening, proven by biopsy to be *Rhizopus* sp.

Scedosporium

Lung transplant recipients are at increased risk for infection with *Scedosporium*, particularly those with underlying cystic fibrosis. Identification of *Scedosporium* in BAL specimens of lung transplant recipients without evidence of infection and identification in patients with either fungal pneumonia or those with evidence of disseminated disease demonstrate the spectrum of manifestations possible with these organisms. Both hyphae and conidia can be present on histopathology specimens of infected tissue using methenamine silver fungal stains. Lowering immunosuppressive therapy and using voriconazole and surgical debridement (when possible) to treat the underlying infection is the most common strategy. Use of a second concomitant antifungal agent (e.g., terbinafine) is controversial (►**Table 2**).

Dimorphic Fungi

Blastomycosis

Blastomyces causes disease via direct inhalation of spores and can disseminate to a variety of organ systems with a predilection for skin, genitourinary tract, and lungs.[33] Blastomycosis infection may present with a range of clinical syndromes that includes asymptomatic pulmonary nodules, necrotizing pneumonia, acute respiratory distress syndrome, prostatitis, and central nervous symptom infections. Host risk factors do not seem to play a significant role in the type of clinical syndrome that develops, and lung transplant recipients do not tend to be more susceptible to Blastomycosis infections. Blastomycosis always requires antifungal treatment. The type and duration of antifungal treatment depends on the clinical syndrome.[63]

►**Fig. 4** illustrates the spectrum of respiratory disease caused by Blastomycosis. ►**Fig. 4A** represents an incidentally discovered pulmonary nodule in a 24-year-old woman undergoing evaluation for liver transplantation for cryptogenic cirrhosis. Blastomycosis antibody and antigen testings were negative; however, Blastomycosis was identified on sputum culture. ►**Fig. 4B** is the CXR of a previously healthy 34-year-old woman with 7-day history of pleuritic chest pain cough. Blastomyces antibody to A surface antigen by EIA and Histoplasma urinary antigen were positive. Sputum smear and culture confirmed the diagnosis of Blastomycosis pneumonia. She was treated with 6 weeks of itraconazole and made a full recovery. The CXR in ►**Fig. 4C** is that of a previously healthy 26-year-old man who presented with fevers, cough, and 4-week history of pneumonia refractory to outpatient antibiotic management. His respiratory status rapidly deteriorated within 24 hours of admission requiring intubation and eventual veno-venous extracorporeal membrane oxygenation. Blastomyces antibody to A surface antigen by EIA and Histoplasma urinary

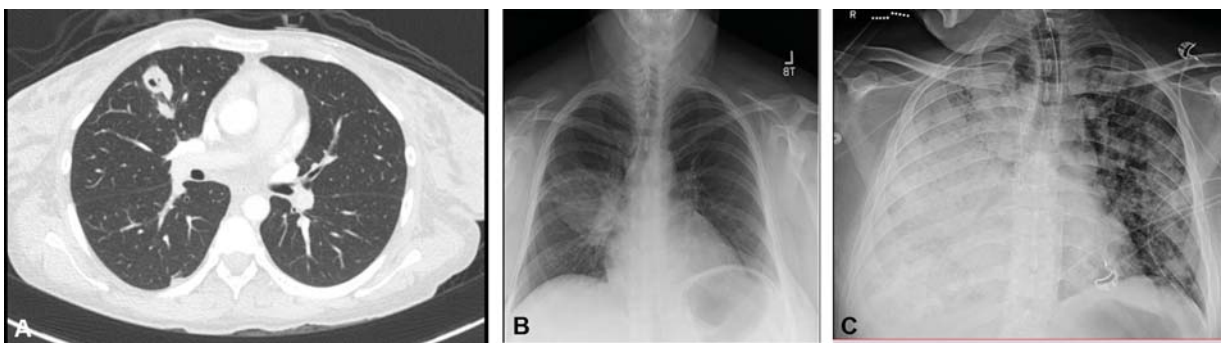

Fig. 4 (A) Computed tomography (CT) illustrating an incidentally discovered right upper lobe cavitary nodule in a 24-year-old woman undergoing evaluation for liver transplantation. Sputum culture grew *Blastomycosis dermatitidis*. (B) Chest roentgenogram (CXR) demonstrating right middle lobe pneumonia in a previously healthy 34-year-old woman. Diagnosis of *Blastomyces* pneumonia was confirmed on sputum smear and culture. (C) CXR demonstrating dense right lung consolidation and patchy left lung infiltrates in a previously healthy 26-year-old man. *Blastomycosis dermatitidis* was identified on bronchoalveolar lavage fungal smear.

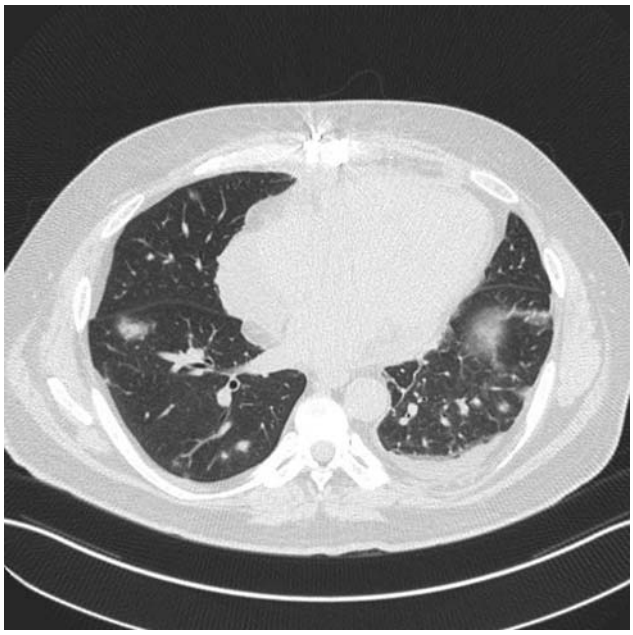

Fig. 5 Multifocal nodular opacities in lungs with surrounding ground-glass halos due to coccidiomycosis 3 months after undergoing heart transplantation.

antigen were positive. BAL Blastomyces PCR, smear, and culture were also positive. After he demonstrated clearance of Blastomyces on BAL cultures, he underwent double-lung transplant for postinflammatory fibrotic lung disease. The timing of transplantation listing was decided based on culture clearance as means of identifying any remaining viable organism rather than utilizing PCR or antigen testing. Unfortunately, he succumbed to intra-abdominal complications of his prolonged critical illness.

Coccidiomycosis

C. immitis and *C. posadasii* are the species which cause this disease process. Disease is most commonly acquired via inhalation of spores from the environment. Endemic areas include the Southwestern United States, Mexico, and certain areas of desert regions of Central and South America. This IFI can pose numerous challenges in management, diagnosis, and prevention.

Coccidiomycosis does not always require treatment in the immunocompetent host, as it is generally a mild and self-limited infection. In immunosuppressed patients, such as those on immunosuppressive agents or with diminished cellular immunity, coccidiomycosis can present a significant challenge. Reactivation of previous latent infection can be encountered, years or even decades after the initial infection (example of posttransplant reactivation is shown in ►**Fig. 5**). Active infection is associated with significant morbidity and mortality.

Screening of lung transplant candidates at risk for coccidiomycosis pretransplantation (via serology testing, and history and clinical questions) is important to identify those at risk and in need of antifungal prophylactic therapy.[64] Similarly, lung transplant recipients residing in areas endemic for coccidiomycosis should receive primary prophylaxis after lung transplantation, for a minimum of 6 to 12 months, as well as when being treated for allograft rejection with intensification of immunosuppressive therapy. Optimal duration of prophylaxis in transplant recipients is not well established, although in some situations, such as cases of central nervous system infection, lifelong antifungal prophylaxis is generally recommended.[64] Infection developing after lung transplantation most commonly presents in the first 6 to 12 months after transplant as severe pulmonary or disseminated infection. Clinicians should also be aware of uncommon but reported cases of donor-derived infection. In such situations, the lung transplant recipient should be managed with lifelong antifungal suppression.[64,65]

Even mild cases of infection should be investigated and treated to prevent progression to more severe or disseminated disease. Fluconazole remains the main drug for treatment and prophylaxis with the exception of severe disseminated disease, where use of lipid formulation amphotericin is recommended.[64]

Histoplasma

Histoplasmosis, caused by *H. capsulatum*, a dimorphic fungus that is endemic to the Midwestern United States, Mexico, and regions of South America with typical exposures attributed to bird or bat droppings.[64] Immunocompromised lung transplant recipients can suffer an opportunistic histoplasmosis infection usually secondary to posttransplant exposure. Diagnosis of histoplasmosis infection includes respiratory samples for culture, tissue histopathology with fungal stains (►**Fig. 6**), and/or Histoplasma urine antigen. Severe disease warrants initiation of liposomal amphotericin; however, mild to moderate infections are typically responsive to itraconazole therapy. As such, histoplasmosis infections while taking post-transplant antifungal prophylaxis are exceedingly rare. Of note, guidelines do not endorse the need for prophylaxis for history of remote infection, although it can be considered for those with histoplasmosis within 2 years of transplant.[64]

Pneumocystis

Pneumocystis is a fungal organism that exclusively causes infection in immunocompromised hosts, and most commonly affects those with reduced T-cell immunity. While human immunodeficiency virus (HIV) is the most notable risk factor for the development of *Pneumocystis* pneumonia (PCP), PCP can occur in lung transplant recipients and has a high overall mortality rate.[12] PCP differs in non-HIV patient populations in that there appears to be a lower organism burden but greater neutrophil response.[66] This is important for two reasons: (1) the lower organism burden may decrease the sensitivity of diagnostic tests such as PCR and silver stain[66] and (2) traditionally employed high-dose corticosteroids may not be effective.[67]

Treatment

Treatment of fungal infections after lung transplantation is multimodal based on three basic principles. First, antifungal drug therapy should be provided targeting the specific offending pathogen. Second, bronchoscopic and surgical

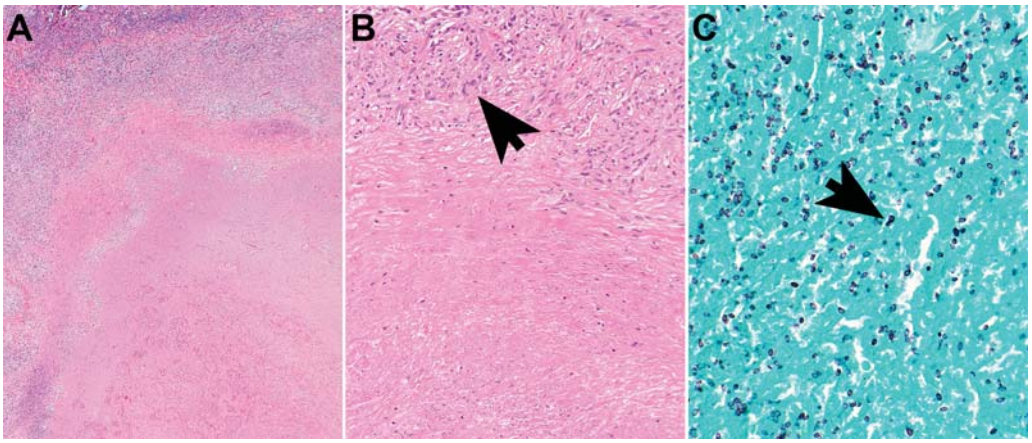

Fig. 6 Necrotizing granuloma with fungal organisms consistent with Histoplasma. (**A**) Necrotic tissue (right lower side) is lined by a rim of chronic inflammatory cells (left and upper side). (**B**) Epithelioid histiocytes, a few lymphocytes, and multinucleated giant cells (arrow) rim the necrosis (lower side). (**C**) A Grocott methenamine silver (GMS) stain highlights small (2–4 μm) fungal organisms that show narrow based budding (arrow). Magnification ×40 (**A**, hematoxylin and eosin), ×200 (**B**, hematoxylin and eosin), ×600 (**C**, GMS). (Contributed by Dr. Anja C. Roden, Mayo Clinic Rochester.)

debridement, resection of infected tissue, or removal of infected devices should be performed, as much as feasible, to reduce fungal load. Third, the intensity of immunosuppression should be alleviated to allow for a functional immune system to resolve an opportunistic mycosis.

The choice of antifungal therapy depends on the offending pathogen. In general, antifungal drugs are classified into three different categories: the polyenes, the triazoles, and the echinocandins. ►**Table 2** is a summary of general antifungal treatments for specific fungal infections. Voriconazole is the treatment of choice for invasive aspergillosis, since it has been proven to be more effective than liposomal amphotericin B. Alternative agents for invasive aspergillosis include caspofungin, posaconazole, and isavuconazole. The echinocandins are now the preferred initial empiric treatment of disseminated *Candida* sp. infections, pending species identification, and antifungal susceptibility testing. High-dose liposomal amphotericin B, up to 10 mg/kg daily as tolerated, is recommended for the initial empiric treatment of invasive mucormycosis. Liposomal amphotericin B with flucytosine is the initial induction treatment of cryptococcal meningitis, and this is followed by a consolidation and maintenance phase of high-dose fluconazole. Nebulized amphotericin B may help in the treatment of invasive bronchial infections. The antifungal drug terbinafine may be used in selected infections caused by multidrug-resistant fungi.

Therapeutic drug monitoring is recommended for most azole therapies. Suboptimal absorption is a major concern with oral itraconazole. Real-world data with use of SUBA-itraconazole (improved bioavailability itraconazole) shows most patients are able to achieve therapeutic trough concentrations at a median of day 7, thereby circumventing the issues related to standard formulation itraconazole.[68] In addition, there are ongoing questions of best dosing strategies in areas where there is concern for sub- or supra-therapeutic levels, such as obesity, patients in the intensive care units, or those who are receiving extracorporeal membrane oxygenation.

The potential for drug interactions and synergistic toxicities should be considered when using these antifungal agents in lung transplant recipients. The azoles increase the levels of calcineurin inhibitors, such as tacrolimus and cyclosporine, and it is recommended to monitor drug levels of the azoles (to document absorption and prevent toxicities) and the calcineurin inhibitors. Preemptive dose reduction and careful monitoring of serum concentrations of calcineurin inhibitors are needed when azoles are used. Monitoring for renal function is also imperative when an amphotericin B product is prescribed, since it may potentiate the renal toxicities of calcineurin inhibitors. As pharmacologic immunosuppression is reduced to complement the antimicrobial treatment of IFIs, one should be extra cautious to minimize the risk of allograft rejection.

Novel Antifungal Therapies

An encouraging and promising area of development is the evaluation of the safety and efficacy of novel antifungal therapeutic agents. There are many studies in the early clinical and preclinical phases, with some investigating novel mechanisms of antifungal activity.[69] These novel compounds are showing promising activity for various IFI, including agents with activity against yeasts, endemic fungi and molds, and resistant fungi.

Fosmanogepix (APX001) is an inhibitor of fungal enzyme Gwt1 with broad anti-yeast, endemic and some mold in vitro activity, and high bioavailability.[70] It is currently undergoing phase II clinical trials.

Ibrexafungerp is first in class oral triterpenoid inhibitor of *β-1,3*-D glucan synthase inhibitor. It is undergoing phase II and III trials, and promises to be a potential option for oral antifungal therapy with mechanism of action similar to echinocandins. This drug is being evaluated against candida, including *Candida auris*, and *Aspergillus* sp.

Olorofim is the first member of a class of antifungals known as orotomides. This class inhibits dihydroorotate dehydrogenase, a key enzyme in the biosynthesis of pyrimidines. It has

shown broad-spectrum antimold in vitro activity including *Scedosporium* sp., *Lomentospora prolificans*, and thermally dimorphic fungi. However, no activity against yeasts or *Mucorales* sp. is expected.[71–73] A phase IIB clinical trial is underway for invasive mold infections with limited treatment options (ClinicalTrials.gov identifier: NCT03583164).

Oteseconazole (VT1161) is a tetrazole with an antifungal drug highly specific for CYP51 with expected broad anticandida activity. A phase II trial that compared otesaconazole with fluconazole for vulvovaginitis showed good safety and efficacy results.[74]

Rezafungin is a novel 1,3-beta-D glucan synthase inhibitor with good activity against a broad range of fungal pathogens. A phase II study for invasive candidiasis showed promising results in terms of safety and efficacy.[75] A phase III clinical trial for candidemia, and another trial evaluating it as a prophylactic agent for *Candida* spp., *Aspergillus* spp., and *Pneumocystis jirovecii* in immunocompromised hosts, is ongoing.[76] Rezafungin is long-acting and only requires weekly dosing.

Conclusion

Invasive fungal infections remain important in lung transplant recipients due to immunosuppressed state, impaired natural defenses, and environmental vulnerability. Antifungal prophylaxis is the main strategy for the prevention of IFI following lung transplantation. Although *Pneumocystis* prophylaxis is continued lifelong, the duration and type of antifungal prophylaxis utilized to prevent most invasive mold infections remains controversial. Furthermore, although some programs practice universal prophylaxis for all recipients, other programs reserve prophylaxis for recipients identified as at increased risk for IFI due to primary diagnosis or pretransplant colonization with fungal organisms. Fungal infections in lung transplant recipients are increasingly diagnosed with the assistance of advanced molecular techniques, which complements (and sometimes circumvents the need for) histopathology. Although common fungal pathogens have not changed considerably, practitioners need to be ever mindful of evolving resistance patterns, as they influence the choices for empiric and targeted therapies. Several new antifungal agents are in the research development pipeline promising improved outcomes.

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Conflict of Interest
None declared.

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