

Special Article

Cryptococcosis in Solid Organ Transplantation

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Abbreviations: BAL, bronchoalveolar lavage; CGB, canavanine glycine bromothymol blue; CNS, central nervous system; CSF, cerebrospinal fluid; IRIS, immune reconstitution inflammatory syndrome; MIC, minimum inhibitory concentration; SOT, solid organ transplant.

Epidemiology and Risk Factors

Cryptococcosis is the third most commonly occurring invasive fungal infection in solid organ transplant (SOT) recipients. Approximately 8% of invasive fungal infections in SOT recipients are due to cryptococcosis (1). The overall incidence of cryptococcosis in SOT recipients ranges from 0.2% to 5% (1,2). Cryptococcosis is typically a late-occurring infection; the median time to onset usually ranges from 16 to 21 months posttransplantation (1,3,4). The time to onset is earlier for liver and lung (<12 months) compared to kidney transplant recipients and this may be due to a higher intensity of immunosuppression in the former subgroups (4).

As in most other hosts, cryptococcal disease in SOT recipients is considered to represent reactivation of quiescent infection (5,6). Epidemiological investigations suggest that acquisition of primary infection following transplantation also occurs (7,8). Isolates from a pet cockatoo and a renal transplant recipient with cryptococcosis showed identical genotypic profile suggesting recent acquisition of this yeast (9). Infection with *Cryptococcus* is thought to be caused by inhalation of the organism, either in yeast form or perhaps as basidiospores, from an environmental source such as bird droppings or soil. Although rare, cases of transmission from donor organ and tissue grafts have also been recognized (10–13). Donor-derived cryptococco-

sis should be considered when diagnosis occurs in the recipient within 30 days of transplant, cryptococcosis is diagnosed in more than one organ recipient from a single donor or *Cryptococcus* is documented at the surgical or graft site (14).

Calcineurin-inhibitors remain the mainstay of immunosuppression in SOT recipients in the current era. These agents do not appear to influence the incidence, but may affect the manifestations of cryptococcal disease (3). Patients receiving a calcineurin-inhibitor-based regimen were less likely to have disseminated disease and more likely to have cryptococcosis limited to the lungs (4). Anticryptococcal activity of these agents that target the fungal homologs of calcineurin was considered to account for these findings (4,15). Corticosteroids are associated with an increased risk of cryptococcosis in all non-HIV infected hosts (16–19); however, the precise daily dose that confers a higher risk in SOT recipients remains unknown (20). Also, cirrhotic patients are at an increased risk for disseminated cryptococcosis rather than local pulmonary infection (16). T-cell depleting antibodies such as alemtuzumab are increasingly employed as induction therapy or as treatment of rejection in SOT recipients (21). Alemtuzumab causes profound lymphocyte depletion of CD4+T cells which may last several months. Employment of more than one dose of alemtuzumab or antithymocyte has been associated with an increase in the risk for cryptococcosis (21). The cumulative incidence of cryptococcosis was 0.3% in SOT recipients who did not receive alemtuzumab or antithymocyte globulin, 1.2% in those who received a single dose, and 3.5% in the patients who received ≥ 1 doses of these agents ($p = 0.04$) (21). Invasive fungal infections occurred more frequently in SOT recipients who received alemtuzumab as antirejection as opposed to induction therapy (22).

While *C. neoformans* var *grubii* (serotype A) has no particular geographic predilection and causes most infections in SOT recipients, (23) *C. neoformans* var *neoformans* (serotype D) is prevalent in Northern Europe (18). *Cryptococcus gattii*, previously regarded as a tropical and subtropical fungus, has emerged in the Pacific Northwest in the United States and British Columbia, Canada (24,25). *C. gattii* infects mostly nonimmunocompromised hosts, causes cryptococcomas more frequently than *C. neoformans*, and may require prolonged antifungal treatment (24,25). The organism has been characterized into four genotypes through multilocus sequence typing: VG1, VGII, VGIII, and VGIV (26). The VGII genotype has been further characterized into VGII a, b and c. The *C. gattii* that is endemic in

tropical and subtropical regions is mostly VG1 (27). The outbreaks in Oregon and Vancouver island are genetically different isolates of VGIIa (28). The incubation period of *C. gattii* disease in Vancouver Island and Pacific Northwest US has been documented to be ~6 months (29). The Oregon subtype (VGIIc) has currently been associated with 70% mortality in SOT recipients and is likely to have a high fluconazole minimum inhibitory concentration (MIC) (30,31).

Clinical findings

Cryptococcosis usually manifests as CNS disease (meningitis) or pneumonia, but can affect multiple sites, including skin and soft tissues, the prostate gland, liver, kidney, bones, and joints. Pulmonary disease ranges from asymptomatic colonization or infection to severe pneumonia with respiratory failure. Radiographic findings are nonspecific and include nodular opacities or masses and less often consolidation or effusions (32–34). Isolated pulmonary disease may occur in 33% of SOT recipients (4). Approximately 50–75% of SOT recipients with cryptococcosis have extrapulmonary disease or CNS involvement (3,4,20,35). It has been reported that 61% of SOT recipients had disseminated disease; 54% had pulmonary and 8.1% had skin, soft-tissue or osteoarticular disease (4). Liver as opposed to other types of SOT recipients had a sixfold higher risk for developing disseminated disease. Up to 33% of the SOT recipients with cryptococcosis had fungemia (3,4,36). Patients with CNS disease in one report were more likely to be fungemic than those without CNS disease (37). Cutaneous cryptococcosis may present with papular, nodular, or ulcerative lesions or as cellulitis, with the majority found on the lower extremities and associated with CNS disease (38,39). While cutaneous lesions largely represent hematogenous dissemination, the skin has also been identified as a portal of entry of *Cryptococcus* and a potential source of subsequent disseminated disease in SOT recipients (8).

Mortality in SOT recipients with cryptococcosis has ranged from 33% to 42% and may be as high as 49% in those with CNS disease (3). Overall mortality in SOT recipients with cryptococcosis in the current era is 14% (4). In a case series of 28 SOT recipients with cryptococcal meningitis, mortality was associated with altered mental status, absence of headache, and liver failure; the latter was an independent predictor for death (36). In contrast, receipt of calcineurin-inhibitor agents was independently associated with a lower mortality, but renal failure at baseline with higher mortality (4). Improved outcomes with the use of calcineurin-inhibitor agents may be attributable in part to their synergistic interactions with antifungal agents (40).

Diagnosis

An important aim of diagnosis of cryptococcosis in SOT recipients is to determine the site and extent of disease, as this will help to dictate management (41). All SOT

recipients with suspected or documented cryptococcosis should undergo a thorough evaluation for extrapulmonary cryptococcosis, including lumbar puncture and blood and urine cultures (II-2). Blood cultures for *Cryptococcus* may be positive in up to 45% of patients with meningitis (37). If isolated pulmonary disease is suspected, a bronchoalveolar lavage (BAL) with or without biopsy should be considered and is important when eliminating other causes (42) (II).

A lumbar puncture should be performed in all SOT patients with suspected cryptococcosis. Opening pressure should be recorded, and large volume (>1 mL or 20 drops) cerebrospinal fluid (CSF) removal should occur and be sent for Gram's stain, cell count, protein, glucose and cryptococcal antigen testing (37,42,43) (II-1). CSF cryptococcal antigen testing is more sensitive and specific than India ink staining or fungal culture. Titers are higher with leptomeningeal than intraparenchymal brain lesions (3,4,36). Cryptococcal antigen testing from serum is also a very sensitive test (90%) for initial diagnosis of infection, but titers among SOT recipients are normally lower (usually <1:1024) than in HIV-infected patients (36,44). Cryptococcal antigen testing can identify *C. gattii* infections but has lower titers than with *C. neoformans* (30,45) and its sensitivity decreases to less than 25% with other nonneoformans species such as *C. laurentii* and *C. albidus* (46–48). Serum cryptococcal antigen titers are higher in patients with disseminated and CNS disease than in those with isolated pulmonary disease (33,34,37). Serologic tests such as *Aspergillus* galactomannan and β -D-glucan are not effective for the diagnosis of cryptococcosis.

Brain CT imaging may be performed prior to lumbar puncture to determine the presence of mass lesions or hydrocephalus, but has suboptimal sensitivity for evaluating cryptococcomas (42). Up to 33% of patients may have cryptococcomas on presentation and MRI is more sensitive than CT imaging for detecting these lesions. Cerebral cryptococcomas are more common in patients with *C. gattii* infection than in patients with *C. neoformans* infection. Mortality is higher in patients with intraparenchymal lesions than with meningeal disease alone (30,37,49).

Extraneural cryptococcosis can occur in the skin, prostate gland, liver and kidney. Biopsies with culture of tissues will confirm the diagnosis. On routine hematoxylin and eosin staining of tissues, *C. neoformans* is difficult to identify. However, Gomori-methenamine silver or periodic acid-Schiff staining allows for identification; the organism can be recognized by its oval shape, and narrow-based budding. With the use of mucicarmine staining, the cryptococcal capsule will stain rose to burgundy in color and help differentiate *C. neoformans* from other yeasts, especially *Blasotomyces dermatiditis* and *Histoplasma capsulatum* (50).

Prostatic and kidney disease may present as yeast in the urine and clinical suspicion is frequently needed to make

this diagnosis (41,51). Diagnosis of pulmonary disease is frequently made by the detection of the yeast in BAL specimens. Cryptococcal antigen testing is also useful, with a recent study in SOT recipients reporting positivity in 83% of patients with any pulmonary involvement (34). Patients with cryptococcosis limited to the lungs were less likely to have a positive antigen than those with concomitant extrapulmonary disease (34).

Identification of cryptococcal species

With the emergence of *C. gattii* in the Pacific Northwest USA, Canada, and other areas, the importance of identification of the cryptococcal species has increased, as it may affect the choice of antifungal therapy (52). Use of canavanine glycine bromothymol blue (CGB) agar will help to differentiate *C. neoformans* from *C. gattii* colonies and should be considered for patients with endemic exposure or clinical findings suspicious for *C. gattii* infection (II). Presently, serotyping of isolates is being performed using agglutination test kits or immunofluorescence assays (53,54). Currently under investigation at research laboratories are the experimental use of rapid technologies such as polymerase chain reaction (PCR), loop-mediated isothermal DNA amplification, high resolution melt analysis and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) (52,55–57) (III).

Susceptibility testing

Antifungal susceptibility testing of *C. neoformans* has been standardized by the Clinical and Laboratory Standards Institute (CLSI), but to date the relationship of MIC to clinical outcomes has not been well studied (58). Presently, the routine use of antifungal susceptibility testing for cryptococcal infections is not recommended. However, several *C. gattii* genotypes may be associated with increased fluconazole MICs. In patients who have documented *C. gattii* infection, or for those with relapsed infection, testing is recommended for flucytosine and azole medications (31,42,53,59). Testing is usually available in a specialty laboratory.

Treatment

There have been no randomized controlled trials of cryptococcosis treatment in SOT recipients. Treatment recommendations have been extrapolated from clinical trials among HIV patients and from data collected retrospectively in SOT recipients. The recommendations herein are consistent with revised guidelines from the Infectious Diseases Society of America (IDSA) (42). Choice of antifungal therapy is dependent on site and extent of disease, net state of immunosuppression and severity of illness. Distinguishing between disseminated disease and localized pulmonary and asymptomatic disease is important prior to initiating therapy. This requires a thorough evaluation for CNS disease with a lumbar puncture (37,42,49,60,61) (II-2). Table 1 summarizes antifungal therapy in SOT recipients.

Table 1: Antifungal therapy for cryptococcal disease in solid organ transplant recipients

Meningoencephalitis or disseminated disease	
Induction	Duration
Preferred therapy	
Liposomal amphotericin B 3–4 mg/kg/day or Amphotericin B lipid complex 5 mg/kg/day plus flucytosine 100 mg/kg/day ¹	Minimum of 2 weeks
Alternative therapy	
Liposomal amphotericin B 3–4 mg/kg/day or Amphotericin B lipid complex 5 mg/kg/day	Minimum of 4–6 weeks
Consolidation	
Fluconazole 400–800 mg/day	8 weeks
Maintenance	
Fluconazole 200–400 mg/day	6–12 months
Pulmonary Disease	
Asymptomatic or mild-to-moderate disease	
Fluconazole 400 mg/day	6–12 months
Severe pulmonary disease, or azole use not an option	
Same as for CNS disease	

¹Dosages of flucytosine and fluconazole outlined above are in the absence of renal insufficiency. Both require dose reduction for renal insufficiency. Monitoring of flucytosine levels is recommended (58,58).

Note: Patients with asymptomatic pulmonary disease require antifungal therapy. Disseminated disease must be excluded in all patients. Those with disseminated disease, diffuse pulmonary infiltrates, and acute respiratory failure should be treated with the same regimen as Cryptococcal meningoencephalitis. Synergistic interactions of antifungal agents with a calcineurin inhibitor may improve outcomes (4,40).

In patients with CNS disease, disseminated disease or severe respiratory disease, fungicidal therapy with a polyene and flucytosine is recommended (41,42,62) (I). A lack of flucytosine as induction therapy has been shown to be an independent risk factor for mycologic failure at week 2 in SOT patients (62). The use of a lipid formulation of amphotericin B is preferred over amphotericin B deoxycholate, as nephrotoxicity is a more common complication in patients receiving amphotericin B deoxycholate. Moreover, many transplant recipients already have baseline renal dysfunction and may be receiving other nephrotoxic agents such as calcineurin inhibitors or antibiotics such as vancomycin or aminoglycosides (37,63–65). Additionally, mortality at 90 days in SOT recipients with CNS cryptococcosis was lower with the use of lipid formulations of amphotericin B compared to amphotericin deoxycholate (65). To avoid adverse effects of flucytosine, including bone marrow suppression and nephrotoxicity, monitoring and maintenance of flucytosine levels (2-h postdose level of 30–80 µg/mL) are recommended (58,62).

The recommendations for the management of neurological disease, disseminated cryptococcosis or severe pulmonary disease is induction therapy with liposomal amphotericin B (3–4 mg/kg/day) OR amphotericin B lipid complex (5 mg/kg/day) plus flucytosine (100 mg/kg/day)

in 4 equally divided doses every 6 hours based on creatinine clearance) for a minimum of 14 days followed by consolidation with fluconazole (400–800 mg/day) for 8 weeks and, finally suppression with fluconazole (200–400 mg/day) for 6 to 12 months (42) (II-2). Extended courses of fluconazole suppression may be required for patients based on clinical progress or net status of immunosuppression (III).

The recommendation for patients with focal pulmonary and incidentally detected pulmonary disease in otherwise asymptomatic patients is fluconazole 400 mg/day for 6–12 months. (II-3) Disseminated disease should always be excluded with a lumbar puncture and blood and urine cultures (3,42) (II-2). *C. neoformans*-positive cultures from sterile and nonsterile sites (i.e. sputum) warrant treatment even if the patient is asymptomatic. This is true in lung transplant recipients where *Cryptococcus* may be colonizing the donor allograft and without treatment, may become invasive disease in the presence of immunosuppression (11). Relapse of cryptococcosis after 6 months of fluconazole maintenance is very uncommon based on available data and thus the recommendations are for 6–12 months of therapy (66) (II-3). However, discontinuation of therapy must be made on the basis of signs, symptoms and level of immunosuppression. With careful monitoring of the drug interactions between fluconazole and calcineurin inhibitors, long-term fluconazole therapy in SOT recipients has proven to be very safe (67).

The use of extended-spectrum azoles, such as voriconazole, itraconazole, and posaconazole do not offer benefit over fluconazole for treatment of *C. neoformans* infection. These agents are more expensive, have more potential drug interactions with immunosuppressive agents and data in HIV-infected patients showed that itraconazole was inferior to fluconazole in the clearance and maintenance phases of cryptococcosis (68–70). In contrast, some genotypes of *C. gattii* appear to have reduced susceptibility to fluconazole. *In vitro* data suggest that the extended-spectrum azoles have excellent potency against with this species and may offer an oral alternative when transitioning from induction to maintenance therapy (31,53) (III). Close monitoring of tacrolimus levels is needed with co-administration of azoles, and dose-reduction should be considered at the time of azole initiation (see chapter 32 for specific recommendations). Voriconazole or posaconazole should preferably not be co-administered with sirolimus given potential for significant elevation of sirolimus levels (68,71,72).

Adjunctive therapies

Interferon- γ has been utilized as an adjunct to antifungal therapies in HIV-infected patients; however, other than one case report there are no large randomized clinical trial data available. Interferon- γ cannot be recommended due to concerns that it may induce organ rejection in this popu-

lation (4). Heat shock protein 90 (hsp90) recombinant antibodies are in development and *in vitro* studies show that in combination with amphotericin B may increase killing of organisms. Currently there are no clinical trial data to support its use in treating SOT recipients (73).

Immunosuppression

An important factor in the management of cryptococcosis is the concurrent attention to the degree of immunosuppression. Whenever possible, a reduction in the net state of immunosuppression should occur during therapy, but this can be complicated if the patient has received profound T-cell depleting agents such as alemtuzumab or thymoglobulin (74). The aim is a gradual tapering of immunosuppression, preferably with corticosteroids first, while on antifungal therapy such that there is eradication of infection with preservation of allograft function. A rapid reduction in immunosuppression may cause adverse acute organ rejection or emergence of IRIS, although no data are available to suggest the optimal methods of reduction in immunosuppression (75).

Complications

Immune reconstitution inflammatory syndrome (IRIS)

It is increasingly appreciated that restoration of host immunity, particularly if abrupt, may have adverse sequelae and when a threshold is reached, the host can become gravely ill with symptomatic disease due to immune reconstitution (76). Rapid reduction of immunosuppressive therapy in conjunction with initiation of antifungal therapy in SOT recipients may lead to the development of immune reconstitution inflammatory syndrome (IRIS), the clinical manifestations of which mimic worsening disease due to cryptococcosis (Table 2) (66,77). IRIS may present as lymphadenitis, cellulitis, aseptic meningitis, cerebral mass lesions, hydrocephalus or pulmonary nodules (66,77). Clinically, CNS IRIS appears to have less inflammation and has been found on lumbar puncture to be associated with protein levels ≤ 50 mg/dL and less than 25 white cells/ μ L (78). In kidney transplant patients, development of IRIS has been temporally associated with allograft loss (79). The overall probability of allograft survival following cryptococcosis in kidney transplant recipients was significantly lower in patients who developed IRIS compared to those who did not (79).

Immunosuppressive agents administered to transplant recipients such as calcineurin-inhibitors and corticosteroids exert their effect by preferentially inhibiting Th1 (IL-2 and IFN- γ) compared to Th2 (IL-10) responses (80,81). Tacrolimus inhibits Th1 to a greater extent than cyclosporine A (82,83). The biologic basis of IRIS in SOT recipients is believed to be reversal of a Th2 to Th1 proinflammatory response upon withdrawal or reduction of immunosuppression. A potential role of Tregs and Th17 regulatory pathways in the pathogenesis of IRIS in SOT

Table 2: Features of IRIS in patients with cryptococcosis (III)

1. New or worsening appearance of any of the following manifestations:
 - (a) CNS: Clinical or radiographic manifestations consistent with inflammatory process, such as contrast enhancing lesions on neuroimaging studies (CT or MRI); CSF pleocytosis, defined as >5 white blood cells; or increased intracranial pressure, that is, opening pressure ≥ 20 mm of water (with or without hydrocephalus).
 - (b) Lymph nodes, skin or soft tissue lesions, for example, cellulitis or abscesses.
 - (c) Pulmonary, for example, nodular, cavitory, mass lesions, pleural effusions (detected by chest radiography or CT).
 - (d) Other focal tissue involvement with histopathology showing granulomatous lesions
- and
2. Symptoms occurred during receipt of appropriate antifungal therapy and could not be explained by a newly acquired infection.
- and
3. Negative results of cultures for *C. neoformans* during the diagnostic workup for the inflammatory process.

Note: Table constructed from references (75,78,79).

recipients has also been proposed (84). Potent T cell lymphocyte depleting agents such as alemtuzumab have also been recognized as a risk factor for IRIS (85).

An estimated 5–11% of SOT recipients with cryptococcosis may develop IRIS, typically between 4 and 6 weeks after initiation of antifungal therapy (66,86). In one study, patients who developed IRIS were more likely to have received potent immunosuppression comprising a combination of tacrolimus, mycophenolate mofetil, and prednisone when compared to patients without IRIS ($p = 0.007$). Additionally, cases with IRIS versus those without were more likely to have disseminated cryptococcosis (79). These data are consistent with those in HIV patients where more profound immunosuppression at the onset of infection and greater severity of infection (or disseminated disease) correlated with an increased likelihood of IRIS after antiretroviral therapy. There are no laboratory markers or clinical criteria that can diagnose IRIS reliably or distinguish this entity from worsening cryptococcosis (49,75).

There is no proven therapy for IRIS. Minor manifestations may resolve spontaneously within a few weeks. Modifications in antifungal therapy are not warranted unless viable yeasts are documented in culture. Anti-inflammatory drugs such as corticosteroids have been employed anecdotally with success in *Cryptococcus*-associated IRIS in SOT recipients (77,86). Corticosteroids in doses equivalent to 0.5 to 1 mg/kg of prednisone may be considered for major complications related to inflammation in the CNS or severe manifestations of pulmonary or other sites (77) (III). The efficacy of thalidomide and other nonsteroidal anti-inflammatory agents remains unproven.

Elevated intracranial pressure (ICP) management

Cryptococcal infection of the brain causes a significant inflammatory response with the development of a film over the pial layer preventing the absorption of CSF, with subsequent elevation of intracranial pressure potentially leading to hydrocephalus, blindness, deafness or death (43). A significant factor related to patient morbidity and mortality is failing to address the raised intracranial pressure. Initial opening pressure should be recorded and if >25 mmHg, a large volume fluid removal should be performed to reduce the intracranial pressure to normal levels. If the initial opening pressure is >25 mmHg, lumbar puncture should be performed daily until opening pressure is < 25 mmHg (III). If the ICP remains high (>25 mmHg) and symptoms persist, consider temporary lumbo-peritoneal or external ventricular drains to monitor CSF pressure (III). Permanent ventriculo-peritoneal shunting should be considered in patients who have received appropriate antifungal therapy and if other conservative measures to reduce ICP have failed (42,43,61).

Antifungal prophylaxis

We do not currently recommend that SOT recipients receive routine antifungal prophylaxis against cryptococcosis, as there is no specific high-risk group that has been identified. In SOT recipients with previous cryptococcosis needing enhanced immunosuppression, consideration for resuming secondary prophylaxis can be made on an individual basis. For SOT recipients who experience graft failure after cryptococcosis, the ideal timing of retransplantation is unknown. However, in kidney transplant recipients, where there is the possibility of a hemodialysis bridge, it is reasonable to consider if they have received a year of antifungal therapy, have no signs or symptoms attributable to active cryptococcal disease and negative cultures from the original site of infection. In those SOT populations where no bridging option is available, we recommend that induction therapy is completed, all sites that yielded positive cultures have cleared and the cryptococcal antigen titer should be optimally declining. In these cases, secondary fluconazole prophylaxis should be considered for at least 1 year period (34,67). For pretransplant patients with active cryptococcal disease, our recommendations are the same as for those patients with graft failure (III).

Future research directions: The emergence of *C. gattii* infections has led to many future research endeavors. These include the development of rapid diagnostic techniques such as PCR testing and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) to differentiate *C. gattii* and *C. neoformans* more rapidly and accurately from a variety of clinical specimens. These tests are currently only performed in major research laboratories. Also, additional clinical studies are underway to better define the clinical outcomes of SOT recipients with *C. gattii* versus *C. neoformans* infections. Lastly, a new water soluble azole antifungal agent, isavuconazole, is currently in

phase 3 studies (Clinicaltrials.gov identifier: NCT00634049) as salvage therapy of invasive fungal infections. This compound may have activity against fluconazole resistant cryptococcal strains, making it a potential therapeutic option in the future.

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