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Cryptococcosis in Solid Organ Transplantation- Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice

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Abstract:

These updated guidelines from the Infectious Diseases Community of Practice of the American Society of Transplantation review the diagnosis, prevention and management of cryptococcosis in the pre- and post-transplant period. The current update now includes a discussion of cryptococcosis, which is the third most common invasive fungal infection in SOT recipients. Infection often occurs a year after transplantation, however early infections occur and donor derived infections have been described within 3 months after transplant.

There are two main species that cause infection, *Cryptococcus neoformans* and *C. gattii.* Clinical onset may be insidious, but headaches, fevers and mental status changes should warrant diagnostic testing. The lateral flow cryptococcal antigen assay is now the preferred test from serum and cerebrospinal fluid due to its rapidity, accuracy and cost. A lumbar puncture with measurement of opening pressure is recommended for patients with suspected or proven cryptococcosis. Lipid amphotericin B plus 5-flucytosine is used as initial treatment of meningitis, disseminated infection and moderate to severe pulmonary infection, followed by fluconazole as consolidation therapy. Fluconazole is effective for mild to moderate pulmonary infection. Immunosuppression reduction as part of management may lead to immune reconstitution syndrome that may resemble active disease.

Etiology and Pathogen Description

Cryptococcosis is caused primarily by two fungal species, (1, 2) *Cryptococcus neoformans* and *Cryptococcus gattii*. These species were initially distinguished by their antigenic diversity; *C. neoformans* strains are of serotypes A and D and *C. gattii* strains are of serotypes B and C. With the emergence of molecular testing, it has become clear that there is a large diversity within the species complex. As such, since 2014, new taxonomy has been proposed based on amplified fragment length polymorphisms: *C. neoformans* has been divided into *C. neoformans* (serotype A, formerly *C. neoformans* var. *grubii*), and *C. deneoformans* (serotype D, *C. neoformans* var. *neoformans*); *C. gattii* would be recognized as five separate species, namely, *C. gattii* (VGI), *C. deuterogattii* (VGII), *C. bacillisporus* (VGIII), *C. tetragattii* (VGIV), and *C. decagattii* (VGIV). (2) There are several other relevant cryptococcal species that have been known to rarely cause human disease, including *C. albidus* and *C. laurentii*.

Epidemiology and Risk Factors

Cryptococcosis is the third most common invasive fungal infection in SOT recipients. Approximately 8% of invasive fungal infections in SOT recipients are due to cryptococcosis. (3) The overall incidence of cryptococcosis in SOT recipients ranges from 0.2-5%. (3, 4) Cryptococcosis is typically a late-occurring infection; with the median time to onset ranging from 16 - 21 months post-transplantation. (3, 5, 6). The time to onset is typically earlier (< 12 months) for liver and lung compared to kidney transplant recipients.

Cryptococcus is an environmental fungus found in the soil, trees and bird droppings. (7) It can infect both immunocompetent as well as immunosuppressed hosts. (8) In general. *C.neoformans* has no particular geographic predilection and causes most infections in SOT recipients. (9) *C. gattii* complex has previously been regarded as a tropical and subtropical fungus, but now is prevalent in the Pacific Northwest in the US and British Columbia, Canada. (10, 11) . With more awareness and use of molecular testing, *C. gattii* is increasingly been identified in areas around the world, with marked genotypic diversity. (12) The incubation period of *C.gattii* disease in Vancouver Island and Pacific Northwest US has been documented to be ~6 months. (13) The Oregon subtype (VGIIc) has currently been associated with 70% mortality in solid organ transplant recipients and has been found to have a high fluconazole minimum inhibitory concentration (MIC). (14, 15)

Cryptococcosis in SOT recipients is considered primarily to represent reactivation of quiescent infection. (16, 17). However, epidemiological investigations suggest that acquisition of primary infection following transplantation also occurs. (18, 19) For example, isolates from a pet cockatoo and a renal transplant recipient with cryptococcosis showed identical genotypic profile suggesting recent acquisition of this yeast. (20). Infection with *Cryptococcus* is usually 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 been

described. (21-24). Donor-derived cryptococcosis should be considered when the diagnosis occurs in the recipient within 30-days of transplant; when cryptococcosis is diagnosed in more than one organ recipient from a single donor, or if *Cryptococcus* is documented at the surgical or graft site. (25)

Immunosuppression is an important risk factor for cryptococcosis. Corticosteroids are associated with an increased risk of cryptococcosis in all non-HIV infected hosts; (26-28) however, the daily dose that confers increased risk in SOT recipients remains unknown. (29) T-cell depleting antibodies such as alemtuzumab and antithymocyte globulin cause profound lymphocyte depletion of CD4+T cells and have been associated with an increase in the risk for cryptococcosis. (30) 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 $\geq$1 doses of these agents (p=0.04). (30) Invasive fungal infections occurred more frequently in SOT recipients who received alemtuzumab as antirejection as opposed to induction therapy. (31) 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 and outcomes of cryptococcal disease. (5, 6) For example, in patients receiving a calcineurin-inhibitor-based regimen infection was associated with a lower odds of disseminated disease when compared to patients who received a non-calcineurin-based regimen (6) Anti-cryptococcal activity of these agents that target the fungal homologs of calcineurin was considered to account for these findings. (6, 32)

Recently, George and colleagues evaluated risk factors for cryptococcosis among 42,634 SOT patients by using administrative data. (33) In multivariable analyses, patients of older age, those with diabetes or those with Medicaid/self-pay had an increased risk of cryptococcosis. Receiving a lung transplant, when compared to other organ type, was independently associated with an increased risk of cryptococcosis. (33)

Clinical Manifestations

The clinical symptoms of cryptococcal infections in SOT recipients are often non-specific; they can be insidious or severe. (8) In patients with meningitis, prolonged headache, altered mental status, fevers and malaise are usually prominent symptoms compared to photophobia and nuchal rigidity. (34, 35). In patients with pulmonary infection, manifestations range from asymptomatic colonization or infection to severe pneumonia with respiratory failure. Symptoms are often non-specific and include, fever, chills, cough, malaise, night sweats, dyspnea and weight loss. Radiographic findings of pneumonia are frequently solitary (33% of patients) (6) or multiple nodules, so the differential for causative agents should include other fungal infections. Other less common radiographic findings include mass lesions, lobar consolidations or effusions. (36-38) Among SOT patients, cryptococcal infections are usually disseminated (extrapulmonary) at time of presentation with both pulmonary and neurologic findings being common. Approximately 50-75% of SOT recipients with cryptococcosis have extrapulmonary or disseminated disease. (5, 6, 39) A recent study identified 61% of SOT recipients with disseminated disease: 54% had pulmonary and 8.1% had skin, soft-tissue or osteoarticular disease. (6) Other sites where cryptococcal lesions may occur include the skin and soft tissues, the prostate gland, liver, kidney, bones and joints. (40, 41) Liver transplant, as opposed to other types of SOT recipients had a 6-fold higher risk for developing disseminated disease. Up to 33% of the SOT recipients with cryptococcosis have fungemia. (5, 6, 42) In one report, patients with CNS disease were more likely to be fungemic than those without CNS disease. (43) Cutaneous cryptococcosis may present as cellulitis, papular, nodular, or ulcerative lesions with the majority of lesions found on the lower extremities and associated with CNS disease. (44) While cutaneous lesions largely represent hematogenous dissemination, the skin may also be a portal of entry for *Cryptococcus* and a potential source of subsequent disseminated disease in SOT recipients. (19)

Overall mortality in SOT patients with cryptococcosis in the current era ranges from 5-20%. However, some *C. gattii* infections in SOT recipients have shown mortality up to 70%, despite use of treatment based on current guidelines. (15) In a case series of 28 SOT recipients with cryptococcal meningitis, increased mortality was associated with altered mental status, absence of headache, and liver failure; the latter was an independent predictor for death. (42) A lack of inflammation on CSF examination, with a low glucose and high cryptococcal antigen has been associated with increased mortality. (1) . In contrast, receipt of calcineurin-inhibitor agents was independently associated with a lower mortality, but renal failure at baseline with higher mortality. (6) The improved outcomes associated with the use of calcineurin-inhibitor agents may be attributable in part to their synergistic interactions with antifungal agents. (45)

Diagnostic Strategies

An important aim of diagnosis of cryptococcosis in SOT recipients is to determine the site and extent of disease, as this helps to determine choice of antifungal agent and duration of therapy. (8, 46) All SOT recipients with suspected or proven cryptococcosis should undergo a thorough evaluation for extrapulmonary sites if infection, including a lumbar puncture and cultures of blood, urine or other tissues, as indicated **.** Routine blood cultures for *Cryptococcus* are positive in up to 45% of patients with meningitis. (8, 43) If only pulmonary lesions/infiltrates have been documented, after meningitis has been excluded, a bronchoalveolar lavage with or without biopsy should be considered as it is important to eliminate other causes of pneumonia. (8)

For evaluation of potential CNS infection, an opening pressure should be measured and the CSF should be sent for Gram's and fungal stains, cell count, glucose, protein, fungal culture and cryptococcal antigen testing. Culture of the organism is helpful and will allow for species identification (*C. neoformans* or *C. gattii*) and susceptibility testing if indicated. India ink testing on the CSF is no longer recommended due to cost, difficulty in obtaining the product

and lack of sensitivity and specificity when compared to cryptococcal antigen testing. (47) The role of quantitative colony counts from CSF to monitor cryptococcal clearance on therapy has been mostly performed in clinical studies in controlled settings. (48, 49) Due to the lack of reproducibility and lack of data in SOT setting, we do not recommend this or the use of flow cytometry. (50, 51)

Cryptococcal antigen testing from CSF and serum is the preferred strategy to diagnose infection. Serum cryptococcal antigen titers are typically higher in patients with disseminated and/or CNS disease than in those with isolated pulmonary disease. (38), but for diagnosis of cryptococcal pulmonary disease, cryptococcal antigen testing is also useful. For example, there is up to 83% positivity in SOT recipients with any pulmonary involvement. (38). Unlike studies in HIV-infected patients, there have been no large clinical trials evaluating screening SOT recipients with serum cryptococcal antigen to exclude CNS disease. It does appear that a positive serum cryptococcal antigen in an SOT recipient does highly correlate with CNS infection. (35, 52) However, even with more sensitive assays, a negative screening serum cryptococcal antigen may not exclude CNS disease in small percentage of patients. If there is a high suspicion for CNS infection, a CSF cryptococcal antigen should be measured.

There are currently two cryptococcal antigen tests commercially available, the latex agglutination (LA) test and the recently approved lateral flow assay (LFA). The LA test, although a sensitive and specific test (>90%) for initial diagnosis of cryptococcal meningitis, is less sensitive for diagnosis of *C gattii* infections.(11, 15, 47) Titers with the LA test are higher with leptomeningeal than intraparenchymal brain lesions, but are generally lower among SOT recipients (usually <1:1024) than in HIV-infected patients. (42) The LA test requires more “hands on” time, and is less available in resource limited areas. (47) The LFA is a point-of-care test that has a 2-year shelf life at room temperature and requires no specimen preparation. In addition, it is in the order of 100-fold more sensitive to polysaccharide across the four cryptococcal serotypes than the older LA tests. This

increased sensitivity may lead to false positive results in patients with a first-time positive cryptococcal antigen LFA titers of 1:2 and therefore before diagnosing a cryptococcal infection, careful evaluation of the SOT recipient should be performed. (53) Given this and its low cost, it is the preferred method recommended for the diagnosis of cyptococcosis. (54, 55) However, a prozone effect can occur in high cryptococcal burden states and recognition of this with appropriate dilution of the specimen may be required. (56, 57)

Multiplex polymerase chain reaction (PCR) technology offers the ability to identify a panel of organisms from CSF in under 3 hours. The Filmarray ME panel (Biomerieux, North Carolina) is approved for the rapid diagnosis of *C. neoformans* and C. *gattii* with only one specimen. (58) . Other CSF testing methods, including real time PCR (60), may be helpful but are center specific and need to be interpreted in context of the patient. Among HIV-infected Ugandan and South African patients, high concentrations of Beta D glucan have been detected in patients with cryptococcal meningitis (59).

Histopathology

The diagnosis of pulmonary cryptococcosis is made frequently by detection of the yeast in bronchoalveolar lavage (BAL) specimens or from lung tissue biopsy specimens. Cryptococcosis can occur at other sites, including the skin, prostate gland, liver and kidney. Prostatic and kidney disease may present as yeast in the urine and clinical suspicion is needed to make this diagnosis. (40) . Biopsies with culture of tissues will confirm the diagnosis of cryptococcosis. 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, including *Blastomyces dermatiditis* and *Histoplasma capsulatum. (61)*

Identification of cryptococcal species

The identification of cryptococcal species has become increasingly important because it may impact the choice of antifungal therapy as well as affect clinical outcomes. (12, 15, 62, 63) Histopathologic methods and cryptococcal antigen testing cannot distinguish between *C neoformans* and *C gattii*. Use of canavanine glycine bromothymol blue (CGB) agar will help to differentiate *C. neoformans* from *C. gattii* colonies and should be considered. Where possible, isolates should be sent to a laboratory where genotypic identification can be determined using either PCR, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) or loop-mediated isothermal DNA amplification, high resolution melt analysis or multilocus sequence typing. (64-67)

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 validated. (68) Presently, the routine use of antifungal susceptibility testing for cryptococcal infections is not recommended. (8) In patients with *C. gattii* infection where some genotypes have been associated with increased fluconazole minimum inhibitory concentrations (MICs), for those with *C. neoformans* in whom primary therapy has failed or who have relapsed infection, or in patients with cryptococcal infection and recent antifungal exposure (i.e. fluconazole prophylaxis), antifungal susceptibility testing for fluconazole at a minimum is recommended. (8, 14, 15, 63, 69, 70)

Radiologic diagnosis

Brain CT imaging is recommended prior to lumbar puncture to determine the presence of mass lesions or hydrocephalus, but has suboptimal sensitivity for evaluating cryptococcomas compared to MRI with up to 33% of SOT recipients having mass lesions. (8, 11) Cerebral cryptococcomas are more common in patients with *C. gattii* infection than in

patients with *C. neoformans* infection. (11, 15) Mortality is higher in patients with intraparenchymal lesions than with meningeal disease alone. (43)

Chest imaging in pulmonary cryptococcosis is non-specific, but most commonly demonstrates solitary or multiple nodules, and can appear like other fungal infections or *Nocardia* infection. . Diffuse pulmonary disease with interstitial infiltrates can mimic *Pneumocystis jiroveci* or *Histoplasma* infection.

Summary Points

- **Patients with suspected or proven cryptococcosis should have a thorough evaluation for extrapulmonary disease, including lumbar puncture, blood and other relevant tissue cultures (Strong, moderate)**
- **Cryptococcal antigen testing from blood and CSF should be performed preferably using the lateral flow assay over latex agglutination assay. (Strong, moderate)**
- **All sources should be cultured for and identified at the species level (*C. neoformans* vs *C. gattii*) (weak, moderate)**
- **Susceptibilty testing is recommended for patients who fail primary therapy, have relapse of disease, who develop cryptococcosis with prior antifungal exposure (i.e. fluconazole prophylaxis), or in patients with *C. gattii* genotypes that have been associated with elevated fluconazole MICs. (Strong, high)**
- **Imaging of lungs and CNS should be performed in patients with suspected or proven cryptococcosis. (Strong, high)**

Treatment

There have been no randomized controlled trials of antifungal therapy for cryptococcosis in SOT recipients. Current treatment recommendations are extrapolated from clinical trials among HIV patients and from data collected retrospectively in SOT recipients (8, 71). The

recommendations herein are consistent with revised guidelines from the Infectious Diseases Society of America (IDSA). (8)

There are four key components to managing cryptococcosis in SOT recipients: 1) lumbar puncture to identify CNS disease and evaluate and manage intracranial pressure; 2) antifungal therapy; 3) adjunctive therapies; and 4) immunosuppression reduction.

1) Lumbar Puncture

Overall, 50–70% of patients with cryptococcal meningitis have elevated intracranial pressure (ICP). (72) A lumbar puncture should be performed to identify CNS disease and to evaluate ICP. The opening pressure should be recorded, and cerebrospinal fluid (CSF) removal should be performed and sent for appropriate diagnostic studies. Among HIV patients, performance of therapeutic lumbar punctures was associated with 69% relative survival protection. (73) If the CSF pressure is ≥ 25 cm of CSF and there are increased symptoms of intracranial pressure during induction therapy, lumbar punctures should be performed as necessary, with a goal of reducing the CSF pressure by 50% if it is high, or to a normal pressure (<20 cm of CSF). Repeat CSF studies at two weeks post-induction may be useful to determine organism clearance with antifungal therapy, where sterility has been associated with favorable outcome among HIV patients (8, 72, 73)

2) Antifungal Therapy

The choice of antifungal therapy is dependent on site and extent of disease, net state of immunosuppression and severity of illness. (8, 74, 75) Distinguishing between disseminated disease and localized pulmonary and asymptomatic disease is important prior to initiating therapy. This requires a thorough evaluation for extrapulmonary disease as described in the diagnosis section. Table 1 summarizes antifungal therapy in SOT recipients.

In patients with CNS disease, disseminated disease or mild-to-moderate respiratory disease, fungicidal therapy with a lipid formulation of amphotericin B and 5-flucytosine is recommended. (8, 76) The use of a lipid formulation of amphotericin B is preferred over amphotericin B deoxycholate due to decreased nephrotoxicity. Additionally, mortality at 90 days in SOT recipients with CNS cryptococcosis was lower with the use of lipid formulations of amphotericin B when compared to amphotericin deoxycholate. (77) A lack of 5-flucytosine as induction therapy is an independent risk factor for mycologic failure at week 2 in SOT patients. (43, 48, 78) To avoid adverse effects of flucytosine, including bone marrow suppression and nephrotoxicity, monitoring and maintenance of flucytosine levels (2-h post-dose level of 30-80 µg/mL) are recommended. (8, 68, 78)

The recommendation 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 2 weeks followed by consolidation with fluconazole (400-800 mg/day) for 8 weeks and, finally maintenance therapy with fluconazole (200-400 mg/day) for 6 to 12 months. (Table 1)(8) Longer courses of induction therapy should be considered for patients who are clinically deteriorating, if a patient remains comatose, if a patent has not improved and has persistently elevated intracranial pressure, or if the results of CSF cultures after two weeks are expected to remain positive. (8) Extended courses of fluconazole maintenance therapy may be required for patients based on clinical progress or net status of immunosuppression.

The recommendation for patients with pulmonary disease in otherwise asymptomatic patients or patients with mild-to-moderate disease is fluconazole 400 mg/day for 6-12 months. Extrapulmonary disease should be excluded . (5, 8) *C. neoformans*-positive

cultures from sterile and non-sterile 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. (22) Relapse of cryptococcosis after 6 months of fluconazole therapy is uncommon based on available data and thus the recommendations are for 6-12 months of therapy. (79) 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, as well as monitoring of the QTc interval, long-term fluconazole therapy in solid organ transplant recipients has proven to be very safe. (80)

The use of extended-spectrum azoles such as itraconaole, voriconazole, posaconazole and isavuconazonium do not offer advantages over fluconazole for the treatment of *C. neoformans* infection. In general, these agents are more expensive, less safe and may have more potential drug interactions with immunosuppressive agents. Itraconazole was shown to be inferior to fluconazole with the clearance and maintenance phases of cryptococcosis in HIV-infected patients. (81) There are only small case series available evaluating voriconazole, posaconazole or isavuconazonium for the treatment of cryptococcosis. While in most cases these drugs are effective, it is unclear what role they may play as a step down treatment other than an alternative when fluconazole cannot be used. (82, 83) However, these compounds may have some role in the treatment of some *C. gattii* genotypes that have reduced susceptibility to fluconazole. *In-vitro* data suggest that these extended-spectrum azoles have potency against this species and may offer an oral alternative when transitioning from induction to maintenance therapy. (15, 69) Close monitoring of tacrolimus and sirolimus levels is necessary with co-administration of azoles, and dose-reduction should be considered at the time of azole initiation (see chapter 32 for specific recommendations). (84, 85)

3) Adjunctive therapies

The role of dexamethasone as an adjunctive therapy for the management of acute cryptococcal meningitis does not appear to be effective. In a large randomized controlled trial in HIV-infected patients with cryptococcal meningitis, the addition of dexamethasone to standard therapy resulted in slower clearance of the CSF, increased serious infections and no impact on mortality compared to placebo. (49) However, there is no clinical data in SOT recipients and some data suggest there may be some clinical benefit after clearance of infection. (86) Sertraline, an antidepressant with *in vitro* activity against *Cryptococcus* demonstrated increased clearance of the yeast in one clinical study, but it is unclear if this will be of benefit in SOT recipients. (87-89)

4) Immunosuppression reduction

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. (90) The aim is a gradual tapering of immunosuppression 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 immune reconstitution inflammatory s qyndrome (IRIS), although no data are available to suggest the optimal methods of reduction in immunosuppression. (91)

- **A lumbar puncture should be performed for diagnosis of CNS disease and evaluation and management of intracranial pressure (Strong, high)**
- **A lipid formulation of amphotericin B plus 5- flucytosine is preferred as induction therapy for CNS disease, disseminated disease or moderate-to-severe pulmonary disease (Strong, high)**

- **Fluconazole is preferred for consolidation and maintenance therapy for CNS disease (strong, moderate)**
- **Fluconazole is the preferred therapy for asymptomatic or mild-to-moderate pulmonary disease (strong, moderate)**
- **Newer triazoles should be reserved as alternative agents when fluconazole cannot be used or for patients with isolates that may be resistant to fluconazole (Weak, low)**
- **Reduction of immunosuppression should be performed at time of diagnosis of cryptococcosis (Weak, low)**

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. (92) 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 frequently until opening pressure is normalized, if possible. If the ICP remains high (>25 mmHg) and symptoms persist, consider temporary lumbo-peritoneal or external ventricular drains to normalize and monitor CSF pressure. (8, 73, 74) 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. (8, 74).

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. (93) The 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 cryptococcal disease (Table 2). (41, 79) IRIS may present as lymphadenitis, cellulitis, aseptic meningitis, cerebral mass lesions, hydrocephalus or pulmonary nodules. (41, 79) Clinically, CNS IRIS appears to have less inflammation and has been found on lumbar puncture to be associated with protein levels ≤ 50mg/dl and less than 25 white cells/μL. (94) The immunology behind IRIS in the CSF has gradually evolved to explain this phenomena. In evaluating HIV-infected patients with IRIS after starting antiretroviral therapy, there was lower interferon gamma (IFN-γ), interleukin (IL-10) and CXCL10 production in patients with higher mortality. (95) These chemokines might enhance T and myeloid cell trafficking into the CNS, resulting in the excessive TH1-type immune responses seen during CM-IRIS. (96)

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. (97, 98) Tacrolimus inhibits Th1 to a greater extent than cyclosporine A. (99, 100) 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 recipients has also been proposed. (101) Potent T cell lymphocyte depleting agents such as alemtuzumab have also been recognized as a risk factor for IRIS. (102)

In SOT recipients with cryptococcosis IRIS is relatively uncommon, with a incidence of 5-12%.(103). Risk factors independently associated with IRIS are CNS disease (Odds Ratio 6.23) and discontinuation of the calcineurin inhibitor (OR 5.11). (103) Typically the onset of IRIS is between 4-6 weeks after initiation of antifungal therapy. (79) The impact of IRIS on allograft function is just as important with high rates of graft rejection and allograft failure. (103, 104) 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. Clinically, IRIS in an SOT recipient may present with worsening of clinical symptoms, especially headache. The LP may have elevated opening pressure, and white cells, but no growth on cultures and a low level cryptococcal antigenemia. It's a diagnosis of exclusion of relapsed disease. (105) Currently there are no laboratory markers or clinical criteria that can diagnose IRIS reliably, but exclusion of recurrent or worsening cryptococosis is important before treatment if IRIS ensues. (91)

There is no proven therapy for IRIS associated with cryptococcosis. Modifications in antifungal therapy are typically not warranted unless viable yeasts are documented in culture. Corticosteroids have been employed anecdotally with success in *Cryptococcus*-associated IRIS in SOT recipients. (41, 103) 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. (41) The efficacy of thalidomide and other non-steroidal anti-inflammatory agents remains unproven. (106)

- **Serial lumbar punctures should be performed for the management of elevated ICP (Strong, moderate)**
- **Temporary or permanent CSF drainage should be considered for in patients where serial lumbar punctures fail to normalize ICP (strong, low)**
- **Immune reconstitution syndrome can occur within weeks of start of antifungal therapy and reduced immunosuppression. Exclusion of clinical failure with repeat cultures is warranted before initiating corticosteroid treatment. (Weak, low)**

Donor Derived infections

An SOT recipient diagnosed with cryptococcosis less than 30 days after transplantation is an unsual event. (23, 24) A recent case report demonstrated that a donor derived infection with cryptococcosis transmitted via liver and kidney organs could occur up to 12 weeks after transplantation, and demonstrated the need to be in touch with the regional coordinating center to identify these cases. (107) An unexplained early meningitis within 30 days after transplantation should give cause for a donor derived cryptococcal infection. (24) Unfortunately, pre-screening donors has not been shown to be successful in prevention. A high clinical suspicion is required, especially when considering organs from donors with unexplained neurologic illness or meningoencephalitis.

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. We also do not recommend routine screening of transplant recipients or donors before transplantation.

In SOT recipients with previous resolved cryptococcosis needing enhanced immunosuppression, consideration for resuming secondary prophylaxis should 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 at least several months of antifungal therapy, have no signs or symptoms attributable to active cryptococcal disease and negative cultures from the original site of infection. In SOT populations where no bridging option is available, we prefer that the candidate at least has completed induction antifungal therapy, has no signs or symptoms attributable to active cryptococcal disease, and has negative cultures from the original site of infection. For SOT candidates with active cryptococcal disease at the time of listing, limited data are available to guide timing of transplant, or appropriate candidacy (108). Our recommendations are the same as those for patients with graft failure.

Although the cryptococcal antigen titer should ideally decline with therapy and eradication of organisms, there are no data supporting monitoring cryptococcal antigen testing once induction therapy is completed and consolidation or maintenance therapy has begun. In these cases, secondary fluconazole prophylaxis should be considered for at least 1-year period. (38, 80)

Future Research Directions:

An important area of research is utilizing cryptococcal genomics to identify virulence factors by understanding the evolutionary pathways that have lead the species to adapt to the host and antifungal therapy. (109, 110) With recent advances in whole genome sequencing, it is becoming easier to distinguish genomic variations within species and determine virulence.

This includes the identification at a molecular level of copy number variants (CNVs). These are a class of structural variants (SVs) that are present and have been shown to alter gene expression in fungal organisms. Researchers have used these CNV's with *C. gattii* to identify virulence genes that overlap between species. (111) The use of WGS and real-time PCR to identify virulence genes may lead to newer better directed treatments and more rapid diagnostics.

Other research includes novel compounds for the treatment of cryptococcal infections with candidates that include an oral encochleated Amphotericin B which is a proprietary cochleate lipid-crystal nano-particle drug delivery technology. This offers promise of an effective oral anti-fungal agent for the treatment of cryptococcal meningoencephalitis (Cinicaltrials.gov identifier: NCT03196921). This may be required with more emerging cryptococcal resistance.

Lastly, CSF extracorporeal filtration (Neurapheresis™) therapy of yeasts from cerebrospinal fluid (CSF) may be a novel, one-time therapy for cryptococcal meningitis. This has been demonstrated in an in-vivo rabbit model where there was a 1.6 log reduction in yeast in the CSF when the filtration platform was performed for 6 hours.

(112) If effective in humans, this would be a tremendous therapeutic innovation in the management of this infection.

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Table 1. Treatment recommendations

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

[§] Dosages 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. (6, 67)

*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. (15,18)

Table 2: Criteria for diagnosis of IRIS in patients with cryptococcosis

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, i.e., opening pressure ≥20 mm of water (with or without hydrocephalus).

   b) Lymph nodes, skin or soft tissue lesions e.g., cellulitis or abscesses.

   c) Pulmonary e.g., nodular, cavitary, 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.