

Cryptococcosis in Solid Organ Transplant Recipients: Current State of the Science

Nina Singh,¹ Francoise Dromer,³ John R. Perfect,² and Olivier Lortholary^{3,4}

¹University of Pittsburgh, Pennsylvania; ²Duke University, Durham, North Carolina; and ³Institut Pasteur, Molecular Mycology Unit, National Reference Center for Mycoses and Antifungals, and ⁴Université Paris Descartes, Centre d'Infectiologie Necker Pasteur, Hôpital Necker-Enfants Malades, Paris, France

Cryptococcosis remains a significant opportunistic infection in solid organ transplant recipients. Disease presentation and outcomes may be affected by, among other factors, the use of calcineurin inhibitor immunosuppressive agents. It is being increasingly recognized that rapid reversal of immunosuppression in transplant recipients treated for cryptococcosis incurs the risk of immune reconstitution inflammatory syndrome, which resembles worsening disease or relapse. This review summarizes the current state of knowledge regarding cryptococcosis in transplant recipients and highlights areas where future investigations are needed to further optimize outcomes for these patients.

Invasive fungal infection is a significant complication in solid organ transplant (SOT) recipients [1–3]. Improvements in transplantation practices and wider use of antifungal prophylaxis have led to a decrease in the overall incidence of invasive fungal infection, particularly infections due to *Candida* and *Aspergillus* species [3–5]. The trends in the incidence of cryptococcosis among transplant recipients are less well delineated, despite ongoing surveillance in some countries [6]. However, it is plausible that major paradigm shifts in the approach to immunosuppression—for example, increasing use of T cell–depleting antibodies—may lead to more frequent occurrence of cryptococcosis in these patients [7]. In addition, our ability to rapidly manipulate the immune system in SOT recipients receiving potent immunosuppressive agents has led to growing appreciation that complications, such as *Cryptococcus*-associated immune reconstitution inflammatory syndrome (IRIS)—which, until recently, had only been consistently recognized in HIV-infected patients [8, 9], can also occur in SOT recipients [10, 11]. Within the past few years, a series of reports have contributed to our evolving knowledge base of cryptococcosis in SOT recipients. This review summarizes the topical devel-

opments in the pathogenesis, epidemiologic characteristics, emerging syndromes, and management of cryptococcosis in SOT recipients.

PATHOPHYSIOLOGY

Cryptococcal disease is generally considered to represent reactivation of quiescent infection, although this has remained unproven [12, 13]. In a study of SOT recipients, pretransplantation and posttransplantation serum samples were tested for cryptococcal antibodies using an immunoblot assay [14]. Most patients who developed cryptococcosis exhibited serologic evidence of cryptococcal infection before transplantation [14]. In fact, these patients developed cryptococcosis significantly earlier after transplantation than did patients without preexistent cryptococcal antibodies [14], suggesting that, indeed, a substantial proportion of cases of transplant-associated cryptococcosis results from reactivation infection. On the other hand, evidence-based epidemiologic investigations also suggest acquisition of primary infection after transplantation [15, 16]. Furthermore, isolates recovered from a pet cockatoo and a renal transplant recipient with cryptococcosis showed identical genotypic profiles, suggesting recent acquisition of the yeast [17]. Finally, rare cases of transmission from donor organ and tissue grafts have been reported [2, 18, 19].

EPIDEMIOLOGY

Cryptococcosis is the third most commonly occurring invasive fungal infection in SOT recipients. Cryptococcosis represents 8% of invasive fungal infections in SOT recipients in the Trans-

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Reprints or correspondence: Dr. Nina Singh, Infectious Diseases Section (111E), VA Medical Center, University Drive C, Pittsburgh, PA15240 (nis5@pitt.edu), or Dr. Olivier Lortholary, Centre National de Référence Mycologie et Antifongiques, Unité de Mycologie Moléculaire, CNRS URA3012, Institut Pasteur, 25, rue du Dr. Roux, 75724 Paris Cedex 15, France (olivier.lortholary@nck.aphp.fr).

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plant Associated Infection Surveillance Network database [20]. The overall incidence of cryptococcosis in SOT recipients is ~2.8% (range, 0.3%–5%) [21]. An estimated 20%–60% of cases of cryptococcosis among non-HIV-infected patients in the United States [22] and 17.4% in France [6] occur in SOT recipients. Surveillance data from 1985–2007 from France have documented a stable proportion of SOT recipients among HIV-uninfected patients with cryptococcosis (French Cryptococcal Study Group, unpublished data).

Calcineurin inhibitors are the mainstay of immunosuppression in SOT recipients in the current era. These agents do not appear to influence the incidence but may affect the extent of cryptococcal disease [21]. On the other hand, corticosteroids are associated with an increased risk of cryptococcosis in all non-HIV-infected hosts [6, 22–25]; however, the precise daily dose that confers a higher risk in SOT recipients remains unknown. T cell-depleting antibodies, such as alemtuzumab, are increasingly used as induction therapy or as treatment of rejection in SOT recipients [7]. This agent causes profound and lasting depletion of CD4⁺ T cells and was associated with a dose-dependent increase in the risk for cryptococcosis [7]. 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 dose of these agents ($P = .04$) [7]. Invasive fungal infections occurred more frequently among SOT recipients who received alemtuzumab as antirejection therapy as opposed to induction therapy [26].

A male predominance has been shown among HIV-infected and HIV-uninfected patients with cryptococcosis [6, 27–29]. Among SOT recipients with cryptococcosis in France, 70.1% were male, a distribution matching the 78% rate among SOT recipients with cryptococcosis in the United States [21]. The sex difference in the susceptibility to cryptococcosis can be explained by influence of the *Xid* locus on the X chromosome in mice or altered immune responses [30]. Cryptococcosis in SOT recipients is typically a late-occurring infection; the median time to onset was 16–21 months after transplantation in 3 studies [20, 21, 31]. The time to onset was earlier for liver and lung transplant recipients than for kidney transplant recipients, possibly because patients in the former subgroups received higher-intensity immunosuppression [21].

Unlike *Cryptococcus neoformans* var *grubii* (serotype A), which has no particular geographic predilection [32] and which causes most infections in SOT recipients, *C. neoformans* var *neoformans* (serotype D) is prevalent in Northern Europe. In France, 18% of the cryptococcal isolates in SOT recipients and in HIV-infected patients are serotype D (French Cryptococcal Study Group, unpublished data). Until recently, *Cryptococcus gattii* had been regarded as a tropical and subtropical fungus. Its ecologic niche, however, has expanded to temperate regions,

and acquisition of cases within the United States has been documented, including in SOT recipients [33]. The incubation period of *C. gattii* disease in Vancouver Island and the Pacific Northwest has been documented to be ~6 months [34].

CLINICAL MANIFESTATIONS

A total of 53%–72% of cases of cryptococcal disease among SOT recipients are disseminated or involve the CNS [21, 22, 31, 35]. Overall, 61% of the SOT recipients in 1 report had disseminated disease, 54% had pulmonary disease, and 8.1% had skin, soft-tissue, or osteoarticular cryptococcosis [31]. Patients receiving a calcineurin inhibitor–based regimen were less likely to have disseminated disease and more likely to have cryptococcosis limited to the lungs [31]. The anticryptococcal activity of these agents that target the fungal homologs of calcineurin [36] was considered to account for these findings [31]. Clinical strains of *C. neoformans* in SOT recipients, however, remain susceptible to calcineurin inhibitor agents, suggesting that breakthrough infection is primarily due to their immunosuppressive effect and not due to the selection of drug-resistant strains [37].

Overall, 33%–39% of the SOT recipients with cryptococcosis have fungemia [21, 30, 38]. Patients with CNS disease in 1 report were more likely to be fungemic than were those without CNS disease [39]. Other studies have documented lower rates of fungemia; of 11 SOT recipients in the French Cryptococcal A/D study [29], 8 had meningoencephalitis, 3 had urinary tract infection, and none had fungemia.

Approximately 33% of the SOT recipients with cryptococcosis have disease limited to the lungs [31]. Pulmonary cryptococcosis may be detected as an incidental finding in asymptomatic patients [40]. These patients presented with cryptococcal disease later in the posttransplantation period and were receiving lower maintenance dosages of prednisone than were symptomatic patients [31]. Pulmonary cryptococcosis manifesting as acute respiratory failure is associated with a grave prognosis [41].

Cutaneous cryptococcosis can present with papular, nodular, or ulcerative lesions or as cellulitis [42, 43]. Although cutaneous lesions largely represent hematogenous dissemination, skin has also been identified as a portal of entry of *Cryptococcus* species and a potential source of subsequent disseminated disease in SOT recipients [16]. Such cutaneous infections in France are often due to serotype D [44].

DIAGNOSIS AND NEUROIMAGING FINDINGS

As in all immunocompromised patients, a complete evaluation, including collection of large-volume CSF specimens (≥ 1 mL or 20 drops) and blood and urine analysis, should be performed to delineate the extent of disease and to determine optimal treatment [29]. The CSF characteristics typically documented

in SOT recipients with cryptococcal meningitis are outlined in table 1.

Positive serum cryptococcal antigen results have been reported in 88%–91% of the SOT recipients with cryptococcal meningitis [21, 38]. However, the serum and CSF antigen titers are generally lower in non-HIV-infected hosts, including SOT recipients, than in HIV-infected patients with CNS cryptococcosis [45]. On the other hand, in SOT recipients with CNS cryptococcal lesions, CSF antigen titers were significantly higher in patients with leptomeningeal as opposed to parenchymal lesions and hydrocephalus [46]. In contrast to studies primarily in HIV-infected patients [30, 47], high serum or CSF antigen titers did not correlate with mortality at 90 days or CSF sterilization at 2 weeks in SOT recipients [39].

Up to 33% of patients with CNS cryptococcosis may have CNS parenchymal lesions due to *Cryptococcus* species [35, 39]. Abnormal brain imaging results were identified as a poor prognostic factor in a population comprising HIV-infected and HIV-uninfected patients [29]. MRI was more sensitive than CT for the evaluation of CNS cryptococcosis in HIV-infected patients [48]. In certain parts of the world, up to 7% of the pulmonary nodules in SOT recipients may be due to cryptococcosis [49, 50]. Serum antigen positivity was documented in 83% of the SOT recipients with pulmonary cryptococcosis [46]. Patients with concomitant extrapulmonary disease were more likely to have a positive antigen test result and had higher antigen titers [46]. Up to 38% of SOT recipients may have pulmonary cryptococcosis detected as an incidental finding on

imaging studies [46]. Nodular densities were more likely than pleural effusions and infiltrates to present as incidentally detected pulmonary cryptococcosis [46].

TREATMENT PRINCIPLES AND PRACTICES

No randomized, prospective trials of antifungal treatments have been devoted to cryptococcosis in SOT recipients. However, on the basis of recommendations extrapolated from clinical trials in other hosts [51], the following strategies are proposed, which are provisionally consistent with the upcoming revised cryptococcal Infectious Diseases Society of America guidelines. It is important to separate treatment for disseminated cryptococcosis from that of extrameningeal nondisseminated and mild to asymptomatic disease. For the management of meningoencephalitis or disseminated cryptococcosis, induction therapy with liposomal amphotericin B (3–4 mg/kg per day) or amphotericin B lipid complex (5 mg/kg per day) plus flucytosine (100 mg/kg per day) for 14 days, followed by a consolidation phase with fluconazole (400–800 mg per day) for 8 weeks and, finally, maintenance or suppression therapy with fluconazole (200–400 mg per day) for 6–12 months (provided the immunosuppression is not augmented) is recommended (table 2).

Several important issues are addressed. Fungicidal therapy with a polyene and flucytosine is highly recommended, because transplant recipients can possess a high burden of yeasts on initial presentation. Lack of flucytosine therapy during induc-

Table 1. Clinical and laboratory characteristics in solid organ transplant recipients with CNS cryptococcosis.

Variable	Study		
	Wu et al. [38]	Husain et al. [27]	Singh et al. [31]
No. of patients	28	172	61
CSF characteristics			
Opening pressure, median cm H ₂ O (range)	...	33 (14–70)	27 (9–36)
WBC count, median cells/mm ³ (range)	188 (0–1464)	33 (0–485)	78 (0–1200)
Glucose level, median mg/dL (range)	52 (20–62)	36 (4–113)	47 (2–181)
Protein level, median mg/dL (range)	226 (69–1015)	74 (16–715)	83 (26–559)
Cryptococcal antigen test			
Percentage of patients with positive result	100	100	98
Mean antigen level	1:2	1:256	1:64
Median antigen level	1:238
Positive India ink test result, percentage of patients	50	80	69
Positive culture result, percentage of patients	77	93	86
Serum cryptococcal antigen test			
Percentage of patients with positive result	91	88	98
Mean antigen level	1:3	1:128	1:512
Median antigen level	1:48
Percentage of patients with fungemia	39	33	36
Mortality rate, %	50	40	20

Table 2. Management of cryptococcal disease (meningoencephalitis) in solid organ transplant recipients.

Therapy	Dosage	Duration
Induction therapy		
Preferred therapy	Liposomal amphotericin B, 3–4 mg/kg per day; or amphotericin B lipid complex, 5 mg/kg per day, plus flucytosine, 100 mg/kg per day ^a	2 Weeks
Alternative therapy	Liposomal amphotericin B, 3–4 mg/kg per day; or amphotericin B lipid complex, 5 mg/kg per day	4 Weeks
Consolidation therapy	Fluconazole, 400–800 mg per day ^a	8 Weeks
Maintenance therapy	Fluconazole, 200 mg per day	6–12 Months
Therapy for isolated pulmonary cryptococcosis ^b	Fluconazole, 400 mg per day ^a	6–12 Months

^a Dosages of flucytosine outlined are in the absence of renal insufficiency.

^b Disseminated disease must be excluded in all patients. Persons with disseminated disease, diffuse pulmonary infiltrates, and acute respiratory failure should be treated with the same regimen as cryptococcal meningoencephalitis.

tion therapy has been shown to be an independent risk factor for mycologic failure at week 2 [29]. Furthermore, receipt of <14 days of flucytosine therapy was associated with an increased risk of treatment failure at 90 days [52]. The use of the lipid formulations of amphotericin B is favored over amphotericin B deoxycholate (≥ 0.7 mg/kg per day), because many transplant recipients receiving calcineurin inhibitors have renal function, and any further worsening of organ dysfunction should be avoided. In addition, exacerbation of renal dysfunction may predispose patients to toxic effects from flucytosine; therefore, maintenance of drug levels (2-h postdose level of 30–80 $\mu\text{g}/\text{mL}$) and monitoring for bone marrow suppression are recommended.

Because the relapse rate of cryptococcosis after 6 months of maintenance therapy is minimal, 6–12 months of maintenance therapy is appropriate [53]. Drug interactions with fluconazole should be monitored; however, long-term fluconazole therapy has proven to be relatively safe in SOT recipients. It is unlikely that there is any added benefit to routine substitution of fluconazole with extended-spectrum azoles, such as itraconazole, voriconazole, or posaconazole [54, 55]. In fact, in HIV-infected patients, itraconazole was inferior to fluconazole during the consolidation and clearance phases [51].

For the treatment of mild-to-moderate pulmonary cryptococcosis, fluconazole (400 mg per day) is optimal (table 2). Disseminated disease should be excluded, and this requires a lumbar puncture and blood and urine cultures. Severe pulmonary disease should be treated the same as CNS disease. Pulmonary cryptococcosis, with or without serum antigen positivity, can be treated similarly if disseminated disease is ruled out [31]. *C. neoformans*-positive cultures of specimens from sterile and nonsterile body sites, such as sputum, warrant treatment, even if the patient is asymptomatic. In lung transplant recipients, this yeast may colonize the donor allograft, and without treatment, the infection will likely become invasive during immunosuppression, which requires early therapy [2].

Another issue specific to SOT recipients with cryptococcosis

is the management of immunosuppression. The net immunosuppression should be reduced during therapy [10, 56], but precisely how this should be performed must be individualized 1 case at a time. Rapid reduction of immunosuppressives can have adverse effects, such as development of organ rejection and/or IRIS [10, 11, 57]. Thus, it is prudent to plan a gradual reduction while antifungal therapy is administered. The goal is eradication of infection but also preservation of allograft function. Although challenging at times, this can be successfully accomplished in a high proportion of SOT recipients. The use of IFN- γ as adjunctive therapy will need to be further scrutinized, because it has the potential adverse effect of inducing graft rejection in this patient population [31].

Routine antifungal prophylaxis for cryptococcosis is not recommended in SOT recipients, because no evidence-based studies support this, and there is no precision in identifying a specific high-risk group. Currently, adequate treatment for primary cryptococcosis should obviate the need for secondary antifungal prophylaxis during treatment for any subsequent allograft rejection episodes. However, close clinical follow-up is necessary in this case.

An important issue is timing of retransplantation in SOT recipients who experience graft failure after cryptococcosis. In kidney transplant recipients in whom the option of bridging dialysis exists, it is reasonable to consider retransplantation if the patient has received 1 year of antifungal therapy, has no signs or symptoms attributable to active cryptococcal disease, and has negative cultures at the site of original infection. In other SOT recipients in whom there is no bridging option, we recommend that the induction therapy should be completed, all sites that yield positive culture results should be rendered negative, cryptococcal antigen titer should be stable or ideally decreasing, and the patient should be receiving a stable regimen of fluconazole. In this case, secondary prophylaxis with fluconazole after retransplantation should be used for an arbitrary duration, but 1 year after transplantation should be considered.

These recommendations are largely based on authors' expert opinion, because data for guidance on these issues are scant.

IRIS

IRIS due to opportunistic pathogens has emerged as a major complication in patients with AIDS receiving HAART. Its incidence is estimated to be 4–16 cases per 100 person-years in AIDS-associated cryptococcosis [58]. IRIS has also been reported in SOT-related cryptococcosis, with a prevalence of 4.8% [10]. IRIS comprises a constellation of clinical manifestations due to an inflammatory tissue response in patients experiencing improvement in cellular immunity after reduction or cessation of immunosuppressive therapy [10].

Calcineurin inhibitor agents and corticosteroids exert their immunosuppressive effect by preferentially inhibiting T_H1 (IL-2 and IFN- γ), compared with T_H2 (IL-10) responses [59, 60]. Tacrolimus inhibits T_H1 to a greater extent than does cyclosporine [61, 62]. Previous alemtuzumab therapy has also been recognized as a risk factor for IRIS [63, 64]. The biologic basis of IRIS in SOT recipients is believed to be reversal of a T_H2 to T_H1 proinflammatory response on withdrawal or reduction of immunosuppression. A potential role of T-regs and T_H17 regulatory pathways in the pathogenesis of IRIS in SOT recipients has also been proposed [65].

In HIV-infected patients, fungemia, an extremely low CD4 cell count, cryptococcosis as an AIDS-defining illness, lack of CSF sterilization at week 2, introduction of HAART within 1–2 months after the diagnosis of cryptococcosis, and a rapid decrease in HIV load after HAART have been recognized as risk factors for IRIS [9]. In SOT recipients, IRIS occurred more frequently in patients receiving potent immunosuppressive treatment, such as with tacrolimus, mycophenolate mofetil, and prednisone, and in those with disseminated cryptococcosis [10].

IRIS may occur either early (within a few days) or late (up to several months) after the introduction of HAART [9]. Time of onset of IRIS appears to be shorter in cases of IRIS that involve the CNS. In SOT recipients, IRIS occurred a mean of 6 weeks after the initiation of antifungal therapy [10] and manifested as lymphadenitis, cellulitis, aseptic meningitis, cerebral abscesses, hydrocephalus, or pulmonary nodules [10, 11]. In kidney transplant recipients, development of IRIS has been temporarily associated with allograft loss [57].

IRIS needs to be distinguished from worsening cryptococcosis (although both entities can occur simultaneously) and from other opportunistic infections or drug-related complications. No specific markers can reliably establish the diagnosis of IRIS [48]. Histopathologic examination often reveals granulomas containing macrophages, with or without necrosis, a feature rarely observed at initial diagnosis of cryptococcosis in an immunosuppressed host [10].

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 in doses equivalent to 0.5–1 mg/kg of prednisone, may be considered for major complications related to inflammation in the CNS and severe manifestations of pulmonary or other sites [11]. The efficacy of thalidomide and other nonsteroidal anti-inflammatory agents remains unproven.

OUTCOMES

Mortality rates in SOT recipients with cryptococcosis have typically ranged from 33% to 42% and may be as high as 49% in those with CNS disease [21]. The overall mortality rate in SOT recipients with cryptococcosis in the current era is ~15% [31]. If cryptococcosis is limited to the lungs, the mortality rate can be as low as 2.8% [31]. In a case series of 28 SOT recipients with cryptococcal meningitis, mortality correlated with altered mental status, absence of headache, and liver failure, the last of which was an independent predictor for death [38]. On the other hand, receipt of calcineurin inhibitor agents was independently associated with a lower mortality rate but renal failure at baseline with a higher mortality rate [31]. Improved outcomes with the use of calcineurin inhibitor agents may be attributable in part to their synergistic interactions with antifungal agents [66].

CONCLUSIONS

Cryptococcosis in SOT recipients primarily consists of disseminated disease or meningoencephalitis. Induction treatment in these patients should include a lipid formulation of amphotericin B, preferably with flucytosine. Patients with nonsevere pulmonary cryptococcosis may be treated with fluconazole. Most cases of relapse occur in the first year of management, supporting the use of suppressive therapy with fluconazole for 6–12 months. A rapid reduction of immunosuppression may be associated with IRIS that mimics worsening disease due to cryptococcosis. Characterization of risk factors for IRIS, discerning its immunologic basis, identifying diagnostic markers, and optimal management of IRIS in SOT recipients merit future investigations [66].

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