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Title

Cryptococcal infection in lung transplant recipients: a five-year retrospective review at an Australian transplant centre

Running title

Cryptococcal infection in lung transplant

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Abstract

Introduction. Cryptococcosis is a common invasive fungal infection (IFI) in solid organ transplant (SOT) recipients. Little is known about cryptococcosis in lung transplant (LTx) recipients despite having one of the highest risks of infection.

Aim. To describe demographic and clinical features of cryptococcal infection in LTx recipients.

Materials and Methods. We performed a retrospective, observational study of cryptococcal infection in LTx recipients at The Alfred Hospital in Melbourne, Australia from 2012 to 2017.

Results. 11 cases were identified. Seven patients (64%) were male and the median age was 54.7 years (range 34 – 69 years). Diagnosis occurred at a median of 233 days (range 1-3650 days) post-transplant. Nine patients (82%) had isolated pulmonary infection of whom 7 (78%) were asymptomatic. All were treated with oral antifungal therapy and one required surgical resection of infected lung.

Two patients (18%) had disseminated infection; one with pulmonary and central nervous system (CNS) infection and one with isolated CNS infection. Both patients presented with headache and brain imaging demonstrated cerebral oedema, myelinosis and leptomeningeal enhancement. One of these patients died.

Conclusion. This study highlights the fact that cryptococcal infection should remain a consideration in asymptomatic LTx recipients, especially in the presence of non-specific nodules on chest imaging, and that the presence of headache in these patients requires urgent investigation for CNS infection.

Keywords: Cryptococcus, cryptococcal infection, cryptococcosis, lung transplant, solid organ transplant

Introduction

Cryptococcosis is the third most common invasive fungal infection (IFI) in solid organ transplant (SOT) recipients, after *Candida* and *Aspergillus*, and is associated with a significant risk of dissemination and mortality.[1, 2] The incidence of cryptococcal infection in SOT recipients ranges from 0.2-5%, with mortality ranging from 14-19.6%, and up to 50% in those with central nervous system (CNS) infection.[1, 3, 4]

Disease typically occurs due to reactivation of a latent focus of infection, with initial infection occurring via inhalation from an environmental source. Cryptococcal infection in SOT recipients typically occurs later in the post-transplant period,[2, 5] although early post-transplant infection has been described (< 30 days post-transplant), particularly in liver transplant recipients.[6-11]

Given the exposure of the allograft to the external environment, lung transplant (LTx) recipients might be considered to have the highest risk of cryptococcosis compared to other SOT recipients.[12] Despite this, relatively little is known about cryptococcal infection in this group of patients. To our knowledge, this study represents one of the largest series to describe demographic and clinical features of cryptococcal infection in LTx recipients.

Materials and Methods

We undertook a retrospective, observational study of cryptococcal infection in LTx recipients at The Alfred Hospital in Melbourne, Australia, a large adult and paediatric lung transplant centre, with over 1400 lung transplants performed since the program's commencement in 1990. Ethics approval was provided by the Research Ethics Committee of The Alfred Hospital, Melbourne, Australia (project number 156/17).

Cases of cryptococcal infection from April 1 2012 to March 31 2017 were identified from the Alfred Health Pathology database, of the approximately 800 'at-risk' alive lung transplant recipients followed up at our institution over this period. Cases were included if the patient had 1) any positive cryptococcal antigen (Prior to November 2014: Cryptococcal Antigen Latex Agglutination System, Meridian BioSciences, Cincinatti, OH USA; From November 2014: Cryptococcal antigen lateral flow assay, Immuno-Mycologics, Norman, OK USA) and/or 2) a positive *Cryptococcus* culture from a sterile or non-sterile site. LTx recipients were identified and had further clinical information retrieved from electronic medical records.

Please see the appendix 1 for clinical definitions.

Microbiological methods

Clinical specimens were cultured on Sabouraud dextrose agar culture medium with chloramphenicol and gentamicin in 5% CO_2 at 35°C for 2 days, then transferred to air at 30°C for an additional 12 days (non-sterile specimens) or 26 days (sterile specimens). Colonies resembling *Cryptococcus* were identified using VITEK 2 or VITEK MS (BioMérieux, Marcy l'Etoile, France). Isolates were further speciated at one of two reference laboratories, the Victorian Infectious Diseases Reference Laboratory (VIDRL) or South Australia (SA) Pathology.

Perioperative management of LTx recipients was as per protocols described elsewhere.[13] All patients received standard triple immunosuppression with cyclosporine or tacrolimus, azathioprine or mycophenolate mofetil and corticosteroids. Peri-transplant prophylactic antibiotics were administered on the basis of known or suspected donor and recipient microbiology with duration based on clinical course post-transplant. All patients considered at risk of cytomegalovirus (CMV) reactivation (either donor- or recipient-positive for CMV IgG) received prophylaxis with intravenous ganciclovir followed by oral valganciclovir. Anti-fungal prophylaxis was not routinely prescribed, however there was a low threshold to initiate oral azole therapy following the isolation of potentially pathogenic fungi such as *Aspergillus, Lomentospora* or *Cryptococcus spp.,* or the presence of a suspicious clinical scenario (eg. nodules or cavitation on chest tomography). Surveillance bronchoscopy, lavage and transbronchial biopsies are performed according to institutional protocol, at 2, 6, 12, 26, 39, 52 and 78 weeks post-transplant, and as indicated by clinical circumstances. Indefinite long-term follow-up was maintained by the Alfred Hospital's Lung Transplant Service clinics.

Statistics

Data were analysed using Stata Statistical Software, version 15 (StataCorp LP, College Station, TX, USA). Descriptive statistics are presented as means, medians and proportions.

Results

Patient characteristics

A total of eleven LTx recipients were found to have cryptococcal infection. 9 (82%) with isolated pulmonary infection and 2 (18%) with disseminated infection. Seven (64%) were male and the median age was 54.7 years (range 34-69 years). The median time to diagnosis was 233 days (range 1-3650 days), with 8 patients (73%) diagnosed more than 3 months after transplantation, two (18%) diagnosed approximately one month post-transplant and one (9%) diagnosed day one post-transplant. Table 1 and 2 summarise key patient demographics and potential sources of exposure.

Clinical presentation

Pulmonary infection

Seven of nine patients (78%) with pulmonary infection were asymptomatic (table 2). In six (86%), infection was detected by positive *Cryptococcus* culture of respiratory samples taken during routine surveillance and in one patient by a positive serum cryptococcal antigen performed due to the incidental finding of a lung nodule on chest radiograph (CXR) performed for an unrelated reason (chest trauma). Two patients (22%) experienced respiratory symptoms: one presented with cough, chest pain, dyspnoea and lethargy, the second with cough only. *Cryptococcus* was isolated from bronchoalveolar lavage (BAL) specimens in both of these patients.

A CXR was performed in 89% of patients with pulmonary infection and a computed tomography (CT) of the chest in 100%, with nodules being the most common abnormal finding with both modalities (CXR 2/8; 25% & CT chest 5/9; 56%). Serum cryptococcal antigen was positive in two patients (22%) (titre: 8 & 64). Imaging and cerebrospinal fluid (CSF) analysis for CNS disease in this group was all negative.

Disseminated infection

Two patients (18%) had CNS infection with headache being the principal neurological symptom (table 2). The first patient presented with headache, fever and confusion. Magnetic resonance imaging (MRI) of the brain demonstrated leptomeningeal enhancement, and CSF analysis revealed elevated protein (1.53g/L; reference range 0.15-0.40g/L), increased polymorphonuclear (PMN) cell and lymphocyte cell count (3 x 10^6/L; reference range <1 x 10^6/L & 11 x 10^6/L; reference range <5 x 10^6/L, respectively) and a positive cryptococcal antigen (titre: 32). This patient also had symptomatic lung involvement, with cough being the main symptom. Serum cryptococcal antigen was also positive (titre: 128). The second patient experienced a short history of headache, followed by rapid onset of confusion and obtundation. A MRI of the brain demonstrated cerebral oedema and pontine and extrapontine myelinolysis, and CSF analysis demonstrated elevated protein (2.67g/L), an increased PMN cell count (12 x 10^6/L), and a positive cryptococcal antigen (titre: 4096). CSF cultured *C. neoformans.* Serum cryptococcal antigen

was also positive (titre: 2048). Of note, in both of these cases, brain imaging on non-contrast CT was unremarkable.

Other investigations

During further investigation of cryptococcal infection, six patients (55%) were tested for HIV and all were negative. Five of eight patients tested (63%) had mildly low total immunoglobulin G (IgG) levels (5.0-6.1g/L: reference range 7-16.5g/L); IgG subclasses were not tested. One of two patients (50%) tested for CD4 lymphocyte level had a low count of 144 cells/mL.

Treatment and outcome

Table 2 summarises the treatment and outcome for all patients. All patients with isolated pulmonary infection received treatment with oral antifungal therapy. Eight (89%) were treated with fluconazole monotherapy. One patient was switched to posaconazole at ten weeks due to adverse effects attributed to fluconazole. Despite seven months of fluconazole treatment, one patient demonstrated a persistently enlarging pulmonary abscess and proceeded to upper lobe wedge resection and this patient remains on voriconazole therapy eight months following surgery. One patient received voriconazole therapy due to co-infection with *Aspergillus* spp. The majority of patients (89%) completed treatment and were considered cured, having received a mean duration of six and half months of therapy (range 5-11 months).

The single patient that survived with disseminated infection (pulmonary and CNS), received liposomal amphotericin and 5-flucytosine induction therapy followed by fluconazole maintenance therapy for ten months. The patient with isolated CNS infection deteriorated and died due to overwhelming infection within 72 hours of presentation and the diagnosis was made post mortem without the patient receiving specific anti-cryptococcal treatment.

Discussion

To our knowledge, this study represents one of the largest series to describe cryptococcal infection in LTx recipients. In keeping with known epidemiological and exposure factors for

cryptococcal disease,[14, 15] patient-reported exposure to potentially contaminated environments was a significant feature in cases of infection, with 72% of patients able to report an exposure (see table 1).

We have demonstrated that compared to other SOT recipients, LTx recipients have an earlier onset of cryptococcosis,[5, 12] with the median time to cryptococcal infection from transplantation being 233 days. Whilst this still fits the definition of 'late' infection (>3 months post-transplant), it is slightly earlier than that reported in LTx cohorts in other large SOT studies.[2, 12] Furthermore, three patients in our study were diagnosed with early onset cryptococcosis (<3 months post-transplant), with one being diagnosed day 1 post-transplant. We believe this most likely represents donor-derived infection as whilst donor BAL fluid was not available for definitive culture, histopathology of explanted recipient lung found no evidence of cryptococcal infection and the recipient's pre-transplant serum was cryptococcal antigen negative. This case serves to highlight that the screening donor bronchoalveolar lavage specimens should include fungal culture. Although donor-derived cryptococcal infection is rare, it should be considered in the evaluation of very early onset infections in LTx recipients.

In our cohort, the majority of patients were asymptomatic, and in most the diagnosis was made due to a routine post-transplant surveillance BAL with a positive *Cryptococcus* culture. This high rate of asymptomatic disease (with incidental positive BAL culture and without dissemination) adds weight to the value of surveillance bronchoscopy. The authors reinforce the recommendation that surveillance BAL post-transplant should include fungal cultures to aid in the detection of early infection.

The route of acquisition of *Cryptococcus* is via inhalation, followed by localized pulmonary infection which may then disseminate. Despite recent studies demonstrating that a high proportion of SOT recipients have disseminated disease, previous studies have reported that LTx recipients, even when controlled for degree of immunosuppression, had a lower risk of disseminated cryptococcosis.[16] In patients with isolated pulmonary cryptococcosis, serum cryptococcal antigen positivity, which suggests extra-pulmonary involvement, is lower for lung compared to all other types of organ transplant recipients.[17, 18] Our data

supports this, with only 22% of patients with isolated pulmonary infection having a positive serum cryptococcal antigen. In keeping with the Infectious Diseases Society of America (IDSA) recommendations,[19] the majority of patients in our cohort underwent evaluation for CNS infection. Interestingly, the proportion of patients with CNS infection in our study was low (2/11; 18%), compared to prior studies reporting up to 72% of SOT recipients with CNS involvement.[20-23] Relatively infrequent dissemination in this cohort likely reflects the institutional protocol for regular BAL examination, and thus infection may have been detected early in its course before dissemination could occur. Furthermore, all patients in our study were taking tacrolimus based immunosuppressive regimens which are thought to be associated with a lower frequency of CNS involvement.[5, 16]

Cryptococcus gattii is known to present some clinical differences compared to other species of *Cryptococcus*, such as being a more frequent cause of cryptococcomas, requiring longer durations of antifungal treatment and being linked to more neurological sequelae.[24, 25] There was only one case of *C. gattii* in our cohort and thus we were unable to meaningfully describe the clinical differences between the cryptococcal species. However, further research in this area would be beneficial in order to elucidate the characteristics and the potential clinical impact of the various cryptococcal species in the solid organ transplant population.

Despite the low rates of disseminated infection, we believe that all patients (including those with only positive BAL specimens and without pulmonary parenchymal involvement) should undergo routine CSF analysis to exclude CNS infection. The two cases of CNS involvement in our study dramatically demonstrate that CNS infection may present with non-specific symptoms but progress rapidly in the immunocompromised patient. In particular, the patient who died due to cerebral complications of infection had been well and living independently, but then rapidly deteriorated in a few days with an enormous burden of disease. This case highlights that a high index of suspicion is required for infection in these organ transplant recipients.

Immunosuppression is a well-documented risk factor for cryptococcal infection.[26] This includes corticosteroid therapy, with previous studies demonstrating doses of >20mg/day

for 60 days as a significant risk factor for disease.[27] Patients in our group received a mean daily dose of 12mg/kg prednisolone at time of diagnosis with only one patient receiving > 20mg/day, due to concerns about transplant rejection. Others have reported infection in LTx recipients receiving 7-10mg prednisolone per day,[28, 29] serving as an important reminder that LTx recipients are significantly immunosuppressed independent of steroid dosing. Given the small numbers in our study, it is difficult to conclude the significance of prednisolone dosage and its contribution to the risk of infection in our cohort.

Given the ubiquitous distribution of *Cryptococcus* in the environment, the exposure of the transplanted lung to the environment, the levels of immunosuppression in LTx recipients, and the fact that 800 lung transplants were being closely followed by our institution during the study period, the number of cases of cryptococcal infection in this cohort of patients may be considered relatively low, compared to other SOT studies[12, 30]. However, at our centre, approximately 25% of patients are on voriconazole or posaconazole antifungal therapy at some point post-transplant for treatment of *Aspergillus* infection and this may partly explain the small number of cases found in our study.

Although mortality in our cohort was low with one death only (9%), the sudden and catastrophic outcome of CNS infection highlights the importance of early diagnosis and treatment.

This is a small, retrospective, medical record-based review and some caution needs to be exercised with regards to interpretation of the findings. Although ascertainment bias is an important consideration, nonetheless this study represents one of the largest cases series evaluating *Cryptococcus* infection in LTx recipients. Our series highlights the fact that cryptococcal infection should remain a consideration in asymptomatic LTx recipients, especially in the presence of non-specific nodules on chest imaging, and that the presence of headache in these patients requires urgent investigation for cryptococcal CNS infection.

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Appendix 1

Clinical definitions

Cases were defined as having either pulmonary or disseminated infection where disseminated infection included those with infection of the central nervous system (CNS), fungaemia or involvement of ≥ 2 non-contiguous organ sites.[3, 5] Pulmonary infection was defined as a positive *Cryptococcus* culture or suggestive histology or cytology from a respiratory specimen (sputum, bronchoalveolar lavage (BAL), lung lesion biopsy) or a positive serum cryptococcal antigen associated with clinical and/or radiographic findings consistent with pulmonary disease.[31]

CNS infection was defined as a positive *Cryptococcus* culture or suggestive histology or cytology from cerebrospinal fluid (CSF) or a CNS lesion biopsy, a positive CSF cryptococcal antigen, and/or a positive serum cryptococcal antigen associated with clinical and/or radiographic findings consistent with CNS disease.[31, 32]

Cure was defined as a negative *Cryptococcus* culture following a positive culture, accompanied by improvement in clinical symptoms, radiological findings and/or cryptococcal antigen titre (serum or CSF); or improvement in clinical symptoms, radiological findings and cryptococcal antigen titre (serum or CSF) in cases with negative cultures.

Table 1: Key patient demographics

Characteristics	n=11
Age (years), median (range)	54.7 (34-69)
Male gender, n (%)	7 (64)
Residence, n (%)	
Metropolitan Melbourne	6 (55)
Rural Victoria	2 (18)
Metropolitan Adelaide	1 (9)
Rural South Australia	2 (18)
Renal transplant, n (%)	2 (18)
Antimicrobial prophylaxis, n (%)	
Trimethoprim-sulfamethoxazole	10 (91)
Trimethoprim	1 (9)
Valganciclovir	7 (64)
CMV serostatus, n (%)	
IgG positive	8 (73)
Potential source of exposure†, n (%)	
Rural farm residence	3 (27)
Soil exposure	5 (45)
Eucalypt/gum tree exposure	3 (27)
Chopping tree branches/burning wood	5 (45)
Hiking/camping in bushland	1 (9)
Pigeon contact	0 (0)
Budgerigar contact	1 (9)
Diagnosis	

Pulmonary disease	9 (82)
Pulmonary and CNS disease	1 (9)
CNS disease	1 (9)

Abbreviations: CMV, cytomegalovirus; CNS, central nervous system

† Patients reported more than one exposure

Table 2: Demographic, clinical, radiological, biochemical features, *Cryptococcus* identification and management of all patients with cryptococcal infection

Case no	Age (years)	Gender	Reason for lung transplant	Immunosuppression	Relevant Co-morbidities	Clinical	CXR	CT chest	CT Brain	MRI Brain	Serum cryag	CSF cryag	Culture site	Isolate identification	Site of disease	Consolidation therapy	Duration of therapy	Surgical management	Outcome
1	65	Female	COPD	T+A+P	DM	C+Le+CP+D	L) LL consolidation	R) ML nodules	NAD	-	0	0	BAL	*C. laurentii*	Lung	VCZ	5.5 months	Nil	Cure
2	44	Female	CF	T+A+P	DM	As	R) pleural effusion	NAD	NAD	-	0	0	BAL	*C. neoformans*	Lung	FCZ	5 months	Nil	Cure
3	69	Male	IPF	T+A+P	DM	As	-	R) ML ground glass; R) LL nodules	NAD	-	0	0	BAL	*C. neoformans var grubii*	Lung	FCZ	11 months	Nil	Cure
4	34	Male	CF	T+M+P	Renal transplant CKD DM Hypogammaglobulinemia	As	R) UL nodule	R) UL nodule	NAD	-	8	0	Lung nodule	*C. gattii*	Lung	FCZ, VCZ[†]	-	Eight lung upper lobe wedge resection[‡]	Currently on VCZ treatment
5	59	Male	Alpha 1 antitrypsin deficiency	T+A+P		As	R) LL non specific opacity	R) LL nodule	NAD	-	0	0	BAL	*C. neoformans var grubii*	Lung	FCZ	8.5 months	Nil	Cure
6	56	Male	IPF	T+M+P	CKD	C	NAD	Mediastinal LAD	-	-	0	-	BAL	*C. neoformans var grubii*	Lung	FCZ	5 months	Nil	Cure
7	61	Male	IPF	T+A+P		As	NAD	L) LL ground glass; L) pleural effusion	NAD	NAD	0	0	BAL	*C. neoformans var grubii*	Lung	FCZ	7 months	Nil	Cure
8	69	Male	COPD	T+A+P	CKD	As	NAD	NAD	NA	NAD	0	0	BAL	*C.*	Lung	FCZ	6	Nil	Cure

									D					*neoformans*			months		
9	46	Male	CF	T+M+P	Renal transplant DM CF related liver disease	H	NAD	-	NAD	Cerebral oedema, pontine and extrapontine myelinolysis	2048	4096	CSF	*C. neoformans var grubii*	CNS	-	-	-	Died[§]
10	56	Female	COPD	T+P	DM	C+F+Le+H+Co	R) LL & L) ML consolidation; R) ML nodules	R) sided nodules; L) LL consolidation	NAD	Leptomeningeal enhancement	128	32	-	-	Lung and CNS	FCZ	10 months	Nil	Cure
11	43	Female	CF	T+M+P	DM	As	L) UL nodule	L) UL nodule	NAD	-	64	0	-	-	Lung	FCZ, PCZ[¶]	5 months	Nil	Cure

Abbreviations: CXR, chest x-ray; CT, computed tomography; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; cryag, cryptococcal lateral flow assay titre; COPD, chronic obstructive pulmonary disease; CF, cystic fibrosis; IPF, interstitial pulmonary fibrosis; T, tacrolimus; A, azathioprine; M, mycophenolate; P, prednisolone; DM, diabetes mellitus; CLD, chronic liver disease; CKD, chronic kidney disease; C, cough; Le, lethargy; CP, chest pain; D, dyspnea; As, asymptomatic; H, headache; F, fever; Co, confusion; L, left; R, right; LL, lower lobe; ML, middle lobe; UL, upper lobe; NAD, no abnormality detected; BAL, bronchoalveolar lavage; CNS, central nervous system; VCZ, voriconazole; FCZ, fluconazole; PCZ, posaconazole

[†] Fluconazole was changed to voriconazole after 7 months of therapy, and at time of surgical management, due to inadequate clinical and radiological response

[‡] Surgical management of a cryptococcal abscess occurred after inadequate clinical and radiological response to 7 months of fluconazole therapy

[§] Death due to cryptococcal CNS infection, diagnosed post-mortem

[¶] Fluconazole was changed to posaconazole after 10 weeks due to adverse effects