

Teaching Point (Section Editor: A. Meyrier)

Donor transmission of *Cryptococcus neoformans* presenting late after renal transplantation

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Introduction

We describe donor-transmitted *Cryptococcus neoformans* infection in a renal transplant recipient. The donor had no apparent risk factors for cryptococcal infection. The disease in the recipient, who did not receive prophylaxis, manifested relatively late at 50 days post-transplantation. We discuss the epidemiology and management of cryptococcal disease in solid organ transplant recipients and lessons learned from this case.

Case report

A 50-year-old man with end-stage renal failure secondary to diabetic nephropathy received a renal transplant from a donation-after-brain-death donor who died from meningitis of presumed bacterial aetiology. Basiliximab induction was followed by maintenance tacrolimus, mycophenolate and reduced dose prednisolone. Because the donor was cytomegalovirus (CMV) positive and the recipient negative, prophylactic valganciclovir was administered in accordance with British Transplantation Society guidelines.

Five days post-operatively, the transplant team was informed that *Cryptococcus neoformans* had been grown from donor cerebrospinal fluid (CSF) and blood. The donor had no apparent risk factors; he was HIV negative with no exposure to steroids or other immunosuppressants. Fluconazole prophylaxis was not recommended primarily because of the rarity of cryptococcal transmission through solid organ transplant (SOT), weighed against the potential adverse effects of fluconazole, including altered tacrolimus pharmacokinetics.

Graft function was delayed and haemodialysis was required for 1 week post-transplant. Thereafter, plasma creatinine stabilized at 150–170 $\mu\text{mol/L}$. Discharge medications included: tacrolimus 6 mg twice daily (bd), mycophenolate (Myfortic R) 540 mg bd, valganciclovir 450 mg

thrice weekly (reduced dose according to renal function), co-trimoxazole 480 mg once daily (od), prednisolone 7.5 mg od, insulin glargine, aspirin 75 mg od, atorvastatin 10 mg od and ranitidine 150 mg bd.

Eight weeks post-transplantation the patient presented with nausea and vomiting. An *Escherichia coli* urinary tract infection and moderate level CMV replication in the blood were detected. He was escalated to treatment dose valganciclovir and prescribed co-amoxiclav 625 mg bd.

Nine weeks post-transplantation he was readmitted with vomiting and severe frontal headache. He acknowledged 3 weeks of fronto-temporal headaches, worse on coughing or straining. There was no visual disturbance, fever, photophobia or neck stiffness, and he felt otherwise well.

On examination he was afebrile and orientated. Kernig's sign was negative. Fundoscopy revealed left-sided cataract, right-sided proliferative diabetic retinopathy and blurred right optic disc. Neurological examination was normal other than absent ankle reflexes. Laboratory results included the following: creatinine 150 $\mu\text{mol/L}$, tacrolimus concentration 10.8 ng/mL, C-reactive protein 0.6 mg/dL and white cell count (WCC) $8.79 \times 10^9/\text{L}$ (73% neutrophils, 22% lymphocytes).

The differential diagnosis included cerebral venous sinus thrombosis and chronic meningitis. A computed tomography scan of the brain was suggestive of raised intracranial pressure. A magnetic resonance imaging (MRI) venogram showed diffuse cortical enhancement, without venous thrombosis. Neurosurgical services advised that it was safe to proceed with lumbar puncture (LP).

The opening pressure was $>40 \text{ cm H}_2\text{O}$, with clear CSF. CSF was examined for protein, glucose, cytology, viral PCR, cryptococcal antigen (CRAG), and microscopy, staining and culture for fungi, bacteria and mycobacterium. The results included: protein 1262 mg/L, glucose 3.1 mmol/L (serum glucose 14 mmol/L) and WCC $44/\text{mm}^3$ (all polymorphs). Yeasts were seen on microscopy, and CRAG was positive. Intravenous flucytosine (25 mg/kg four times daily) and liposomal amphotericin (4 mg/kg od) were

Table 1. Results of *C. neoformans* genotyping

Isolate	Copy number of nucleotide repeat within the selected microsatellite regions								
	CNA2A	CNA2B	CNA2C	CNA3A	CNA3B	CNA3C	CNA4A	CNA4B	CNA4C
Donor	14	18	23	66	13	9	0	10	10
Recipient	14	18	23	66	13	9	0	10	10
Isolate 1	22	12	8	8	9	16	0	5	9
Isolate 2	11	11	6	40	4	0	0	3	5

prescribed. Daily LPs were performed until the opening pressure was <25 cm H₂O. Mycophenolate was withheld. UK Transplant, the national coordinating centre, was contacted to establish the fate of the other organs. The liver and partner kidney had been successfully transplanted; the other centres had prescribed fluconazole prophylaxis.

After 6 days of treatment, opening pressure was 18 cm H₂O, and the patient's condition was improved. Seventeen days into treatment, renal biopsy was performed for deteriorating graft function (creatinine 250 µmol/L). This showed acute tubular necrosis compatible with amphotericin toxicity. After 21 days of treatment, CSF was sterile, and treatment was switched to oral fluconazole 400 mg od for 8 weeks, to be followed by 200 mg od for 9 months. Reduced dose mycophenolate was restarted, and low blood tacrolimus concentrations (5–6 ng/mL) targeted. Renal function recovered to creatinine 180 µmol. The patient remains well.

Isolates of *C. neoformans* from this patient were sent to the UK National Mycology Reference Laboratory for genotyping by PCR amplification and sequencing of a panel of nine microsatellite markers, a novel method developed from the technique described by Illnait-Zaragoza [1]. Because different strains of *C. neoformans* have different numbers of copies of discrete di-, tri- and tetra-nucleotide repeats, this approach provides an impressive discriminatory power of 0.993 (the average probability of correctly assigning a different type to two randomly sampled unrelated strains of a given taxon). Isolates from the donor were similarly processed, and confirmed to be the same strain. Conversely, the donor and recipient isolates were quite distinct from two further cryptococcus isolates collected from patients in the same area as the donor during the same time period (isolates 1 and 2). The results are displayed in Table 1.

Discussion

Epidemiology

Cryptococcus is the third most common fungal infection among SOT recipients with a lifetime incidence of 1.8% (range 0.3–5.0%) [2, 3]. The mortality rate approaches 20% and is highest in those with central nervous system (CNS) involvement [4]. The disease usually occurs >1 year after transplant and is thought to represent reactivation of latent infection rather than fresh exposure [4–6].

There is scant information on the epidemiology of very early cryptococcosis post-transplant (within a month). It is believed that most cases represent unrecognized pre-transplant infection, but transmission from the donor is also possible [7]. There have only been four cases of definite cryptococcal transmission following SOT [8, 9], three

from a single donor. It is, therefore, an extremely rare event. In both implicated donors, cryptococcal disease was only diagnosed after the transplants had taken place. The organism was isolated from the CNS in one and the urinary tract in the other. Three affected patients were renal transplant recipients, all of whom survived having received appropriate treatment. The fourth was a liver transplant recipient, who died of disseminated disease. In all cases, symptoms manifested within the first 1–4 weeks post transplantation.

The UK Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) advises that diagnosed acute infections and undiagnosed presumed acute infectious disease in a potential donor do not necessarily preclude donation, but that microbiology advice should be sought [10]. However, the risk assessment for donor-transmitted infections is complicated by lack of epidemiological data, limited microbial testing when compared with blood transfusion, varying national standards and lack of availability of suitable screening assays [11]. SaBTO discourages the use of organs from donors with undiagnosed meningitis or risk factors for fungal meningitis [10]. In this case, the donor had no risk factors for atypical causes of meningitis: it had been presumed by the referring team to be bacterial in origin and treated as such. The growth of cryptococcus from donor blood and CSF several days later was unexpected. The decision of several transplant centres, including our own, to proceed without a microbiological diagnosis rendered these transplants relatively high risk.

Risk factors

There are a number of risk factors for the development of cryptococcal disease after SOT. Immunosuppressive agents vary in their effects on the risk of invasive fungal infections. T-cell depleting agents cause profound depletion of CD4+ T cells and are associated with a dose-dependent increase in the risk of cryptococcosis [12].

Calcineurins are highly conserved from man to yeast, and calcineurin inhibitors (CNIs) have potent *in vitro* anti-fungal activity against *C. neoformans* which is mediated through inhibition of fungal homologues of calcineurin [13, 14]. Interestingly, the use of CNIs, in particular tacrolimus, appears to have a protective effect on mortality in SOT patients with cryptococcosis [15].

Cryptococcal disease is more common in liver than renal transplant recipients, and tends to manifest earlier. This may be explained by the greater intensity of immunosuppression [4]. In addition, liver failure causes specific defects in chemotaxis, complement deficiency and depressed cellular immunity [16].

Clinical picture

Fishman recently published a useful overview of infections in SOT recipients, including clinical presentation [6]. Between 53% and 72% of the cryptococcal disease in SOT recipients is disseminated or involves the CNS [4, 15, 17, 18]. Reported rates of fungaemia in SOT patients with cryptococcal infection vary, but may exceed one-third, particularly in those with CNS disease [4, 15, 18, 19]. One investigator found that abnormal neuroimaging as well as abnormal baseline neurology (including disordered mentation) was predictive of mortality in cryptococcal meningitis, but the proportion of SOT recipients in this study was small [20].

Management

There is no strong evidence base to guide prophylaxis or treatment of cryptococcosis in SOT recipients. The Infectious Disease Society of America (IDSA) has extrapolated from clinical trials in other hosts to produce guidelines for transplant recipients [21]. The recommended management of disseminated disease or meningoencephalitis includes induction therapy with amphotericin B 4 mg/kg/day plus flucytosine 100 mg/kg/day for 14 days, followed by a consolidation phase using fluconazole 200–400 mg/day for at least 6 months (provided the immunosuppression is not augmented). LP should be repeated after 2 weeks of induction therapy to ensure that the CSF is sterile. If not, intravenous therapy should be continued with fortnightly LPs until CSF becomes culture negative. LPs may be needed more frequently to control raised intracranial pressure. It is recommended that if there is persistent, symptomatic intracranial pressure of >25 cm H₂O, daily LPs should be performed until stability is achieved. If daily LPs are technically difficult or required for a prolonged period, ventriculostomy or percutaneous lumbar drainage may be considered.

Fluconazole and itraconazole have been shown in randomized controlled trials to reduce the incidence of primary cryptococcosis in HIV-positive patients with a CD4 count of <50 cells/mm³ [22]. However, the IDSA standpoint is that the rarity of cryptococcal disease in resource-rich settings does not justify the risks of drug interactions, drug resistance, increased cost and non-compliance. Therefore, primary prophylaxis is not recommended for either HIV-positive or SOT patients. There is no existing guidance for the highly unusual situation we faced in this case, and it was a difficult decision as to whether to offer prophylaxis. In retrospect we made the wrong decision. In addition, fluconazole is generally a well-tolerated drug. While it does markedly increase tacrolimus blood concentrations (through its effect on the cytochrome P450 3A4 enzyme), this can usually, in our experience, be managed by halving the dose of tacrolimus and close monitoring until stable levels are achieved.

Immune reconstitution syndrome (IRS) has been seen in SOT recipients with cryptococcosis after the commencement of antifungal treatment [23]. This is an inflammatory condition that presents as worsening or reappearance of previous manifestations of cryptococcosis after an initial response, despite appropriate anti-fungal treatment. It is thought to be a result of a switch from a TH2 to a TH1 predominant immune response, and may be associated with allograft loss due to rejection [23]. This has led some to suggest that immunosuppression should

not be reduced at the same time as antifungal therapy is started [24]. No evidence of IRS was observed in this case.

Conclusion

Although transmission of cryptococcus by SOT is rare, we believe that the lessons learned from this case are worth sharing. The few reported cases show presentation early after transplantation (<24 days). This case shows that the presentation may be significantly later. It is possible that transmission of cryptococcus by SOT is under-recognized, especially if the donor does not have specific risk factors. This case emphasizes the importance of characterizing the causative organism when brain death is caused by meningoencephalitis, and the need for administering appropriate prophylactic therapy when causation has been clarified. In retrospect, the advice not to offer prophylaxis was wrong.

Teaching points

- (i) *C. neoformans* can be transmitted via solid organ transplantation
- (ii) The use of organs from donors with microbiologically undiagnosed meningoencephalitis is of high risk
- (iii) Prophylaxis should be offered to transplant recipients when a donor is confirmed to have cryptococcal disease
- (iv) Donor-transmitted disease does not necessarily manifest within the first few weeks

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