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Longitudinal study of cryptococcosis in adult solid-organ transplant recipients

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Abstract While studies in kidney recipients have found meningitis to be the most common clinical manifestation of cryptococcosis (Cry), it is unclear if the clinical presentation of Cry differs among various solid-organ transplant (SOT) recipients and whether the serum cryptococcal antigen (SCA) might predict the site of infection. We report the clinical manifestations and the correlation with a positive SCA among 55 consecutive SOT recipients diagnosed with Cry at the University of Pittsburgh Medical Center. These included: heart ($n=13$), lung ($n=4$), liver ($n=28$), kidney ($n=9$) and small bowel ($n=1$) recipients. While there were no significant differences in the manifestations of Cry in heart and lung recipients, kidney recipients had disseminated disease as the most common presentation ($P=0.02$). In contrast, pneumonia ($P=0.003$) and meningitis ($P=0.02$) were more frequent than disseminated disease in liver recipients. Positive SCA was higher in patients with disseminated disease and meningitis than in patients with isolated pneumonia ($P=0.0001$). Significant differences in the manifestations of Cry were observed among types of SOT populations. A positive SCA may be predictive of dissemination and meningitis, but it may not be sensitive for pulmonary disease.

Keywords Cryptococcosis · Organ transplantation · Serum cryptococcal antigen · Cryptococcus

Introduction

Cryptococcosis is an invasive fungal disease most often caused by *Cryptococcus* var. *neoformans*. The vast majority of the cases of cryptococcosis are thought to result from the failure of host defenses to contain the organism after inhalation of aerosolized particles from an environmental source [1, 17]. Solid-organ transplant recipients (SOT) are recognized to be at increased risk for cryptococcosis [1, 17]. However, in contrast to the

reported decline in the incidence of other invasive fungal infections in SOT recipients [23, 27], recent estimates suggest that about 20 to 60% of the cases of cryptococcosis in non-AIDS patients have occurred in SOT recipients [8, 21, 26].

Previous studies in kidney recipients have found meningitis to be the most common clinical presentation of cryptococcosis [7, 13, 19, 22], and an uncontrolled study in liver transplant recipients [24] suggested that cutaneous or osteoarticular lesions might be more frequent than meningitis. However, the small sample size of some of these studies and the lack of comparison between different types of SOT recipients with cryptococcosis have precluded a comprehensive assessment of this fungal infection in this patient population. The purpose of our study was (1) To investigate the incidence, clinical manifestations, and relative risk of cryptococcosis among the various transplant groups and (2) To examine the correlation with a positive serum cryptococcal antigen to determine whether this test could predict the site of infection in SOT recipients.

Methods

Patients and definitions

Cases of cryptococcosis among SOT recipients were identified from the electronic information database Medical Archival Retrieval System (MARS) at the University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA. A case of cryptococcosis was defined as a positive cryptococcal antigen in blood or cerebrospinal fluid (CSF) or India-ink preparation, or culture from any body site or histopathological findings consistent with *Cryptococcus* together with a compatible clinical manifestation. Disseminated cryptococcosis was defined as involvement of at least two organ systems. *Cryptococcus*-related mortality was defined as death that was directly attributed to *Cryptococcus*, i.e., *Cryptococcus* was recovered from autopsy specimens. Patients who were not autopsied but who died within 4 weeks of the diagnosis of cryptococcosis being made were also considered to have died of a *Cryptococcus*-related disease [3].

Chart review

The medical records of the study patients were obtained and reviewed for data collection from MARS. Included in this electronic medical-record database are the admission history and physical-examination details, discharge summary, dictated progress notes, medications dispensed from the pharmacy, and all laboratory data, including microbiology and pathology reports. Demographic data, including race, birth date, gender, dates of admission and discharge, dates of organ-transplant operations, diagnostic procedures, and outcome of present hospitalization, were obtained. The admission history and physical examination were reviewed for the presence and duration of fever, cough, shortness of breath, headache, nausea/vomiting, seizures, and visual disturbances. Recorded microbiological data included the date, specimen site, and results of cultures of all specimens, and results of cryptococcal antigen in the serum and CSF. The cause of death was recorded from the discharge summary or autopsy report.

Microbiology

Prior to 1 April 1995 all specimens submitted to the microbiology laboratory for fungal culture were inoculated onto Sabouraud's dextrose agar and mycophil agar containing antibiotics (penicillin and gentamicin). Since 1 April 1995, the inoculation of all specimens for fungal culture was done on inhibitory mold agar slants (IMA, Becton Dickinson, Cockeysville, Md., USA) and brain–heart infusion agar slants with 10% sheep blood containing antibiotics (BHI wAb; chloramphenicol and gentamicin, Becton Dickinson). Cultures were incubated at 30 °C and were examined daily during the first week and then weekly for the following 3 weeks. Cryptococcal serum and CSF antigen were prepared with the use of cryptococcal antigen latex agglutination system (Meridian Diagnostics, Cincinnati, Ohio, USA) and performed according to the manufacturer's protocol.

Statistical analysis

Continuous data were presented as the mean ± SD, or median and range, and categorical data as proportions. Differences in proportions among the different groups of SOT recipients with cryptococcosis were tested with the Z test of proportions with the use of the STATA 6.0 software package (College Station, Texas, USA). Significance was defined as $P < 0.05$.

Results

Between January 1990 and October 2000, 55 of 5,377 (1%) SOT recipients were diagnosed with cryptococcosis at the University of Pittsburgh Medical Center. These included: heart ($n = 13$), lung ($n = 4$), liver ($n = 28$), kidney ($n = 9$) and small bowel ($n = 1$) graft recipients. The incidence of cryptococcosis by type of organ-transplant recipient during the study period is presented in Table 1. It was significantly higher in heart-transplant recipients than in other transplant groups ($P = 0.0001$). The mean (± SD) age of patients was 53 ± 13 (range 25–79) years and 98% ($n = 54$) were Caucasian. The male/female ratio was 37/18. All SOT recipients were receiving immuno-

Table 1 The incidence of cryptococcosis by type of organ-transplant recipient over a 10-year period (1990–2000). We calculated the incidence of cryptococcosis per 1,000 patients by dividing the number of cases in each group by the corresponding total number of transplant recipients during the study period

Type of transplant (n)	Number of cases; $n = 55$ (incidence per 1,000 patients)
Heart (372)	13 (34.9)
Lung (471)	4 (8.4)
Liver (2,346)	28 (11.9)
Kidney (2,120)	9 (4.2)
Small bowel (68)	1 (14.7)
Total (5,377)	55 (10)

suppressive therapy at the time of the diagnosis of cryptococcosis. This included tacrolimus ($n=47$) or cyclosporin A ($n=8$). Of these 55 patients, 48 (87%) were also receiving prednisone in varying dosages. In lung-transplant recipients, the immunosuppressive regimen also included the use of azathioprine or mycophenolate mofetil.

Clinical manifestations

The median time interval between transplantation and cryptococcosis among the different groups of SOT recipients is presented in Table 2. Overall, the clinical syndromes included isolated pneumonia (38%), isolated meningitis (35%), and disseminated disease (24%). Two other (3%) patients presented with cervical lymphadenitis ($n=1$) and a laryngeal mass ($n=1$). While there were no significant differences in the type of clinical presentation of cryptococcosis among heart and lung transplant recipients, kidney recipients had disseminated disease as the most common presenting feature of cryptococcosis, compared with pneumonia (67% vs 11%; $P=0.02$) or meningitis (67% vs 22%; $P=0.05$), respectively. In liver recipients, pneumonia (46% vs 11%; $P=0.003$) and meningitis (36% vs 11%; $P=0.02$) were more frequent clinical presentation than dissemination.

Clinical signs and symptoms were reported in all transplant recipients. The mean duration of symptoms before the diagnosis of cryptococcosis was 17 ± 23 (range 1–97) days. The signs and symptoms among patients with pneumonia were shortness of breath (95%), cough (76%), and fever (62%). In the patients with meningitis, the most frequent symptoms were headache (79%), nausea/vomiting (79%), fever (74%), loss of vision (11%), and seizures (5%).

Microbiology

Cryptococcus was recovered from biopsy samples of the lung ($n=14$), skin ($n=4$) lymph nodes ($n=2$), and larynx ($n=1$). In addition, the fungus was isolated in cultures of the CSF ($n=19$), broncho-alveolar lavage fluid ($n=16$), and blood ($n=6$). Other cultures obtained from the same sites at the time of the diagnosis of cryptococcosis in SOT recipients did not yield any additional pathogen. Serum cryptococcal antigen was performed in 44/55 (80%); 33 (75%) were positive and 11 (25%) negative. Nine (82%) of the patients with a negative serum cryptococcal antigen had cryptococcal pneumonia. The proportion of positive serum antigen was higher among patients with disseminated disease and meningitis than in patients with isolated pneumonia (13/13, 100% and 12/14, 86% vs 6/15, 40%; $P=0.0001$). The serum antigen was positive in both cases of other presentations of cryptococcosis. The mean titer of the positive serum antigen according to clinical presentation of cryptococcosis was $1:3,974\pm9,609$ in disseminated disease, $1:3,403\pm6,157$ in isolated meningitis and $1:103\pm152$ in patients with isolated pneumonia ($P=0.09$). The CSF cryptococcal antigen was positive in 100% of the patients with involvement of the central nervous system, and the mean titer was $1:2,238\pm4,129$.

Treatment and outcome

Most of the study patients were treated with a combination of amphotericin B and 5-flucytosine (65%); ten were treated with amphotericin B only, and nine with fluconazole. The overall *Cryptococcus*-related mortality in SOT recipients was 27% (15/55) and was more commonly associated with meningitis (7/15; 47% $P=0.04$) and disseminated disease (6/15; 40% $P=0.09$) than with pneumonia (2/15; 13%). The mortality rate was 4/13 (31%) heart, 7/28 (25%) liver, and 4/9 (44%) kidney transplant recipients ($P=0.5$). Mortality did not differ among patients treated with the combination of amphotericin B and 5 flucytosine, or amphotericin B alone, or fluconazole ($P=0.2$).

Discussion

Studies [7, 13, 22] in renal-transplant recipients under cyclosporine-based immunosuppression agents suggest-

Table 2 Clinical manifestations of cryptococcosis in adult SOT recipients. Other site included cervical lymphadenitis ($n=1$) andlaryngeal mass ($n=1$)

Type of SOT recipient (*n*)	Diagnosis after transplantation in months Median (range)	Pneumonia *n* (%)	Meningitis *n* (%)	Disseminated disease *n* (%)	Other site *n* (%)
Lung (4)	7 (1–13)	3 (75)	0 (0)	1 (25)	0 (0)
Heart (13)	15 (3–120)	4 (31)	6 (46)	3 (23)	0 (0)
Liver (28)	12 (1–146)	13 (46)	10 (36)	3 (11)	2 (7)
Kidney (9)	21 (4–204)	1 (11)	2 (22)	6 (67)	0 (0)
Small bowel (1)	21	0 (0)	1 (100)	0 (0)	0 (0)
Total (55)	13 (1–204)	21 (38)	19 (35)	13 (24)	2 (3)

ed an incidence of cryptococcosis of 3% to 4%. In heart–lung and liver transplant recipients under cyclosporine-based immunosuppression, the reported incidence of cryptococcosis was 2.3% and 0.26%, respectively [3, 4, 5]. Generally, these reports indicate that the main clinical presentation is with meningitis, often in a sub-acute form. In contrast, while our results show a relatively low overall incidence (1%) of cryptococcosis, the incidence and clinical manifestations vary among different SOT groups. The present analysis points out that heart-transplant recipients have the highest incidence of cryptococcal infection. Although the reasons for this finding are not clear, it might be a reflection of greater immunosuppression in this group of patients. Longitudinal studies [4, 9] after organ transplantation suggest that heart-transplant recipients have more infections and are more affected by them than kidney-transplant patients. Another factor to be considered in the risk of developing cryptococcosis among our patients may be the geographical region where they reside. Early studies conducted in the US [7, 8, 24] among transplant and non-transplant patients have pointed out a higher risk for cryptococcosis in individuals from the eastern part than in those from the western part of the country. The reported frequency of this fungal infection was 6–83% in SOT recipients residing in the eastern continental US, compared to only 1.5% in those residing in the western part. In addition, a recent study [18] of infectious complications among heart-transplant patients over a 16-year period at a transplant center on the west coast of the US reported an incidence of cryptococcosis of only 0.6% (4/620). Therefore, it is possible that unidentified environmental sources of infection may affect the rate of cryptococcosis among SOT recipients.

It has been recognized that the primary contributing factor to cryptococcosis in transplant patients is the immunosuppressive regimen used to prevent allograft rejection. In particular, prednisone has been associated with an increased risk for cryptococcal infection [1, 17]. A high proportion of our patients (87%) was receiving prednisone at the time of the diagnosis of the fungal infection. In a recent review [11] of cryptococcosis in some SOT recipients (145 kidney, 20 liver and 10 heart), it was speculated that the type of immunosuppression after transplantation (i.e., azathioprine, cyclosporine, and tacrolimus) might influence the clinical presentation of cryptococcosis. Of the 127 transplant recipients assessed according to their immunosuppressive regimen, those receiving tacrolimus were less likely to develop cryptococcal meningitis and more likely to develop skin or osteoarticular cryptococcal disease than patients who received a non-tacrolimus-based regimen [12]. However, the majority of patients in this review (78, 61%) received prednisone, and only nine (7%) received tacrolimus, a fact that makes the comparison difficult to interpret. According to the same review, meningitis was the most common presentation (55%) followed by skin and/or osteoarticular involvement (13%) and pneumonia (6%). This is similar to the early reports of cryptococcosis after renal transplantation.

Our current study with tacrolimus as the most frequently used primary immunosuppressive agent, demonstrates a broader spectrum of cryptococcosis not previously appreciated. Indeed, pneumonia, an infrequent manifestation of cryptococcosis according to previous literature [1, 2, 8, 17], was a common presentation in this study. More importantly, some unique differences in the characteristics of cryptococcosis were observed among the various types of SOT recipients. Renal-transplant recipients had disseminated disease as their most common presentation of cryptococcosis (67%). A rapid clinical progression may be a function both of immunosuppressive regimens and the underlying illness of this patient population. Indeed, uremia has been shown to decrease lymphocyte transformation in animal models of cryptococcosis [6, 10]. Furthermore, the production of tumor necrosis factor, interleukin (IL)-12, IL-18 and other cytokines, are implicated in the development of Th-1-type anti-cryptococcal immune response [16, 20]. This response may be altered in these patients as a consequence of uremia and immunosuppression. However, our findings corroborate the fact that non-specific symptoms are the most important manifestation of cryptococcosis, regardless of the site of initial involvement. Thus, a high index of suspicion for cryptococcosis should remain in the differential diagnosis of infections in SOT recipients.

The detection of cryptococcal polysaccharide capsular antigen is a useful test to establish the diagnosis of cryptococcosis [15]. A previous study by Kauffman et al. [14] reported that *Cryptococcus* antigen was present in both the serum and spinal fluid in 86% of 330 confirmed cases of cryptococcal meningitis. Antigen was detected in CSF specimens in 99% of these cases but in only 87% of serum samples. Similarly, our results showed that a positive serum test is suggestive of disseminated disease or meningitis. However, it is noteworthy to point out that the cryptococcal serum antigen was positive in only 40% of the patients with pneumonia. This finding suggests that screening with serum cryptococcal antigen may not facilitate the diagnosis of pulmonary disease. This observation is important, since an increasing number of cases of cryptococcosis may present as pneumonia in SOT recipients. Some reports [2, 25] have suggested the unreliability of the serum latex agglutination test for the diagnosis of pulmonary cryptococcosis. Therefore, the diagnosis of pulmonary cryptococcosis may require invasive procedures. Accurate diagnosis is crucial, since the natural history of this disease in immunocompromised patients is the development of disseminated infection in the absence of antifungal therapy [1, 2, 17].

In summary, our analysis indicates significant differences in the incidence and clinical manifestations of cryptococcosis among various types of transplant recipients. Positive serum cryptococcal antigen may be predictive of meningitis and dissemination but cannot be used reliably in the diagnosis of pulmonary cryptococcosis.

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