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Letter to the Editor

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Cryptococcal infection in renal transplant: two case reports and a literature review

Maria Dolores Rojo-Martin[1]
Inmaculada de Toro Peinado[1]
Juan Diego Ruiz Mesa[2]
Begoña Palop Borrás[1]

[1]Microbiology Department. Universitary Regional Hospital of Malaga. Malaga. Spain.
[2]Infectious Diseases Department. Universitary Regional Hospital of Malaga. Spain.

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Sir,

Cryptococcosis is a potentially life-threatening opportunistic mycosis caused by *Cryptococcus neoformans* and *Cryptococcus gattii*. Exceptionally, cases of infection in humans by *C. laurentii* and *C. albidus* have also been reported [1]. The capsule of these yeasts is considered an important factor of pathogenicity. Serotypes are classified according to capsule epitopes: A and D for *C. neoformans* strains and B and C for *C. gattii* strains. [2]. *C. neoformans* is an ubiquitous environmental yeast that inhabits the soil contaminated with bird excrements and nests, especially pigeons. *C. gattii* has been associated with different species of eucalyptus, with tropical and subtropical distribution, although its prevalence has increased in other geographic areas [1,2].

C. neoformans infection can cause disease in immunocompetent individuals, although it most commonly affects immunocompromised patients, including HIV patients, transplant recipients, and other patients with impaired cell-mediated immunity [1,3]. Cryptococcosis, with a prevalence of 0.5%-2.8%, is the third most common mycotic infection in solid organ transplant recipients (SOT) [4,5]. About 20-60% of cases of cryptococcosis in negative-HIV patients occur in SOT recipients [6]. In kidney recipients, this infection mainly occurs after the first year, with a very high rate of mortality reaching up to 40% [7]. Early diagnosis is crucial to improving prognosis. In this paper, we report two cases of cryptococcal meningitis (CM) diagnosed in our center in kidney transplant recipients to illustrate the peculiarities of *C. neoformans* infection in this risk group. Table 1 includes a summary of the clinical and microbiological characteristics of each patient.

Correspondence:
Maria Dolores Rojo Martin
Microbiology Department, Universitary Regional Hospital of Malaga. 29010, Malaga. Spain.
Phone: +34 951291028
E-mail: md.rojo.sspa@juntadeandalucia.es

The first case was a 64-year-old woman with chronic kidney failure secondary to nephroangiosclerosis and nonsteroidal anti-inflammatory drug use, who received a kidney transplant in July 2017. Immunosuppressive therapy included prednisone, tacrolimus, and mycophenolate. In October 2018, the patient was admitted for a two-week history of progressive cephalea, diplopia and occasional speech problems, although the patient remained afebrile. Routine serum chemistry was normal, except for creatinine 2.75 mg/dL and a glomerular filtration (GF) of 18 mL/min. Brain CT scan was normal. Findings in cerebrospinal fluid (CSF) were consistent with lymphocytic meningoencephalitis (Table 1). Bacterial and fungal culture was negative, as were nucleic-acid amplification tests (NAATs) for bacteria, mycobacteria, virus, and fungi. Empirical treatment with acyclovir (350 mg/24 h, IV), ceftriaxone (2 g/12 h IV), and antituberculous drugs was started. Mycophenolate was suspended, whereas tacrolimus and prednisone were maintained at previous doses. After a week, the patient developed neurological deterioration. A new lumbar puncture was performed and NAAT was positive for *Cryptococcus neoformans/gatti*. A yeast was isolated from culture and identified as *C. neoformans* by mass spectrometry (MALDI-TOF). Antifungal therapy with liposomal amphotericin B (250 mg/24h IV) and fluconazole (200 mg/12 h IV) was initiated and neurologic function of the patient improved significantly. Simultaneously, tacrolimus was suspended. After 10 days of antifungal treatment, the viral load of CMV progressively increased, and ganciclovir (115 mg/24 h) was added. Tacrolimus at low doses (1mg/day) was restarted at 14 days.

India-ink staining of CSF obtained by control lumbar punctures performed weekly was persistently positive and *C. neoformans* was isolated again from culture. Four weeks after diagnosis, the patient developed pancytopenia, which was probably linked to drug toxicity and ganciclovir was withdrawn. After this finding, the patient had fever, dyspnea, and rapid progressive general deterioration resulting in death 34 days after admission. *Streptococcus pneumoniae* was isolated in blood culture.

Table 1 Demographic, clinical and microbiological characteristics of CM patients

Variable	Patient 1	Patient 2
Sex	Female	Female
Age	64	56
Time after transplant at the admission	15 months	17 months
Duration of symptoms before diagnosis[a]	21 days	14 days
Immunosuppressive therapy	PR, MF, TC	PR, MF, TC
CSF[b]		
Leukocytes/µL)	263 (98% MN)	31 (90% MN)
Glucose (mg/dL)	33	34
Proteins (mg/dL)	221,8	66,1
Initial anti-infective empirical therapy	Acyclovir (350 mg/24 h), ceftriaxone (2 g/12 h), antituberculous drugs	Ganciclovir (90 mg/24h)
Initial immunosuppressive therapy adjustment (type and time)	MF suspension at the admission TC suspension 1 week after admission	MF suspension at the admission TC dose decrease 5 days after admission
Initial cultures and microbiological results	CSF culture and NAAT negative, BC negative	CSF culture and NAAT positive
First positive microbiological test (time)	NAAT (CSF) (1 week after admission)	Gram and India ink staining/NAAT (CSF) (5 days after admission)
Antifungal therapy	Amphotericin B (250 mg/24 h), fluconazole (200 mg/12 h)	Amphotericin B (150 mg/24 h), fluconazole (200 mg/12h)
Time delay of adequate antifungal therapy[c]	7 days	5 days
Last immunosuppressive therapy	TC (0,75mg/24 h) PR (25 mg/24 h)	PR (5mg/24 h)
Weekly evolution of CMV viral load (cop/mL)[c]	91-420-3060-1024	208-158-317-52

PR: prednisone; MF: mycophenolate; TC: tacrolimus; CSF: cerebrospinal fluid; BC: blood culture; NAAT: nucleic-acid amplification tests; MN: mononuclear
[a]From the beginning of symptoms; [b]First lumbar punction; [c]From admission

The second patient was a 56-year-old woman with advanced chronic kidney disease Stage 5 D secondary to hepatorenal polycystic disease, who received a kidney in June 2017. Standard immunosuppressive therapy included steroids, mycophenolate, and tacrolimus. From March 2018, the patient exhibited a mild deterioration of kidney function (creatinine 2-2.5 mg/dL) and GF of 20 mL/min. In November 2018, the patient presented in emergence room with a several-day history of vomits, peripheral vertigo and holocraneal headache. Laboratory test revealed leukopenia (1.7 x109/L) and a kidney function like baseline. CMV load was 208 copies/mL. Ganciclovir therapy was started and maintained for 5 days (90 mg/24h). Mycophenolate was withdrawn, whereas prednisone (5mg/24 h) and tacrolimus (2.5mg/12h) were maintained. The patient was admitted to the Nephrology Unit with a diagnosis of CMV infection.

Five days after admission, the patient exhibited neurologic deterioration with severe headache, increased instability, and the appearance of dysarthria. Brain CT scan showed a slight right-sided occipital hypodensity. CSF obtained by lumbar puncture had rock water appearance (31 leukocytes/µL, 90% mononuclear, glucose 34 mg/dL and protein 66.1 mg/dL). Gram staining was positive for yeasts, and typical encapsulated yeast were observed in the India ink staining. *C. neoformans* was isolated in CSF culture and multiple NAAT detected *Cryptococcus neoformans/gattii* DNA. Antifungal therapy with liposomal amphotericin B (150 mg/24 h) and fluconazole (200 mg/12h IV) was started. CSF was analyzed weekly, persistently showing encapsulated yeasts in India staining and growth of *C. neoformans* in culture.

Fourteen days after admission, the patient presented general deterioration that required transfer to the ICU. Blood cultures were negative, whereas *Klebsiella pneumoniae* and *Enterococcus faecalis* were isolated in urine. Meropenem (1g/8 h) and linezolid (600mg/12 h) was started and tacrolimus was suspended. After initial improvement, the patient presented fever, pancytopenia, and respiratory failure. *Aspergillus flavus* was isolated from bronchoalveolar lavage fluid. Fluconazole was replaced with voriconazole (200mg/12h). The patient underwent progressive deterioration to death occurred 28 days after ICU admission.

Based on the immunologic state of the host, inhalation of *C. neoformans* spores or poorly encapsulated yeasts can originate asymptomatic pulmonary infection, localized pneumonitis, or disseminated infection [8]. Transplant recipients frequently develop central nervous system complications (50-75%) [9], being cryptococcal meningoencephalitis (CM) the most common problem. Although the incidence of CM among kidney transplant recipients is 0.2-5%, mortality rates are high (20-49%) [4,7,10,11]. In the two cases reported in this paper, infection was associated with neurologic symptoms suggestive of CM, and despite antifungal therapy, evolution was torpid. The patients developed pancytopenia probably secondary to drug toxicity, which favoured the appearance of new hospital-acquired infections with fatal outcomes. Factors associated with severity and poor prognosis of CM are high output CSF pressure, a leukocyte count in CSF < 20/mm3, persistent positive culture after 2 weeks of induction therapy and recently, the presence of pulmonary nodules has been associated [10,12,13].

C. neoformans infection in transplant recipients typically appears in the late post-transplantation period (> 12 months) [10]. This infection originates from primary acquisition after transplantation or the reactivation of a latent infection [6,14]. However, very early reactivation and donor-derived transmission have also been reported. In such cases, the infection generally occurs within the first month after transplantation, [15,16]. In the two cases described, infection appeared more than one year after transplantation. This leads us to think that infection occurred because of the reactivation of a previous latent infection or primary infection. In transplant recipients, immunosuppressive therapy includes glucocorticoids, which reduce cellular immunity and facilitate *C. neoformans* infection. In contrast, other drugs such as calcineurin inhibitors (tacrolimus) and mycophenolate, especially the former, have antifungal activity and, along with azoles, can exert a synergic effect against *C. neoformans* [17-19]. Evidence has been provided of a possible relationship between a rapid reduction of tacrolimus and the appearance of cryptococcosis in transplant recipients receiving hematopoietic precursors [20]. Our two patients were initially administered prednisone, tacrolimus, and mycophenolate. After the appearance of CM symptoms, mycophenolate was withdrawn, and the dose of tacrolimus was reduced progressively until its withdrawal, although it was resumed later in the first patient. Additionally, the two patients exhibited an increase in CMV viral load parallel to CM presentation. In a case review, Marques et al [11] suggested a possible association between CMV infection and cryptococcosis.

For the management of disseminated cryptococcosis, current guidelines recommend induction therapy with liposomal amphotericin plus flucytosine (can be replaced with fluconazole) followed by fluconazole in the consolidation and maintenance phase [11,21]. However, itraconazole, voriconazole and isavuconazole have been successfully used in the induction phase [9]. In the two cases reported, liposomal amphotericin plus flucytosine therapy was started after diagnosis, with an initially favourable evolution. Another crucial aspect is the management of immunosuppression. A rapid reduction of immunosuppression may result in organ rejection and/or in immune reconstitution inflammatory syndrome, which increases morbi-mortality in some patients [22]. In case of persistent severe infection, the suspension of immunosuppressive drugs would be considered.

A determinant factor in the prognosis of CM is the time from the onset of symptoms to diagnosis. Some authors suggest that high mortality rates in non-HIV patients as compared to HIV patients may be due in part to delayed diagnosis due to low clinical suspicion [23,24]. Diagnosis of cryptococcosis is relatively easy and involves searching the typical rounded capsulated yeast by India ink staining of CSF, histological analysis by specific staining to identify the presence of capsules or melanin, direct detection of cryptococcal antigen by latex agglutination, and microbiological culture of samples. Lateral flow immunoassay detection of polysaccharide capsular antigen in body fluids has a high sensitivity and specificity [14]. However, the utility of serial determinations of cryptococcal antigen in blood and CSF to monitor the evolution of infection after treatment in non-HIV patients is a matter of controversy [25]. The detection of CM in patients with low clinical suspicion, generally non-HIV patients, has improved with the use of new multiplex molecular tests with a battery of meningitis-encephalitis-producing pathogens, including *C. neoformans-C. gattii*. Although these tests have proven to have very good sensitivity and specificity [26], false negatives and false positives may occur [27] which makes culture necessary. In the cases described here, molecular detection was the key to establishing the diagnosis of CM, though, in patient 1, NAAT was negative at the first. This negative result could be due to low fungal counts below the lower detection limit of the panel (100 CFU / mL). In fact, *C. neoformans* was not recovered from CSF culture, despite prolonging the incubation time and use of enrichment broths.

In conclusion, cryptococcosis should be considered in the differential diagnosis when neurological symptoms appear in kidney transplant recipients. Given the severity of this infection, all available phenotype- and genotype-based, methods should be used, as early diagnosis is crucial for prognosis of CM.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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