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.

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 cephalalgia, diplopia and occasional speech problems, although the patient remained afibrile. 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 (1 mg/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.
The second patient was a 56-year-old woman with advanced chronic kidney disease Stage 5 D secondary to hepato renal 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 emergency room with a several-day history of vomits, peripheral vertigo and holocranial headache. Laboratory test revealed leukopenia (1.7 x10^9/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/24h) 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) was started. CSF was analyzed weekly, persistently showing encapsulated yeasts in India staining and growth of C. neoformans.

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.

| 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* | 21 days | 14 days |
| Immunosuppressive therapy | PR, MF, TC | PR, MF, TC |
| CSF | | |
| Leukocytes (µL) | 263 (89% 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 (80 mg/24h) |
| Initial immunosuppressive therapy adjustment (type and time) | MF suspension at the admission | MF suspension at the 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 therapyc | 7 days | 5 days |
| Last immunosuppressive therapy | TC (0,75mg/24 h) | PR (5mg/24 h) |
| Weekly evolution of CMV viral load (cop/mL) | 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

*From the beginning of symptoms; †First lumbar punctation; ‡From 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 morbidity-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.

**FUNDING**

None to declare

**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

**REFERENCES**

1. Perfect J R. Cryptococcosis. In: Bennett J E, Dolin R and Blaser M J editors. Mandell, Douglas and Bennett's Principles and Practice of
Infectious Disease. 8th Ed. Philadelphia: Elsevier; 2015. p. 2934–48

2. Baddley JW, Forrest GN; on behalf of the AST Infectious Diseases Community of Practice. Cryptococcosis in solid organ transplantation—Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13543. doi:10.1111/ctr.13543.

3. Martin Mazuelos E, Aller García A I. Aspectos microbiológicos de la criptococosis en la era post TARGA. Enferm Infecce Microbiol Clin. 2010;28(Supl 1):40–5. doi: 10.1016/S0213-005X(10)70007-0.

4. Gassiep I, McDougall D, Douglas J, Francis R, Playford E G. Cryptococcal infections in solid organ transplant recipients over a 15-year period at a state transplant center. Transpl Infect Dis. 2017;19:e12639. doi:10.1111/tid.12639.

5. Muñoz P, Aguado J M. Enfermedades invasoras por hongos levaduriformes en el receptor de un trasplante de órgano sólido. Rev Iberoam Micol. 2016;33(3):152–9. doi:10.1016/j.riam.2016.02.005.

6. Ventura Aguiar P, Lopes V, Martins I S, Santos J, Almeida M. Cryptococcal infection in non-HIV immunosuppressed patients – Three cases in a nephrology setting. Medical Mycology Case Reports. 2014;3:14–6. doi:10.1111/mmc.12003.

7. Pappas PG, Alexander BD, Andes DR, Hadley S, Kaufman C A. Invasive Fungal Infections among Organ Transplant Recipients: Results of the Transplant-Associated Infection Surveillance Network (TRANSNET). CID 2010;50(8):1101–11. doi:10.1086/651262.

8. Maziarz EK, Perfect JR. Cryptococcosis. Infect Dis Clin North Am. 2016;30(1):179–206. doi:10.1016/j.idc.2015.10.006.

9. Yang Y, Chen M, Gu J, Zhu F, Xu X, Zhang C et al. Cryptococcosis in kidney transplant recipients in a Chinese university hospital and a review of published cases. International Journal of Infectious Diseases. 2014;26:154–61. doi:10.1016/j.ijid.2014.05.028.

10. Husain S, Wagener MM, Singh N. Cryptococcus neoformans infection in organ transplant recipients: variables influencing clinical characteristics and outcome. Emerg Infect Dis. 2001;7(3):375–81. doi:10.3201/eid0703.010302.

11. Marques S, Carmo R, Ferreira I, Bustorff M, Sampaio S, Pestana M. Cryptococcosis in Renal Transplant Recipients: A Single-Center Experience Transplantation Proceedings. 2016;48(7):2289-93. doi:10.1016/j.transproceed.2016.06.006.

12. Cao W, Jian C, Zhang H, Xu S. Comparison of Clinical Features and Prognostic Factors of Cryptococcal Meningitis Caused by Cryptococcus neoformans in Patients With and Without Pulmonary Nodules. Mycopathologia. 2019;184:73–80. doi:10.1007/s11046-018-0263-8.

13. Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ et al. Clinical Practice Guidelines for the Management of Cryptococcal Disease: 2010 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2010;50(3):291–322. doi:10.1086/649858.

14. Wald-Dickler N, Blodgett E. Cryptococcal disease in the solid organ transplant setting: review of clinical aspects with a discussion of asymptomatic cryptococcal antigenemia. Curr Opin Organ Transplant. 2017;22(4):307–13. doi:10.1097/MOT.0000000000000426.

15. Camargo JF, Simkins J, Schain DC, Gonzalez AA, Alcaide ML; Anjan S et al. A cluster of donor-derived Cryptococcus neoformans infection affecting lung, liver, and kidney transplant recipients: Case report and review of literature. Transpl Infect Dis. 2018;20:e12836. doi:10.1111/tid.12836.

16. Marinelli T, Anagnostou N, Daniel S, Wigg AJ, Teh J. Very early-onset of Cryptococcus neoformans disease following liver transplantation: Report of two cases and a review of the literature. Transpl Infect Dis. 2020;22:e13227. https://doi.org/10.1111/tid.13227.

17. Iyer SP, Movva K, Wiebel M, Chandrasekar P, Alangaden G, Caron M et al. Cryptococcal meningitis presenting as sinusitis in a renal transplant recipient. Transpl Infect Dis. 2013:15: e187–e190. doi:10.1111/tid.12128.

18. Fortún J, Ruiz I, Martín-Dávila P, Cuenca-Estrella M. Fungal infection in solid organ recipients. Enferm Infecce Microbiol Clin. 2012;30(Supl 2):49–56. doi:10.1016/S0213-005X(12)70082-4.

19. Singh N, Alexander BD, Lortholary O, Drometer F, Gupta KL, John GT et al. Cryptococcus neoformans in organ transplant recipients: Impact of calcineurin-inhibitor agents on mortality. J Infect Dis. 2007;195(5):756–64. doi:10.1086/511438.

20. Mitsui A, Kimura M, Araoka H, Kageyama K, Takagi S, Yamamoto G. Cryptococcal meningitis following umbilical cord blood transplantation, association between the occurrence of cryptococcal infection and tacrolimus discontinuation among allogeneic hematopoietic stem cell recipients. J Infect Chemother. 2019;25(4):289-92. doi:10.1016/j.jiec.2018.09.006.

21. Perfect JR, Bicanic T. Cryptococcosis diagnosis and treatment: What do we know now. Fungal Genetics and Biology. 2015;78:49–54. doi:10.1016/j.fgb.2014.10.003.

22. Pontello N, Gleicheranet E, Facundo M, Sinay V. Cryptococcosis meningea in immunosuprimidos: rol del síndrome inflamatorio de reconstitución inmune. Neurologia Argentina. 2012; 4(1):31-4. doi:10.1016/j.neuarg.2011.07.002.

23. Brizard KE, Baddley JW, Pappas PG. Predictors of Mortality and Differences in Clinical Features among Patients with Cryptococcosis According to Immune Status. PLoS ONE. 2013; 8:e60431. doi:10.1371/journal.pone.0060431.

24. Yoon HA, Felsen U, Wang T, Pirofski LA. Cryptococcus neoformans infection in Human Immunodeficiency Virus (HIV)-infected and HIV-uninfected patients at an inner-city tertiary care hospital in the Bronx. Med Mycology, 2020;58(4):434–43. doi:10.1093/mmy/myza082.

25. Kennedy E, Vanichanan J, Rajapreyar I, Gonzalez B, Nathan S, Gregorie I et al. A pseudo-outbreak of disseminated cryptococcal disease after orthotopic heart transplantation. Mycoses. 2016;59:75–9. doi:10.1111/myc.12433.

26. Leber AL, Everhart K, Balada-Llasat JM, Cullison J, Daly J et al. Multi-center Evaluation of BioFire FilmArray Meningitis/Encephalitis Panel for Detection of Bacteria, Viruses, and Yeast in Cerebrospinal Fluid Specimens. J Clin Microbiol 2016;54(9):2251-61. doi: 10.1128/JCM.00730-16.

27. Chew KL, Lee CK, Cross GB, Lum LH, Yan B, Jureen R. Culture-confirmed cryptococcal meningitis not detected by Cryptococcus PCR on the Biofire meningitis/encephalitis panel®. Clinical Microbiology Infection. 2018;24(7):791-792. doi:10.1016/j.cmi.2018.02.024

Rev Esp Quimioter 2021;34(2): 158-161