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Bronchoalveolar Lavage Cytokine Profile in a Renal Transplant Recipient with *C. Deuterogattii* Infection

da Silva PR[1], Mora DJ[*2], Andrade Silva LE[2], Ferreira-Paim K[2], Silva-Vergara ML[2], Silva-Teixeira DN[1]

[1]Internal Medicine Department, Discipline of Clinical Laboratory, Triângulo Mineiro Federal University, Uberaba, Minas Gerais, Brazil

[2]Infectious Diseases Unit, Internal Medicine Department, Triângulo Mineiro Federal University, Uberaba, Minas Gerais, Brazil

[*]**Corresponding author:** Mora DJ, Infectious Diseases Unit, Internal Medicine Department, Triângulo Mineiro Federal University, Uberaba, Minas Gerais, Brazil, Tel: +55 (73) 99161-9377, E-mail: delioj@gmail.com

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Abstract

Cryptococcosis caused by *Cryptococcus gattii* differs from that due to *C. neoformans* by an increased incidence of cryptococcomas in lung and brain, higher neurological morbidity and slower response to antifungal therapy. A case report of a renal transplant recipient with disseminated and fatal cryptoccocosis is presented. For this patient and for 12 controls, bronchoalveolar lavage fluid levels of IL-10, TNF-α, IL-12p40, CXCL-8 and CXCL-10 were measured. Broth microdilution assay, in accordance with CLSI-M27-A3 document was performed to determine antifungal susceptibility. BAL yielded yeasts when cultured that were identified as *C. deuterogattii* through biochemical reactions and *URA5*-RFLP genotype. The strain presented susceptibility to amphotericin B, itraconazole, voriconazole, fluconazole, and ketoconazole. The patient presented significant higher levels of IL-10 (73.22 pg/mL), IFN-γ (257.08 pg/mL), CXCL-8 (161.93 pg/ml) and CXCL-10 (1000.76 pg/mL) than controls. Different from *C neoformans*, scarce data are available about the immune response in patients with *C. deuterogattii* infection, especially cytokine profile on BAL. According to our findings, proinflammatory cytokines are important soluble mediators associated to pulmonary immune response to this *Cryptococcus* species in humans.

Keywords: *C. Deuterogattii*; VGII Genotype; Cytokine; Bronchoalveolar Lavage; Renal Transplant

Introduction

The *Cryptococcus* species complex is a group of related fungal pathogens that cause disease in both healthy and immunocompromised patients. The nomenclature of *C. neoformans/C. gattii* species complexes is continuing to evolve under molecular evidences [1]. In 2014, new taxonomy has been proposed: *C. neoformans* has been divided into *C. neoformans* (serotype A, formerly *C. neoformans* var. *grubii*) and *C. deneoformans* (serotype D, *C. neoformans* var. *neoformans*); *C. gattii* would be recognized as five separate species, namely, *C. gattii* (VGI), *C. deuterogattii* (VGII), *C. bacillisporus* (VGIII), *C. tetragattii* (VGIV), and *C. decagattii* (VGIV) [2].

C. neoformans and *C. deneoformans* usually infects individuals who present an impaired cell immune response, such as HIV infected patients, and those receiving immunosuppressive therapy before organ transplantation [3,4]; whereas the five *C. gattii* species can cause disease both in immunocompetent and immunocompromised individuals [5]. Individuals infected with *C. gattii* can present skin lesions, increased incidence of cryptococomas on lungs and brain, neurological disability and slower response to antifungals therapy [1,5].

In solid organ transplantation recipients, 5% to 59% of recipients may develop invasive fungal infections mainly caused by *Candida* and *Aspergillus* species while *Cryptococcus* spp. are the third agent more isolated [6]. In renal transplant recipients, incidence of cryptococcosis ranges from 0.3 to 5.8%, and overall mortality rates as high as 20–50% [4]. Additional data suggests that some differences in the incidence and clinical features of cryptococcosis may exists, depending on the specific transplanted organ [4,6].

Immune response against cryptococcal infection depends on coordinated interactions between antigen-presenting cells and effector T cells to generate a robust Th1 immune response [3]. As a result, protection may depends on high levels of proinflammatory cytokines such as IFN-γ, IL-17A, IL-6, IL-8, TNF-α and IL-12 production. On the other side, IL-4, IL-10 act as downregulators of the cellular immune response, inhibiting anti-cryptococcal functions leading to uncontrolled fungal infection and a poor outcome [3,7]. Thus, Th1-associated cytokines are essential for natural immunity whereas Th2 cytokines reduce the host's ability to deal with *C. neoformans*, which seems to occur in transplanted patients [6].

Based on this data, the aim of the present report is to describe the antifungal susceptibility, genotype and bronchoalveolar cytokine profile of a transplanted renal patient who developed severe and disseminated cryptococcosis due to a *C. deuterogattii* infection.

Case Report

A 35-year-old Brazilian woman who received a kidney transplant from her mother, five years after the diagnosis of chronic renal failure. Four years after the transplant, she was admitted at the emergence of Teaching Hospital of Triângulo Mineiro Federal University in Uberaba, Brazil complaining about weakness, malaise, asthenia, abdominal and precordial pain. Since the transplant, she received a daily oral dose of prednisone (10 mg). The chest X-ray showed a right perihilar consolidation which was treated with endovenous ceftriaxone (1 g twice a day), and she had a negative HIV test. Due to the chest X-ray image showing intense bilateral base opacity, cefepime and ampicilin/subactam were also prescribed. After two days, patients' clinical status worsened, presenting tachycardia hypotension, dyspnea and later she evolved to hemodynamic instability and respiratory failure. After the clinical worsening, liposomal amphotericin B was administrated, but despite these procedures, the patient presented cardiorespiratory arrest and progress to an irreversible shock.

Postmortem examination revealed fungal dissemination in lungs, lymph nodes, brain, heart, stomach, liver, salivary glands, esophagus, intestines, bone marrow, pancreas, thyroid, kidney, spleen and supra renal gland. Mucicarmim stain detected multiple encapsulated yeasts suggestive of *Cryptococcus* sp on several tissues and organs such brain and lungs, Figure 1. *C. deuterogattii* was identified by L-Canavanine-glycine-bromothymol blue (CGB) agar and *URA*5-RFLP analysis, Figure 2. This strain was susceptible to amphotericin

B (Bristol-Myers Squibb, USA), itraconazole (Janssen Pharmaceuticals, Belgium), ketoconazole (Janssen Pharmaceuticals, Belgium), Voriconazole (Pfizer, USA) and fluconazole (Pfizer, USA) [8,9]. The BAL of the case reported presented significant higher levels of IL-10, IFN-γ, CXCL-8 and CXCL-10 than control subjects ones ($p < 0.05$, Mann-Whitney U test) and ten-fold increased BAL of CXCL-10 levels than controls ($p < 0.001$, Mann-Whitney U test), Table 1.

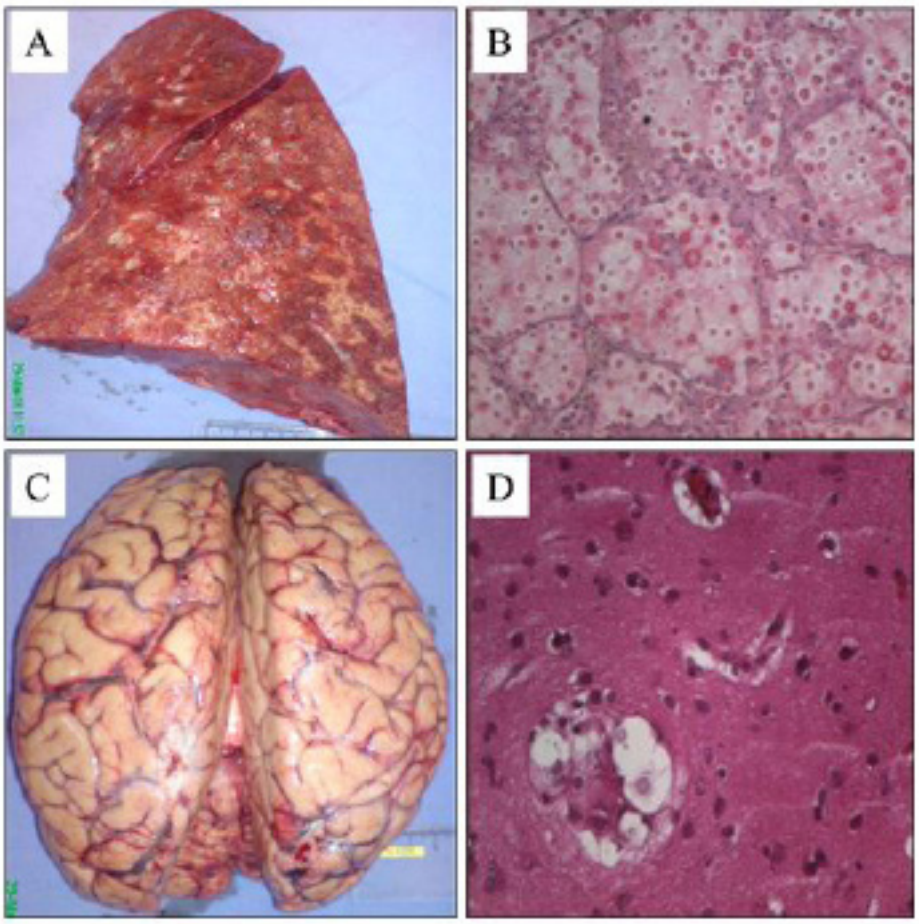

Figure 1: Macroscopic (A, C) and microscopic (B, D) aspects of patient's lung and brain infected by *C. deuterogattii*, respectively (mucicarmin stain 400x)

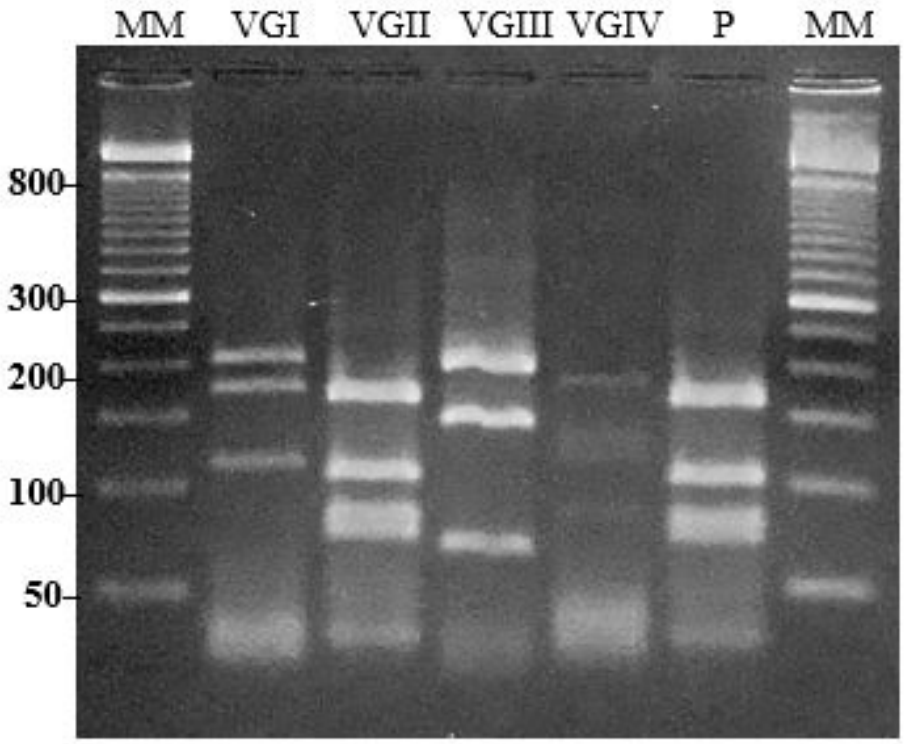

Figure 2: Agarose gel electrophoresis of *URA*5-RFLP after double digestion with *Sau 96*I and *Hha*I from *C. deuterogatti* clinical isolate. Left to right: MM: 50bp DNA ladder (Bionner, USA), VGI (WM 179), VGII (WM 178), VGIII (WM 161) and VGIV (WM 779), P (*C. deuterogatti* clinical isolate)

	Case report [mean]	Controls [mean, range]	P value
IL-10	73,22	14,94 ± 13,42	< **0.05**
IL-4	40,26	26,63 ± 18,85	0.25
TNF-α	32,86	66,87 ± 123,6	0.23
IL-12p40	98,19	53,12 ± 39,69	0.16
IFN-γ	257,08	63,77 ± 22,81	< **0.05**
CXCL-8	161,93	21,15 ± 40,03	< **0.001**
CXCL-10	1000,76	102,55 ± 214,52	< **0.001**

Table 1: Cytokine levels [pg/ml] on BAL from case report and controls (n = 12). The values are expressed in mean ± standard deviation

Discussion

Increasing prevalence of mortality attributed to cryptococcosis in the immunocompromised patients underscores the importance of elucidating the host defense–pathogen interaction. Fungal infections in solid organs transplant recipients are most frequent caused by *Candida* spp. and *Aspergillus* spp. [10]. However, cryptococcal infections can occur in these patients being the central nervous system the most common site, while pulmonary involvement by *C. gattii* has been rarely reported [11]. Patients with pulmonary cryptococcosis frequently present unilateral, nodular or cavity infiltrates at chest X-ray [12]. The case herein described presented bilateral perihilar opacity and was empirically treated as bacterial infection without clinical response, which lead to the fungal etiologic suspicion considering his immunosuppressive condition associated to chronic corticosteroids therapy. The poor outcome was probably due to late diagnosis, lack of 5-flucytosine, as well as the virulence properties of VGII genotype in solid organ transplantation [6].

The interaction between *Cryptococcus* spp. and the host's immune system is a major determinant for the outcome of the disease. Previous studies suggest that the Th1cellular immune response is effective to protect against a cryptococcal infection and it is mediated by high levels of IFN-γ, IL-10 and IL-12p40 production associated to reduced levels of and TNF-α [3,7]. Levels of IL-10, IL-4, CXCL-8, IL-12p40, IFN-γ and CXCL-10, but not TNF-α were markedly elevated on patient's BAL. This finding is in line with previous description relating the low TNF-α level with chronic infections in immunocompetent models [13].

The present case exhibited high levels of IL-12p40 and IFN-γ compared to controls. This is an important fact due to direct relation of levels of Th1 cytokines and fungal clearance [3]. Moreover, IL-12p40 and specially IFN-γ appear to play a central role at inducing chemokines like CXCL-10, also known as IFN-γ–induced protein 10, which has an important role in recruiting activated T cells into sites of tissue inflammation [14]. The CXCL-8 production observed may cause neutrophil recruitment migration to the lungs [14]. A common feature of these protective cytokines and chemokines is that they either induce the Th1 immune response, enhance the activity of other Th1-inducing cytokines, and/or suppressing Th2 immune responses.

High levels of IL-10 and IFN-γ were observed in macrophages/lymphocytes isolated from BAL of a patient with pulmonary cryptococcosis, suggesting that these molecules must be directly involved in local immune response during pulmonary infection [15]. In this context, high levels of the potent anti-inflammatory cytokine IL-10 founded on patient's BAL may have contributed to the disseminated cryptococcal infection evidenced at postmortem examination [16].

Although *C. neoformans* is more frequently associated with cryptococcal infection in kidney of transplant receptors, the actual geographical expansion of *C. deuterogattii* will increase the frequency of infections by this specie.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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