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CASE REPORT

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Fatal *Cryptococcus gattii* genotype VGI infection in an HIV-positive patient in Barranquilla, Colombia

María Clara Noguera[1], Patricia Escandón[2], Elizabeth Castañeda[2]

ABSTRACT

Cryptococcosis is a major invasive fungal disease related worldwide with the AIDS population. New reports of HIV/AIDS cases to the national public health surveillance system (SIVIGILA) in Colombia have shown that there is a growing community at risk of contracting cryptococcosis throughout the country who do not have access to ART. Even though the most prevalent species *Cryptococcus neoformans* is mainly associated with the HIV population, we report a fatal case of cryptococcosis in an AIDS patient in Barranquilla, associated with *Cryptococcus gattii* VGI, isolated from blood culture.

KEYWORDS: Cryptococcus gattii VGI. Cryptococcosis. Colombia. Blood culture.

INTRODUCTION

Cryptococcosis is a life threatening mycosis that affects humans among other vertebrates. Approximately one million cases are annually reported globally, particularly among HIV/AIDS patients in sub-Saharan Africa where the greatest burden of this syndrome is identified (estimated mortality ranges between 50% and 70%, ranking fourth above tuberculosis)[1]. Since a significant number of cases of this mycosis has been reported in the HIV population, it is considered an AIDS-defining condition[2].

The main etiological agents of cryptococcosis are two basidiomycetous yeasts, *Cryptococcus neoformans* species complex and *Cryptococcus gattii* species complex. *Cryptococcus neoformans* can be further classified in two varieties, *Cryptococcus neoformans* var. *grubii* and *Cryptococcus neoformans* var. *neoformans*; both species complex can be differentiated into four serotypes (A and D, or B and C, respectively), several hybrids (AD, AB, and BD), and eight molecular types (molecular types VNI-VNIV, and VGI-VGIV)[3,4]. More recently, a new taxonomy for *C. neoformans* and *C. gattii* comprising seven species has been recently proposed[5].

Different environments in rural and urban settings are the natural habitat for these species and the infection starts after inhaling infectious propagules, desiccated blastoconidia or basidiospores (sexual stage). Studies have suggested that eight molecular types are distributed within South America and at least seven circulate in Colombia[6,7].

Cryptococcosis does not require compulsory notification to public health in Colombia but different reports confirm its importance[8-10]. A national survey designed according to the guidelines established by the European Confederation of Medical Mycology and approved by the Ethics Committee of the Instituto Nacional de Salud (INS) has been carried out voluntarily at medical institutions (public and

(1) Universidad Metropolitana, Barranquilla, Colombia

(2) Instituto Nacional de Salud, Bogotá, Colombia

Correspondence to: María Clara Noguera
Universidad Metropolitana, Programa de Bacteriología, Calle 76, Nº 42-78, 080020, Barranquilla, Colombia
Tel: +57 5 3697012

E-mail: maclarnog@gmail.com

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private) throughout the country since 1997 and health professionals complete the forms. Risk factor information or whenever cryptococcosis has been an AIDS defining illness are inquired in addition to the diagnosis date, clinical findings, type of treatment, outcome, type of diagnostic assays employed (clinical laboratory/image) and treatment.

According to the Colombian Cryptococcosis Study Group, the mean annual incidence rates of cryptococcosis 2006-2010 in the general population was 2.4×10^6 individuals; in AIDS patients the rate was 3.3×10^3 individuals. *C. neoformans* var. *grubii* was prevalent (96.7%) in comparison with *C. gattii* (2.2%)[9,10].

This is the first report of cryptococcosis caused by *Cryptococcus gattii,* VGI, in an HIV/AIDS male patient in Barranquilla, Colombia, with pulmonary manifestations without antiretroviral treatment (ART).

CASE REPORT

A 53-year-old male resident in Barranquilla, Colombia, was referred to a tertiary care hospital facility by his government affiliated medical service in February of 2012 and admitted to the emergency service with poor clinical conditions, a notorious decrease of muscular mass and generalized motor deficit, and reporting three days of intense coughing, copious expectoration, fever, pain in right hemithorax, and dyspnea. Additionally, he reported general weakness, weight loss (20 Kg) during the last three months and several hospital admissions due to dyspnea and pneumonia in the past year. As relevant background, the patient had been previously diagnosed with HIV/AIDS, confirmed through a Western-Blot analytical technique one month prior to this episode. At admission to hospital, he reported to be unemployed and he was not receiving ART.

During the physical examination, the patient appeared very anxious, presented isochoric and photoreactive pupils showing no meningeal signs, left cervical lymphadenopathies and generalized skin blemishes. Preliminary diagnosis included HIV/AIDS, community-acquired pneumonia, pulmonary tuberculosis, protein energy-malnutrition and oral candidiasis.

During hospitalization, clinical tests performed on day 2 confirmed an anemic condition and the metabolic panel testing did not show significant results. The patient underwent several immunologic tests for Hepatitis B and C with negative results; tuberculosis was also ruled out; the cytomegalovirus anti-IgM results were positive, as well as the total CORE antibody. CD4 count and viral load results were not available.

Regarding microbiological studies, two blood cultures were performed on day 1 and at day 3. Both blood cultures (Bactec 9000 PLUS Aerobic/f; Becton Dickinson) were performed in duplicate, following the recommendations for clinical microbiology reported by the Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC)[11]. From the first blood culture, one of two samples tested positive; from the second blood culture, both samples tested positive; a direct exam with India ink showed the encapsulated blastoconidia cells with micromorphological features suggestive of *Cryptococcus* spp. Isolation was possible after culturing the supernatant in 15 ml Columbia Agar (Becton Dickinson) with 5% sheep blood incubated at 37 °C, from which pure creamy yeast like colonies were observed 48 hours later. The urease test was carried out individually in tubes with 2.5 ml Christensen Media from the yeast like colonies with positive results. The clinical laboratory reported *Cryptococcus neoformans* on day 9.

Antifungal therapy with Amphotericin B (40 mg/day) plus Fluconazole (400 mg/day) was initiated after cryptococcosis was diagnosed; however, the patient's hemodynamical condition deteriorated progressively and he died nine days after the onset of treatment and 20 days after hospital admission.

The isolates were forwarded to the departmental public health laboratory in Barranquilla with the correspondent survey, and later to the Instituto Nacional de Salud (INS) in *Bogotá*, D.C. for confirmation (genus and species) and molecular typing. Phenotypic testing included conventional laboratory techniques, such as India ink method, melanin production in *Guizotia abyssinica* media, urease production and use of nitrates. Differentiation between *Cryptococcus neoformans* species complex and *Cryptococcus gattii* species complex was performed using Canavanine-Glycine-Bromothymol Blue agar (CGB) and the isolate was identified as *C. gattii*.

High-molecular-weight DNA was extracted from a pure culture according to modifications proposed by Ferrer *et al*.[12]. RFLP of the URA5 gene grouped the isolate into the molecular type VGI[13].

DISCUSSION

Cryptococcosis associated to *C. neoformans species complex* and *C. gattii* species complex is considered the second most common cause of life threatening mycoses globally, occurring in humans and animals. Since the emergence of AIDS, this fungal disease is more frequently diagnosed among HIV-positive individuals associated with the worldwide distributed species of *C. neoformans* in 80% of the cases and in less proportion with *C. gattii* in 20%[3]. From 1981 to 1990, the reports of cryptococcosis due to the AIDS epidemic reached a 1,500% increase[2,14].

The spectrum of this pathology ranges from self-limiting manifestations to systemic disease, with a wide variety of clinical signs and symptoms. In this case, the patient's signs and symptoms during his admission and physical evaluation were consistent with clinical presentation and characteristics described for this mycosis; lung disease was predominantly found (mainly cough, fever, malaise and dyspnea) over central nervous system infection, which is consistent with findings by different authors[3,15]. This is the first documented case in Barranquilla, Colombia, addressing the clinical and genotypic features of *C. gattii* molecular type VGI, as the etiological agent of cryptococcosis identified from the bloodstream of an HIV/AIDS male patient.

Despite its lower prevalence, *C. gattii* is considered an emergent pathogen with a changing epidemiological behavior not fully understood yet, partly because of its low search in clinical laboratories and partly because it was unusually diagnosed in the American continent before 1999. Taxonomically, *C. gattii* was considered as a variety within the *Cryptococcus* species complex and more recently, it came to be accepted as the sibling of *C. neoformans,* a species that has been the subject of increased attention and epidemiological interest since the emergence of AIDS[15]. From the late 1990s, *C. gattii* won importance for causing the outbreaks in the pacific west coast of North America, starting in the province of British Columbia, Canada, and later expanding South to nearby regions of the United States. Furthermore, new cases have been also identified in the Eastern coast of North America[15]. In South America there have been more cases reported by this pathogen as well as isolates from natural sources[7-10,14]. A study regarding the prevalence of cryptococcosis in *Atlántico,* Colombia, showed that among the clinical isolates, *C. gattii* was responsible in 2.4% of them, while *C. neoformans* was in 90.4%; in the environment, only *C. neoformans* var. *grubii* was isolated in nine of the 2,068 total samples collected[16].

C. gattii is found less frequently worldwide compared with its sibling, and the principal risk factors involved in this circumstance remain unclear, given the fact that this species mainly affects apparently healthy hosts. It is also known that the general distribution of VGI and VGII types from clinical and veterinary sources can overcome VGIII and VGIV, with VGII being more prevalent than VGI (47% over 34% among 980 global isolates)[15]. Even though there is evidence that associates cryptococcosis by *C. gattii*, molecular types VGI and VGII with cerebrospinal involvement in immunocompetent hosts, this clinical case shows that much more needs to be understood about the association between host immunological status and molecular types[15].

In terms of major virulence determinants, both species show similarities but considering the disease outcome in *C. gattii* infections, and taking into account that certain molecular types have proven to be more virulent than others, virulence seems to be the result of complex interactions, many of which are yet unknown[15].

Even though the incidence of cryptococcosis in developed countries is relatively steady since the advent of ART, its prevalence is increasing in the developing world. After sub-Saharan Africa and followed by Southern Asia, Latin America stands in the third place with 54,400 yearly cryptococcal meningitis cases and 29,900 estimated deaths[16]. Isolation of clinical as well as environmental pathogenic species of *Cryptococcus* is not uncommon in Colombia[8-10,16]. In the national report (1997-2014), 1,535 of the isolates from patients with cryptococcosis (79% with AIDS) correspond to *C. neoformans* and 52 to *C. gattii*[17].

Although more cases of cryptococcosis in immunocompromised patients are associated with *C. neoformans* molecular type VNI, it is necessary to continue monitoring this mycosis in Colombia since the SIVIGILA data show there has been an upward trend in the annual reports of HIV/AIDS in Colombia since 2007. Cases reported in 2012 show that Barranquilla (capital city of the department of *Atlántico*) presented an incidence of 33.7 x 10^5 inhabitants, more than twice compared with the national indicator (16.4 x 10^5 inhabitants); furthermore, the incidence reported in the department of *Atlántico* (19.4 x 10^5 inhabitants) was also higher[18]. It is possible that many cases of cryptococcosis in Barranquilla and in *Atlántico* have not been reported suggesting a number of patients at risk. Additionally, it should not be ignored that the World Health Organization (WHO) declared in December 2010 that only 34% of the HIV patients in Colombia were receiving ART, indicating that untreated seropositive population is at higher risk of developing coinfections such as cryptococcosis[19]. These numbers are consistent with those found in a study held in Colombia in a tertiary care hospital, between 1996 and 2010 where only 23.8% of the patients with AIDS were receiving ART[8]. Even though the patient in this case was diagnosed with AIDS prior to his hospital admission, he denied any treatment with ART, which could influence his outcome.

Laboratory diagnosis of cryptococcosis is simple and usually involves microbiological/serological procedures, which have been in use for decades and are still currently valid. Commonly performed tests include direct visualization of the encapsulated blastoconidia from body fluids, such as CSF and sputum using India ink, but also from blood culture supernatant as detailed in this case. Subculturing in Sabouraud Dextrose agar media allows the isolation of the fungus after incubation at 37 °C/26 °C during 24-72 hours,

after which yeast like creamy colored colonies develop. Positive blood cultures have been reported in 47-71% of HIV-infected patients in comparison with 27% of uninfected patients[2]. Sequentially, in addition to common tests within species of this genus (India ink, urease and melanin production, and use of nitrates), CGB phenotypic test allows differentiation between both pathogenic species. Unfortunately, after the development of creamy yeast like colonies at 37 °C and morphologic identification of the encapsulated yeast cells through India ink direct exam, specific CGB test was not available in this hospital facility, only the urease test, which limited the accurate identification of *C. gattii.* Isolates were phenotypically and genotypically confirmed as *C. gattii,* genotype VGI when the isolates were forwarded to the national reference center.

Moreover, beyond the evidence presented regarding this clinical case of cryptococcosis due to *C. gattii* molecular type VGI in an immunosuppressed patient, the first case reported in this part of the country that was included in the results of the study previously developed in *Atlántico*, Colombia, we emphasize the importance of timely diagnosis and follow-up of HIV patients without ART, taking into account the high risk of infection and further development of fatal diseases such as cryptococcosis.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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