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Disseminated and cutaneous cryptococcosis by *C. neoformans* VNI in an immunocompetent patient

Criptococose disseminada e cutânea por C. neoformans *VNI em paciente imunocompetente*

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ABSTRACT

Cryptococcosis is caused by yeasts of the *Cryptococcus neoformans/C. gattii* complex, presenting cutaneous, respiratory and disseminated forms. A 44-year-old immunocompetent male with facial lesion and latent pneumonia was hospitalized and misdiagnosed with paracoccidioidomycosis. Computerized tomography scans showed pulmonary and neurological involvement, and cultures/China ink, cryptococcal antigen test and restriction fragment length polymorphism of urease gene (*URA5*-RFLP) confirmed *C. neoformans* genotype VNI. Hemoculture indicated ampicillin-resistant *Klebsiella pneumoniae* (healthcare-associated infection). Fluconazole was administered, but after resistance detection, amphotericin B was chosen (cumulative dose/1500 mg). The patient was discharged with clinical remission (75 days) and amphotericin for one year (maintenance phase).

Key words: cryptococcosis; cryptococcal meningitis; *Cryptococcus neoformans*; amphotericin B; *Klebsiella pneumoniae*.

RESUMO

A criptococose é causada por leveduras do complexo Cryptococcus neoformans/C. gattii *e se apresenta nas formas cutânea, respiratória e disseminada. Um homem imunocompetente de 44 anos com lesão facial e pneumonia latente foi hospitalizado e erroneamente diagnosticado com paracoccidioidomicose. Tomografias computadorizadas mostraram envolvimento pulmonar e neurológico, e culturas/tinta da China, teste do antígeno criptocócico e técnica de polimorfismo de comprimento de fragmentos de restrição do gene urease (*URA5*-RFLP) confirmaram* C. neoformans *genótipo VNI. Hemocultura indicou* Klebsiella pneumoniae *resistente à ampicilina (infecção relacionada com a assistência à saúde). Fluconazol foi administrado, mas após detecção de resistência, optou-se por anfotericina B (dose cumulativa/1500 mg). O paciente recebeu alta com remissão clínica (75 dias) e administração de anfotericina B por um ano (fase de manutenção).*

Unitermos: *criptococose; meningite criptocócica;* Cryptococcus neoformans*; anfotericina B;* Klebsiella pneumoniae.

RESUMEN

La criptococosis es causada por levaduras del complejo Cryptococcus neoformans/C. gattii *y se presenta en las formas cutánea, respiratoria y diseminada. Un hombre inmunocompetente de 44 años de edad con lesión facial y neumonía latente fue hospitalizado y erróneamente diagnosticado con paracoccidioidomicosis. Tomografías computarizadas mostraron afectación pulmonar y neurológica, y culturas/tinta china, prueba del antígeno criptocócico y* URA5*-polimorfismos en la longitud de los fragmentos de restricción (RFLP) confirmaron* C. neoformans *genotipo VNI. El hemocultivo indicó* Klebsiella pneumoniae *resistente a la ampicilina (infección asociada a la atención en salud). El fluconazol le fue administrado, pero tras detección de resistencia, se optó por anfotericina B (dosis acumulativa/1500 mg). Al paciente le dieron el alta en remisión clínica (75 días) y administración de anfotericina B durante un año (fase de mantenimiento).*

Palabras clave: *criptococosis; meningitis criptocócica;* Cryptococcus neoformans*; anfotericina B;* Klebsiella pneumoniae.

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INTRODUCTION

Cryptococcosis is an opportunistic infection caused by fungi of the *Cryptococcus neoformans/C. gattii* complex, with potential for systemic dissemination[1]. The main risk factor in Latin America is the human immunodeficiency virus (HIV) infection, frequently associated with cryptococcal meningitis in Brazil[2]. Cutaneous lesions are generally seen as secondary focus and an indicator of sepsis, but primary cutaneous cryptococcosis (PCC) can develop after a traumatic inoculation[3].

The etiological agents are distributed worldwide: *C. neoformans* is usually associated with pigeon excreta and related to opportunistic infections, while *C. gattii* causes infections mainly in immunocompetent hosts, and is found on decaying organic matter[1, 2]. Yeasts inhaled from the environment can install themselves in the lung and increase their polysaccharide capsule to inhibit phagocytosis and opsonization, causing symptoms ranging from fever and cough to severe conditions (meningitis)[1, 3].

The *Cryptococcus* species complex contains cryptic species classified into eight genotypes through restriction fragment length polymorphism of urease gene (*URA5*-RFLP) (VNI-VNIV for *C. neoformans* and VGI-VGIV for *C. gattii*)[4]. Thus, we report a case of cryptococcal meningitis by *C. neoformans* with pulmonary and simultaneous cutaneous involvement in an immunocompetent patient, with the use of serological and molecular tests for an accurate diagnosis.

CASE REPORT

A 44-year-old male patient from the urban area of Maceió, a mason seen initially by a dermatologist (day 0) was referred to Hospital Escola Dr. Helvio Auto (Maceió, Alagoas, Brazil), with a suspected fungal disease and no definite underlying comorbidities. The patient affirmed to work in a region with many pigeons, where he suffered an injury on the nose with ceramic material. He evolved with a wound that did not heal, a long-lasting cough and fever for one month and 20 days, 10 years ago.

The patient reported new facial lesions two months ago, persistent headache and fever in the evening period. On physical examination, erythemopapular lesions were observed, with nodular and verrucose lesions located on the face and right ear, evolving to the left auricular region, nose and jaw, besides nodules on the scalp (**Figure 1A/B**). Posteroanterior (PA) chest radiograph showed posterior basal peribronchial infiltrate on the right lung (**Figure1 C/D**).

In laboratory tests (day +2), hemogram showed normal leukocytosis and platelets, and renal and hepatic functions were in normal limits, with serology negative for HIV, herpes simplex virus and syphilis, as well as negative bacilloscopy for tuberculosis.

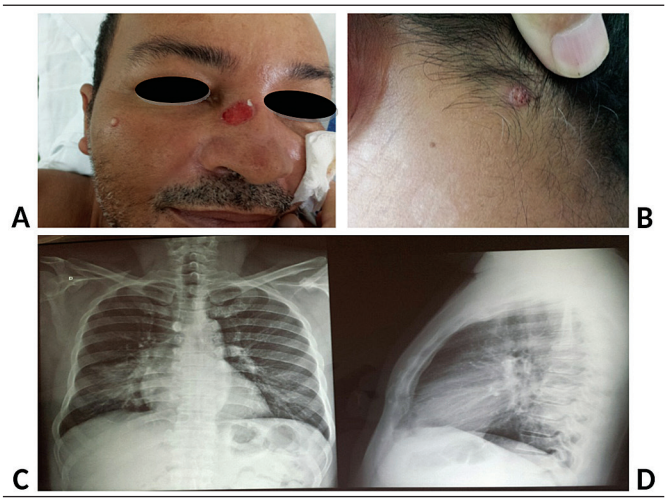

FIGURE 1 – *Clinical and X-ray evaluation of the patient*

A) lesions on the face; B) nodules on the scalp; C and D) chest X-ray posteroanterior indicated right posterior basal peribronchial infiltrate.

After admission for hospital follow-up (day +15), computerized tomography scanning (CT) of the skull detected small areas of undetermined hypodensity (**Figure 2A/B**), while a thorax CT showed nodules and a large consolidation in lung (**Figure 2C/D**).

Anatomopathological examination (biopsy) from face, nose and right ear lesions indicated characteristics of cryptococcosis and yeasts (day +16), but culture performed by an external laboratory in the same day indicated *Paracoccidioides brasiliensis* (paracoccidioidomycosis). Fluconazole treatment was initiated. However, cerebrospinal fluid (CSF) analysis showed slightly turbid, elevated CSF protein of 91 mg/dl and encapsulated yeasts suggestive

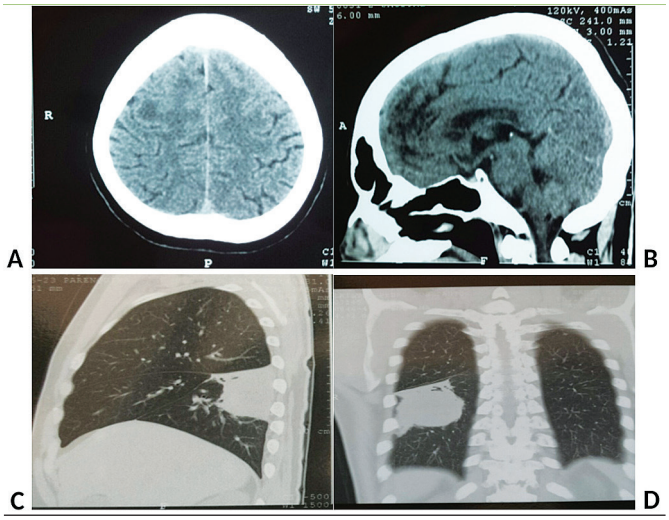

FIGURE 2 – *A and B) CT of the skull detected small areas of undetermined hypodensity, distributed in the subcortical and white matter of both cerebral hemispheres; C and D) CT of thorax showed a lobar parenchymal consolidation of the right lower lobe, with permeable air bronchograms and small non-calcified pulmonary nodules*

CT: computerized tomography scanning.

of *Cryptococcus* sp. through the China ink, as well as fluconazole resistance indicated by the automatized system Vitek®2 (bioMérieux). Treatment was then changed to amphotericin B/50 mg (day +18) on alternated days, with satisfactory clinical response.

Confirmatory CSF analyzes by the Laboratory of Mycology were performed (day +18) for an accurate diagnosis, since two tests indicated different fungi. CSF culture (**Figure 3A**) and China ink test for microscopy were repeated (**Figure 3B**), including growth on canavanina-glycine-bromothymol blue (CGB) agar, phenoloxidase activity in niger seed agar (NSA), urease and serological tests for detection of cryptococcal antigen (CrAg) by immunochromatographic lateral flow assay (CrAg-LFA®, INLAB) (**Figure 3C**). Analysis by *URA5*-RFLP (restriction fragment length polymorphism of urease gene) identified a molecular type VNI (**Figure 3D**).

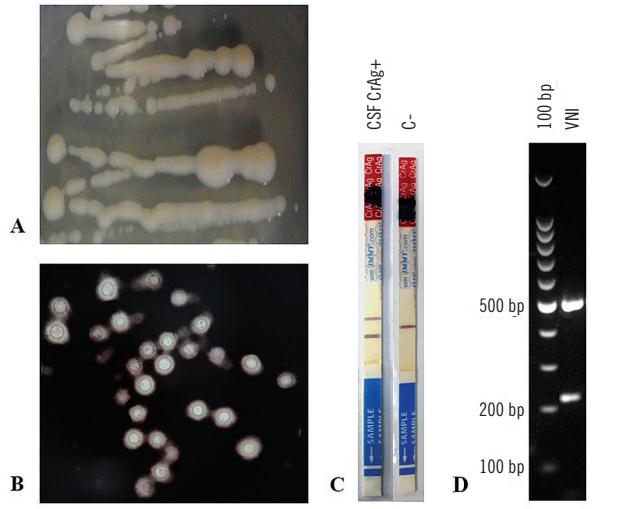

FIGURE 3 – Cryptococcus neoformans *identification*
A) C. neoformans *culture on potato dextrose agar medium (37°C for 48 h); B) China ink preparation with typical encapsulated yeasts; C) CrAg-LFA® positive in CSF from patient and CrAg-LFA® negative (C-); D) electrophoretic profile of* C. neoformans *genotype VNI on 3% agarose gel with 100 bp DNA ladder.*

CrAg-LFA: cryptococcal antigen lateral flow assay; CSF: cerebrospinal fluid; DNA: deoxyribonucleic acid.

Repeated CTs indicated a hypodense subcortical area in the skull, as well as a nodule in the right lower lobe of the right lung of intermediate density and well-defined contours (4.8 × 3.8 cm^2), confirming the presence of persistent lesions and active infection (day +27). Hemoculture was performed in Vitek®2, which detected ampicillin resistant *Klebsiella pneumoniae* (day +28), probably acquired as a healthcare-associated infection (HAI).

After a wrong paracoccidioidomycosis diagnosis and HAI by *K. pneumoniae*, the definitive diagnosis was of disseminated cryptococcosis. The cumulative 1500 mg dose of amphotericin B was completed (consolidation phase) and the patient has made sustained clinical progress, with resolution of symptoms. The patient was discharged after two months (day +75), with amphotericin for one year (maintenance phase). This research was approved by the Research Ethics Committee (no. 19035713.8.0000.5013) and ethical principles were safeguarded through ethical secrecy, with informed consent of patient.

DISCUSSION

In symptomatic patients with cryptococcosis, cough with mucoid expectoration predominates; whereas asymptomatic patients may only have fever, when tissue and immunological reactions limit the infection, which can be reactivated later with hematogenous dissemination reaching the central nervous system due to the fungus tropism[5]. PCC due to direct cutaneous inoculation is rare and usually characterized by the absence of systemic involvement, and in the present case it is not possible to confirm PCC by the wound the patient suffered, since the analysis of the material was not performed[6, 7]. As the patient presented cough and fever, it is more likely that continuous contact with contaminated excreta in the work environment fixed the fungus in his lung (pulmonary consolidation), with reactivation and hematogenous spread after 10 years, causing meningitis.

The genotypes of the complex present ecoepidemiological, physiological and genotypic differences among them, and phylogenetic studies have proposed to divide *C. neoformans* and *C. gattii* into two and three species, respectively[3, 4]. Although *C. gattii* causes cryptococcosis more frequently in healthy individuals and *C. neoformans* affects mostly immunocompromised ones, the latter has been considered emergent in immunocompetent seronegative people[6-8].

Absence of typical symptom comorbidities and risk factors for cryptococcosis (HIV negative or non-transplanted) in the patient delays the correct diagnosis with the suspected bacterial infection, since cellular immunity deficiency and previous diseases can facilitate the cryptococcosis acquisition[7]. The laboratory routine for *Cryptococcus* sp. identification is usually easy through direct examination, culture and histopathologic analysis[5]; however, it relies on technical knowledge, with a possibility of misdiagnosis as in the present case, with indication of *P. brasiliensis*. CrAg-LFA® detects the presence of free capsular antigen in body fluids such as blood, urine and

CSF, being a highly reliable tool and less expensive than other immunochromatographic methods, which provides rapid results regardless of the infrastructure of the laboratory[9]. In Brazil, the VNIV genotype is more associated to PCC, while VGII is the most common in infections of immunocompetent[10] subjects, different from the strain identified, able to spread and manifest different clinical forms during infection after a long latency period.

Other relevant factors should raise suspicion of cryptococcosis in immunocompetent patients, such as work activity close to pigeons and their excreta. We also recommend CrAg-LFA® as a quick, low cost and reliable method for laboratory diagnosis of cryptococcosis, allowing more rapid and efficient clinical interventions and increasing the survival rate of patients affected.

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CONFLICT OF INTEREST

No conflicts of interest declared.

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