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Cryptococcus neoformans in cerebrospinal fluid and blood

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A 56-year-old man was admitted in the emergency room for an episode of aphasia. A preliminary diagnosis of stroke was made, but neurological examination and brain scan did not support this diagnosis and a possible diagnosis of meningitis was made. During the last 3 months the patient lost 15 kg of weight. Laboratory tests revealed: hemoglobin 10.3 g/dl (reference value (RV): 13.0–17.5), leucocytes 8.7×10^9/L (RV: 4.0–11.0), platelets 354×10^9/L (RV: 150–450), creatinine 3.52 mg/dl (RV: 0.7–1.2), lactic acid dehydrogenase 471 U/L (RV: 100–250), C-reactive protein 21.2 mg/dl (RV: <0.5), procalcitonin 2.32 ng/dl (RV: <0.5). The cerebrospinal fluid (CSF) analysis showed: protein 32.4 mg/dl (RV: 15–45), glucose 51 ng/dl (RV: 40–70), <1 leucocytes/mm^3 (RV: <5) and some images of an encapsulated yeast (unicellular and with an abundant capsule), suggestive of *Cryptococcus* spp. (Figure 1). In the peripheral blood film, were observed some isolated black to blue circular inclusions inside of some neutrophils, some of them with a clear white surrounding area (Figure 1). An *inaugural* diagnosis of HIV1 infection was also made. Blood and CSF cultures were collected and 4 days after, the growth of a yeast was obtained and the identification of *Cryptococcus neoformans* was performed by MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization—Time-Of-Flight Mass Spectrometry). The patient started treatment with amphotericin B but deceased the day after admission.

Cryptococcosis have a worldwide distribution and is responsible for wide range of clinical presentations (mainly pulmonary, central nervous system, skin and prostate infections, but bone, peritoneum and urinary system infections are sometimes described).[1,2] Infection start primarily by inhalation of environmental basidiospores or poorly encapsulated yeast cells (with less than 5 μm), that can disseminate, after a latent period within lung lymph nodes.[1–3] There are two principal species of *Cryptococcus* spp related with this disease: *Cryptococcus neoformans* and *Cryptococcus gattii*.[1–3] *Cryptococcus albidus* and *Crytpococcus laurentii* are rarely associated with cryptococcosis in humans.[2]

C. gattii is more commonly found at eucalyptus trees in tropical and subtropical regions, affects more frequently immunocompetent hosts and is less likely disseminated to the central nervous system, causing predominantly pulmonary infection.[1,2]

C. neoformans responsible for 95% of cryptococcal infections is an environmental ubiquitous yeast occurring worldwide, associated with excreta from birds such as pigeons, environmental scavengers (ameba and sowbugs), soil, and hollows of a variety of tree species.[1,2] *C. neoformans* can cause life-threatening infections, especially diffuse meningoencephalitis.[1–4] The most significant predisposing condition is Human Immunodeficiency Virus (HIV) infection, but persons with other immunodeficiency conditions or apparently immunocompetent may also be affected.[1–4] The surface capsule (composed of glucuronoxylomannan and glucuronoxylomannogalactan) is a distinctive cellular phenotype intimately associate with this pathogen that have an antiphagocytic function[1,2,4] and can suppress the proinflammatory nuclear factor kB pathway decreasing levels of proinflammatory cytokines such as tumor necrosis factor.[2] The capsule can increase in size when exposed to body tissues and fluids.[1] Unencapsulated *C. neoformans* cells are rarely observed in clinical samples, what can make the diagnosis by microscopy more difficult. Moreover, specific mutations resulting in capsule defects typically result in a dramatic attenuation of *C. neoformans* virulence.[4] Other virulence factors include melanin pigment production (that protect the yeast from oxidative stress and can explain in part its neurotropism), and thermotolerance.[1,2] Its ability to survive within a phagolysosome allow *C. neoformans* to cross the blood–brain barrier.[1,2]

Cryptococcal meningitis is responsible for a set of neurological symptoms, most frequently headache, altered mental status (lethargy and memory loss), fever, nausea, and vomiting. Patients can also present diplopia and later, reduced acuity, cranial nerve palsies (particularly cranial nerve VI), papilledema and depressed consciousness

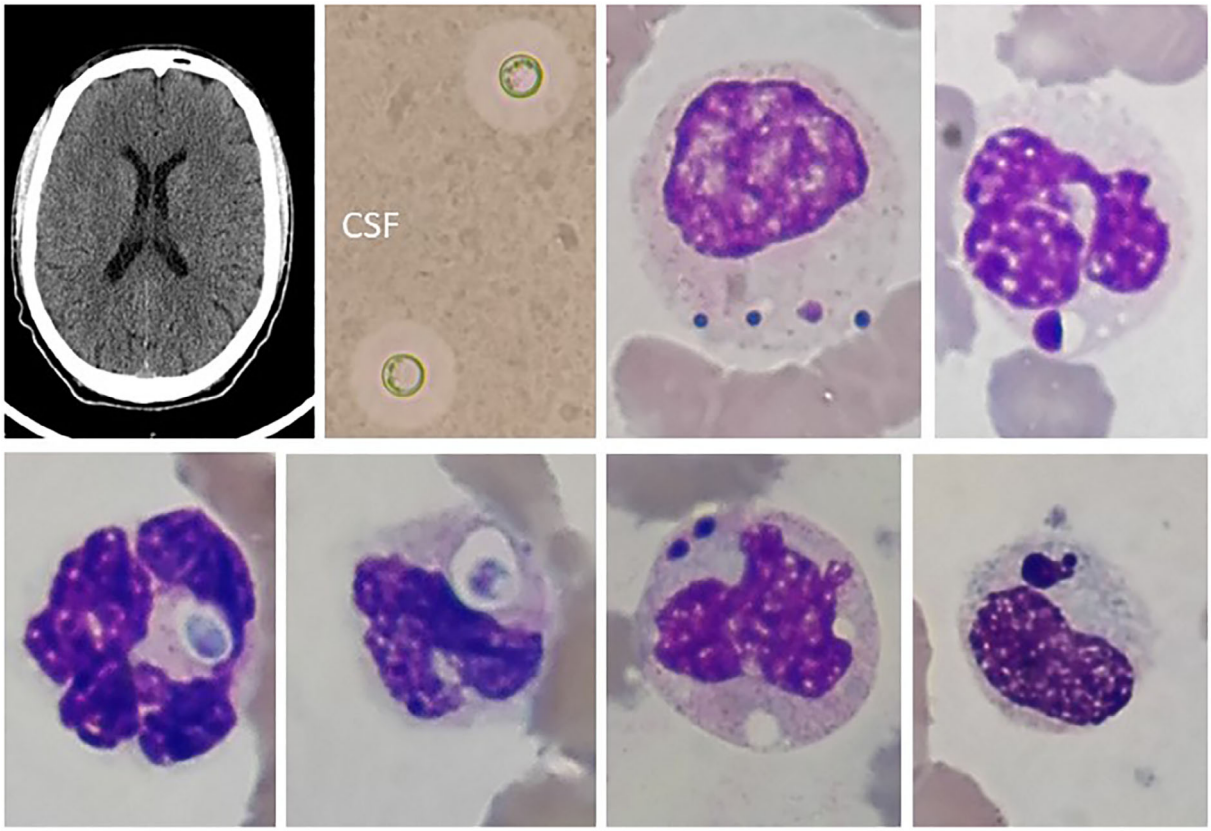

FIGURE 1 Patient's brain computerized tomography scan; *Cryptococcus neoformans* in CSF (India ink, objective ×40) and peripheral blood film (May-Grünwald Giemsa stain, objective ×100)

secondary to high intracranial pressure.[1–3] Cryptococcomas are more common in infections owing to *C. gattii* than *C. neoformans*.[2]

An important aspect related with hematogenous dissemination is secondary cutaneous cryptococcosis that can be responsible for the development of plaques, ulcers, cellulitis, abscesses, umbilicated papules and nodules.[2] These cutaneous lesions can mimic *molluscum contagiosum* and Kaposi sarcoma, as such, a skin biopsy with culture and histopathology are essential for definitive diagnosis.[1,2]

Other important aspect related with cryptococcal meningitis particularly in HIV patients is the concomitant lung involvement, often overlooked.[3] Pulmonary involvement ranges from 10% to 55% of patients with AIDS-associated cryptococcal meningoencephalitis.[1] Pulmonary infection is more frequent and severe in *C. gattii* than *C. neoformans* infection.[2]

The differential diagnosis of cryptococcal meningitis can be made with *Mycobacterium tuberculosis*, *Histoplasma capsulatum*, and *Acanthamoeba* infection; neurosyphilis; pyogenic abscess; meningeal metastases; and intracranial hemorrhages.

The laboratory diagnosis of cryptococcosis can be made by microscopy observation, serology, or culture.[1,2]

India ink microscopy is an inexpensive and rapid technique used to identify *Cryptococcus* spp. particularly at cerebrospinal fluid. When Indian ink is used, the background is stained but the thick polysaccharide capsule is not, allowing that a halo of light can be observed in the light microscopy. This technique is highly specific but lake sensitivity (<86%).[2,3]

In histopathological samples (from lung, skin, bone marrow, brain, and other organs), the observation of encapsulated yeast cells (which are clearly visualized by mucicarmine, periodic acid-Schiff and Alcian blue staining) is sufficient to diagnose *C. neoformans* infections, even in the absence of culture.[1,4]

The diagnosis of cryptococcosis can also be done by serologic tests as latex agglutination, enzyme immunoassay techniques and lateral flow assay, with sensitivities and specificities greater than 90%, but false-positive and false-negative results can occur.[1–3]

Culture is the gold standard for diagnosis of cryptococcal meningitis and fungemia, but the diagnosis can be time consuming and take until 1 week.

Other diagnostic methods include multiplex polymerase chain reaction (multiplex-PCR) or next-generation sequencing (NGS). However, these techniques are expensive.

In conclusion, the presence of unicellular encapsulated yeasts (with an abundant capsule) in CSF or other clinical samples allows the differential diagnosis of *Cryptococcus* spp. with other fungi and allows the rapid diagnose and treatment of this infection.

AUTHOR CONTRIBUTIONS

Marco P. Barros Pinto performed the research, analyzed data and wrote the paper. J. Melo Cristino analyzed data and wrote the paper.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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