

Cryptococcus gattii and its Distinctive Yeast Morphology in Cerebrospinal Fluid Cytology

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ABSTRACT

A 30-year-old immunocompetent male with no significant medical history presented with 4 weeks history of somnolence, fatigue, poor appetite, fever, headache, neck stiffness, and nausea. Subsequent brain magnetic resonance imaging indicated numerous subcentimeter foci of diffusion restriction, mainly in the bilateral putamina and caudates, but also in the bilateral posterior limbs of the internal capsules and periventricular. Cerebrospinal fluid (CSF) cytology evaluation demonstrated clusters of brown-pigmented, oval- and tear-drop-shaped budding yeasts along with elevated lymphocyte and monocyte counts. The morphology was not characteristic of *Cryptococcus neoformans* yeast. CSF was sent for fungal culture and grew *Cryptococcus gattii*. The patient was treated with induction therapy with amphotericin B (4 mg/kg) and flucytosine (25 mg/kg Q6H).

Key words: Cryptococcosis, *Cryptococcus gattii*, *Cryptococcus* morphology, *Cryptococcus neoformans*, *Cryptococcus*, immunocompetent, meningoencephalitis

INTRODUCTION

Cryptococcosis is a potentially life-threatening infection of the lungs and central nervous system (CNS) by *Cryptococcus* genus.^[1-5] The main pathogenic species of *Cryptococcus* include *Cryptococcus neoformans* and *Cryptococcus gattii*, both of which are normally budding basidiomycetous yeasts with thick capsules. *Cryptococcus* infections affect males in greater proportions than females with a male-female ratio of 2.9–1.^[6] Although *C. neoformans* predominantly affects immunocompromised patients, especially those infected with HIV, *C. gattii* is known to infect immunocompetent individuals in greater proportions.^[3-8] *C. neoformans* is the most common cause of fungal meningitis globally and is one of the most prevalent life-threatening infections in AIDS patients.^[8] *C. gattii* has primarily emerged in tropical and subtropical environments, commonly found in red gum variants of Eucalyptus trees and in nearby soil and organic materials.^[1,2,5,9] The emergence of *C. gattii* infections to temperate zones may be related to the importation of the eucalyptus tree from Australia, international travel, and global climate changes.^[10] Several

outbreaks have been identified in the United States, including southern California.^[1-3,6-8,11]

We, herein, present a case of *C. gattii* meningoencephalitis in an immunocompetent 30-year-old male with distinctive yeast morphology.

CASE REPORT

A 30-year-old immunocompetent male with no significant medical history presented with a 4 week history of somnolence, fatigue, poor appetite, fever, headache, neck stiffness, and nausea. He works as a personal trainer and recalls feeling tired at work as well. He denies any sick contacts or travels. He does endorse cannabis use but denies opiate use or illicit IV drug use.

A head computed tomography scan on arrival showed no evidence of intracranial abnormality. A subsequent brain magnetic resonance imaging indicated numerous subcentimeter foci of diffusion restriction, mainly in the bilateral putamina and caudates, but also in the bilateral

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posterior limbs of the internal capsules and periventricular [Figure 1].

On admission, the patient's laboratory values were as follows: White blood cell (WBC) count of 10.8×10^9 per liter (L) with a differential of 81.2% neutrophils, 13.3% lymphocytes, 3.8% monocytes, and hemoglobin of 14.1 g/dL. Blood cultures showed no growth. Lumbar puncture (LP) showed a clear colorless fluid with 46 cm H₂O opening pressure and cell count of 239 nucleated cells consisting of 76% lymphocytes, 18% monocytes, 2% polymorphonuclear leukocytes, glucose of 42 mg/dL, and total protein of 51 g/dL.

The cerebrospinal fluid (CSF) cytology evaluation demonstrated lymphocytosis and many budding yeasts with thick capsules. While a small subset of yeasts was round with dot-like centers and thick refractile capsule, the majority are elongated, rod-like or tear-shaped, an unusual morphology for *C. neoformans* yeasts [Figure 2].

A CSF Gram stain showed few WBC and encapsulated budding yeast. A CSF and serum cryptococcal lateral flow antigen assay were positive with a titer of 2.560 and 10.240, respectively. Fungal cultures of the CSF grew a few cream-colored, flat, moist, and mucoid colonies, morphologically suggestive of *Cryptococcus* spp., which were identified by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) as *C. gattii*. Additional serological tests were negative for West Nile virus, Lyme disease, Creutzfeldt–Jakob disease, herpes simplex virus 1 and 2, and HIV.

The patient was managed by infectious disease specialists. Treatment included amphotericin B (4 mg/kg) and flucytosine (25 mg/kg Q6H). The patient's hospital course was complicated by persistently elevated intracranial pressure requiring several LPs and a ventriculoperitoneal shunt to control the pressure. Following an induction course length of 28 days, the patient was discharged with consolidation therapy of 400 mg fluconazole daily and continued follow-up with neurosurgery. At the time of this writing, the patient is doing well and only continues to have mild headaches that are relieved with acetaminophen. Otherwise, he denies any fever or chills, nausea or vomiting, changes in his vision, or gait instability.

DISCUSSION

C. neoformans and *C. gattii* are the primary agents causing medically important cryptococcosis. *C. gattii* has recently been recognized as a distinct species. It is classified into four unique molecular types consisting of VGI, VGII, VGIII, and VGIV, and four serotypes, including A, B, C, and D.^[1,3,9] Variants VGII and VGIII have been commonly identified in North and South America. Particularly, high proportions of VGIIA were identified in outbreaks in the Pacific Northwest territories of North America.^[1] Once inhaled, *C. gattii* basidiospores can colonize the lung tissue causing pneumonia or surpass lung tissue, enter the bloodstream, and spread to other organ systems, especially the CNS, where *C. gattii* can cause cryptococcal meningitis. The most common clinical presentations of the pulmonary disease include cough, hemoptysis, chest pain, and dyspnea, while common CNS

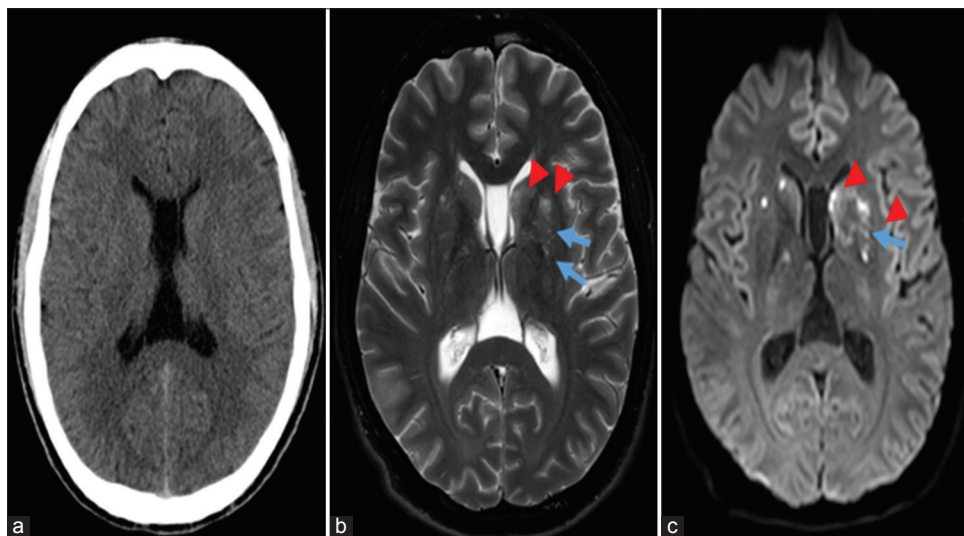


Figure 1: (a) The head computed tomography on presentation demonstrated no abnormality. (b) 3T magnetic resonance imaging performed 6 days later demonstrated T2 hyperintensities in the basal ganglia and internal capsule, some of which are more linear consistent with Virchow–Robin space dilatation (blue arrows) and others more rounded likely representing pseudocysts (red arrowhead). (c) High B-value diffusion restriction sequence shows correlating mild hyperintense signal about the dilated perivascular spaces and more intense hyperintensity associated with pseudocyst

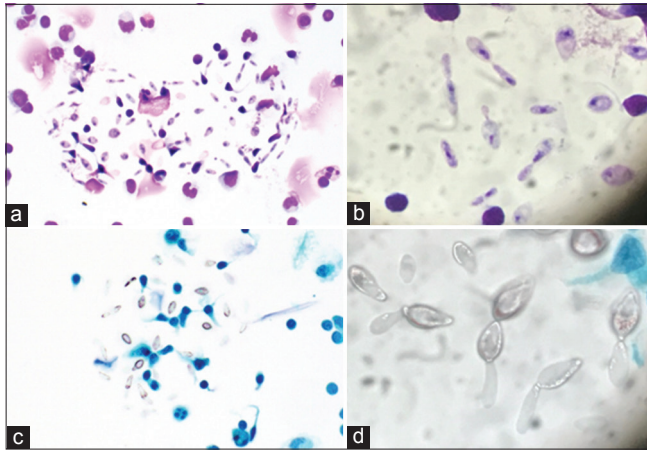


Figure 2: (a) Diff-Quick stain, $\times 40$ and (b) Diff-quick stain, $\times 100$. (c) Papanicolaou stain, $\times 40$ and (d) Papanicolaou stain, $\times 100$ of cerebrospinal fluid cytology showing elevated lymphocyte and monocyte counts, along with clusters of encapsulated oval and tear-drop shaped budding yeasts. The yeasts also show an irregular shape and varied size with narrow-based budding

infection symptoms comprise headaches, neck stiffness, vomiting, and seizures.^[1] The median incubation period for *C. gattii* is 6–7 months, although periods as short as 2 months and as long as 11 months have been recorded.^[11]

Cryptococcosis caused by both *C. neoformans* and *C. gattii* is associated with significant mortality. *Cryptococcus* species complex is considered to be a life-threatening opportunistic pathogen in immunocompromised patients with lung and CNS infections. In immunocompetent patients, *Cryptococcus* infections may still be a possibility causing severe pulmonary and/or neurological symptoms, especially with the *C. gattii* species,^[1-8,12] while *C. neoformans* and *C. gattii* may cause similar clinical presentations in immunocompetent patients, serological testing, phenotypic testing, proteomic (MALDI-TOF) analysis, nucleic acid testing, and CSF cytological evaluation are viable methods for *Cryptococcus* species differentiation.^[1,4,5,13] The two species can be differentiated by their reaction to canavanine glycine bromothymol blue (CGB) agar. *C. gattii* changes the CGB medium from yellow to blue, whereas *C. neoformans* does not induce a color change. Both species are urease positive and produce brown colonies on birdseed agar due to melanin production. In addition, various histochemical stains are utilized for *Cryptococcus* diagnosis. Hematoxylin-and-eosin and Gomori methenamine silver staining are useful for detecting yeast cells within tissue sections. Mucicarmine, alcian blue, Indian ink, and periodic acid–Schiff can stain polysaccharide capsules in cryptococci. Fontana-Masson reagent stains melanin pigments in the *Cryptococcus* cell wall, which is very helpful for acapsular strains.^[1]

In cryptococcal meningoencephalitis, CSF cytology slides with Papanicolaou and Diff-Quick staining typically

reveal round or oval narrow-necked budding yeast with thick mucinous capsules. The capsule can be seen clearly with Indian ink staining. The mucinous capsule is one of the major virulence factors in *Cryptococcus* infection, enabling protection from phagocytosis and facilitating immune evasion within the body. The yeast cells of *C. gattii* can be round, oval to tear-shaped, while *C. neoformans* yeast cells are almost uniformly round.^[10,14] In addition, under environmental stresses such as nutrient limitation, CO₂ concentration alteration, and temperature alteration, *C. gattii* yeast capsule thickness, and diameter can vary, coupled with the formation of morphologically irregular cells.^[15]

In our case, CSF cytology evaluation demonstrated elevated lymphocyte and monocyte counts, along with clusters of encapsulated oval and tear-drop shaped budding yeasts. However, a small subset of yeasts was round with dot-like centers and a thick refractile capsule, an unusual morphology for *C. neoformans* yeasts [Figure 2]. The presence of this morphology can favor the presence of the species *C. versus C. neoformans*. Further immunological testing, histochemical staining, and molecular assays can help further differentiate these species.

CONCLUSION

With hospitalization rates as high as 90% and a case fatality rate over 30% across US hospitals, *Cryptococcus* infections are a significant differential diagnosis for pneumonia and meningitis cases.^[12] Although most Cryptococcal infection occurs in immunocompromised patients and is caused by *C. neoformans*, *C. gattii* can involve both immunocompromised and immunocompetent hosts and cause severe pulmonary and CNS infections. Therefore, physicians should consider screening for *C. neoformans* and *C. gattii* infections in both immunocompetent and immunocompromised patients to ensure earlier detection and treatment. While typically presenting as budding yeasts with a thick capsule similar to *C. neoformans* in CSF cytology specimens, *C. gattii* may exhibit unusual morphology. *C. gattii* agent should be considered in cytology cases when encapsulated pear-shaped yeasts predominate. Definitive diagnosis at the species level can be obtained with specialized agars, proteomic analysis, or molecular assays.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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