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The Brief Case
(For answers to the self-assessment questions and take-home points, see page 1934 in this issue [doi:10.1128/JCM.03254-15].)

Cryptococcus gattii Meningitis with Ventriculitis

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CASE

A 39-year-old previously healthy man was transferred to Emory University Hospital Midtown, Atlanta, GA, USA, from Savannah, GA, USA, with a 1-month history of progressive headaches, drowsiness, blurred vision, and photosensitivity. Magnetic resonance imaging (MRI) revealed a noncommunicating obstructive hydrocephalus at the level of the third ventricle. Endoscopic ventriculostomy was attempted but could not be completed because of marked adhesions, scar tissue, and nodularity throughout the ependymal surfaces of the lateral ventricles. An external ventricular drainage catheter was placed, and a cerebrospinal fluid (CSF) sample was obtained. The CSF was bloody, with a normal glucose level of 40 mg/dl (normal range, 40 to 70 mg/dl), a corresponding serum glucose level of 100 mg/dl (normal range, 65 to 110 mg/dl), and an elevated CSF protein level of 148 mg/dl (normal range, 15 to 45 mg/dl). The CSF white blood cell count was elevated at 25 cells/μl (normal range, 0 to 5 cells/μl) with predominantly polymorphonuclear neutrophils (PMNs) (67%). A CSF Gram stain showed few PMNs but no organisms. A CSF cryptococcal latex agglutination antigen test (Meridian Bioscience Inc., Cincinnati, OH, USA) was negative, but a serum cryptococcal antigen titer performed a few days later was 32. Blood cultures taken at that time showed no growth, but bacterial and fungal cultures of the CSF grew a few cream-colored, mucoid colonies in 3 days (Fig. 1A). Direct microscopic examination of the colonies revealed round yeast cells that tested germ tube negative (Fig. 1B). The organism was able to hydrolyze urea, and a caffeic acid disk test was positive for melanin production, consistent with Cryptococcus neoformans, but upon growth, a blue coloration was produced on 1-canavanine–glycine–bromothymol blue (CGB) agar (Centers for Disease Control and Prevention [CDC], Atlanta, GA, USA), distinguishing the isolate as Cryptococcus gattii (Fig. 1C). The identification was confirmed as C. gattii biotype VGI at the CDC by multilocus sequence typing, with 100% identity to the reference isolate across 4,141 nucleotides.

The patient had recently pressure washed houses along the Georgia coast without wearing a mask. He was presumed to have acquired the C. gattii infection from organisms aerosolized through the action of pressurized wash water. An enzyme-linked immunosorbent assay (ELISA) for HIV type 1 and 2 antibodies and p24 antigen was negative.

The patient was started on a combined treatment of amphotericin B lipid complex (5 mg/kg of body weight/day) and fluconazole (400 mg/day). Multiple negative cultures were achieved, but CSF protein remained high (>300 mg/dl), and repeated MRI showed worsening ventriculitis. Flucytosine (100 mg/kg/day) and steroids (dexamethasone, 6 mg/day) were added to the treatment regimen, which adequately decreased the protein level, and a ventriculoperitoneal shunt was placed. The patient was confused and agitated throughout his hospital course but spoke fluently and was alert, oriented, and responsive to verbal commands at the time of discharge on hospital day 40.

After discharge, the patient was maintained on oral fluconazole (400 mg daily) and remained shunt dependent. He required three shunt revisions over the next 4 months following an intra-abdominal infection with coagulase-negative Staphylococcus and two episodes of shunt malfunction. The patient expired 9 months after initial hospitalization due to a probable shunt blockage.

DISCUSSION

The genus Cryptococcus is a collection of basidiomycetous yeasts, of which Cryptococcus gattii and Cryptococcus neoformans are the primary agents of medical importance. Both species were originally considered varieties of C. neoformans; however, C. gattii has since been recognized as a distinct species composed of four major molecular types: VGI, VGII, VGIII, and VGIV (1, 2).

Although C. gattii was originally thought to be confined to tropical and subtropical regions, the emergence of C. gattii infections in North America provided definitive evidence of spread to temperate climatic zones (1–3). Currently, C. gattii has been documented in Africa, the Americas, Asia, Australasia, and Europe, although the global distributions of the four types vary significantly (1). In North America, all four types and at least three VGII subtypes (VGIIa, VGIIb, and VGIIc) and two VGIII subtypes (VGIIIa and VGIIIb) have been identified (1, 2).

The reservoir of C. gattii is plant matter, in particular, trees and decomposing wood in tree hollows (1, 4). Initially, eucalypts were identified as the environmental source; however, >50 different species of tree (e.g., Douglas fir, coastal western hemlock, and cedar) can provide an ecological niche (1, 4).

Cryptococcal infections due to C. gattii and C. neoformans result from inhalation of basidiospores (1). Infections due to these two species can be loosely differentiated based on the presenting individual: C. gattii infection in immunocompetent persons and...
C. neoformans infection in immunocompromised persons (1, 4). Despite this broadly differentiating characteristic, C. gattii infection in immunocompromised individuals, including those with HIV/AIDS, has been described and often involves VGIII and VGIV isolates, whereas the VGI and VGII types are associated primarily with disease in otherwise-healthy people (2). Infection can involve any body site, but the principal sites of infection are the lungs and central nervous system (CNS), where C. gattii can cause large cryptococcomas (1, 4). The symptoms of pulmonary disease are pneumonia-like and typically include chest pain, cough, and dyspnea, while CNS symptoms often comprise headache, altered mental status, a reduced level of consciousness, seizures, and visual or other focal neurologic symptoms (1, 4). Notable complications of disease include raised intracranial pressure and immune reconstitution inflammatory syndrome (1). A noted complication in our case was ventricular and ependymal scarring. Of particular concern is the fact that C. gattii CNS disease can be associated with high mortality rates, particularly if diagnosis is delayed (1).

Differences between sporadic disease and that observed in case clusters have been described and likely reflect variations in the molecular types/subtypes of the offending isolates and their geographic distributions (1, 5). For example, in the United States and Canada, outbreak cases have been restricted to the Pacific Northwest and are a result of infection with C. gattii VGIIa, VGIIb, and VGIIc, so-called “outbreak strains.” Patients infected with these strains are more likely to present with pulmonary rather than with CNS symptoms. Conversely, patients within the United States infected with nonoutbreak strains present primarily with CNS disease in the absence of pulmonary symptoms (5).

A number of laboratory methods are employed for microbiologic diagnosis and include microscopy, histopathology, cryptococcal antigen (CrAg) tests, and culture. However, only culture on specialized media or culture on routine mycology media followed by interrogation with proteomic (e.g., mass spectrometric) or nucleic acid-based methods can differentiate between species. C. gattii and C. neoformans are encapsulated, round-to-oval, narrow-based budding yeasts, approximately 5 to 10 μm in diameter, that can be visualized in clinical specimens using various stains. Upon Gram staining, cryptococci stain Gram negative, positive, or variable, and in many cases, the polysaccharide capsule impedes uptake of the stain, rendering Gram-negative cells with granular Gram-positive inclusions (4). In CSF specimens, India ink can be used as a negative stain to reveal encapsulated yeasts (1, 4). Several histopathologic stains detect C. gattii and C. neoformans in tissue, and use of multiple stains increases the sensitivity and specificity of identification. Hematoxylin-and-eosin stains yeast cells but not the capsule, thus creating a clearing around the organism. As with all yeasts, Gomori methenamine silver and periodic acid-Schiff stain the cell wall, while Alcian blue and mucicarmine specifically stain the polysaccharide capsule. Acapsular or poorly encapsulated strains can be observed with Fontana-Masson stain, which stains melanin in the cell wall (1, 4).

CrAg tests are antibody-based platforms that detect cryptococcal capsular polysaccharide in serum and CSF specimens. They are extremely useful for the initial diagnosis of cryptococcosis but do not discriminate between species and have limited utility for monitoring responses to therapy (1, 4). These assays display a high sensitivity, including in HIV-positive patients, for C. gattii lung disease (90%) and CNS disease (87 to 100%) (1). False-positive results are uncommon but can occur due to the presence of rheu-
matoid factor and infections due to Trichosporon beigeli, Rothia mucilaginosa, Capnocytophaga canimorsus, and Klebsiella pneumoniae. Likewise, false-negative results are uncommon but can result from low organism burden, from infection with acapsular strains, or as a consequence of prozone effects in overwhelming disease (1, 4). Of note, our patient’s serum and CSF CrAg tests were positive and negative, respectively. The CSF may have been negative due to a prozone effect resulting from his fulminating CNS disease.

Cryptococcus species are routinely recovered on mycology media without cycloheximide and generally yield visible colonies within 48 to 72 h of incubation under aerobic conditions at 30 to 35°C (4). Upon culture, C. gattii can be identified to the species level using proteomic and nucleic acid-based platforms, but commercial biochemical systems are unable to discriminate between the two species (1, 4). Colonies of C. gattii and C. neoformans are urease positive and turn dark brown on caffeic acid (“birdseed”) agar as a result of organism-dependent melanin production, whereas CGB agar provides selective and differential isolation of C. gattii. This is based on the ability of C. gattii to grow in the presence of l-canavanine and use glycine as the sole carbon and nitrogen source, which increases the pH of the medium, causing the bromothymol blue indicator to change from yellow to blue. In contrast, C. neoformans, which is susceptible to l-canavanine, is either completely inhibited or grows poorly, producing no color change (1, 4).

Management of cryptococcosis is centered upon antifungal therapy, supportive care, and, critically, in the setting of CNS disease, control of raised intracranial pressure (1, 6). Surgery may be required to resect cryptococcomas (6). Therapeutic approaches are similar for the two species and depend upon host status, site of infection, and associated complications (1, 6). Antifungal recommendations for CNS disease include induction therapy with amphotericin B plus 5-flucytosine, followed by consolidation and maintenance treatments with fluconazole (1, 6). The length of induction therapy for CNS disease due to C. gattii is recommended to be at least 6 weeks, with a total length of therapy of 18 to 24 months (1).

SELF-ASSESSMENT QUESTIONS

1. What is the reservoir for Cryptococcus gattii?
   (a) Rodents
   (b) Plant matter
   (c) Avian guanos
   (d) Water

2. In the United States and Canada, what clinical presentation was most often associated with outbreak cases due to C. gattii?
   (a) Central nervous system disease
   (b) Osteomyelitis
   (c) Pulmonary disease
   (d) Endocarditis

3. Which of the following statements correctly describes the biochemical basis for the reaction of C. gattii on l-canavanine–glycine–bromothymol blue agar?
   (a) A yellow-to-blue color change occurs because of an alkaline pH shift that occurs because C. gattii can use citrate as a sole carbon source and ammonium ions as the sole nitrogen source.
   (b) A yellow-to-blue color change occurs because of an alkaline pH shift that occurs when the urease enzyme produced by C. gattii hydrolyzes urea to ammonia.
   (c) A yellow-to-blue color change occurs because of an alkaline pH shift that occurs because C. gattii is able to grow in the presence of l-canavanine and utilize glycine as the sole source of carbon and nitrogen.
   (d) There is no color change because, although C. gattii can grow in the presence of l-canavanine, it does not utilize glycine.

REFERENCES