Case Report

# Capsule-Deficient Cryptococcal Meningitis: A Diagnostic Conundrum

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## Abstract

Cryptococcosis is a serious systemic mycosis. Its incidence has escalated in the past four decades. *Cryptococcus neoformans* causes localized or disseminated infection in immunocompromised and immunocompetent patients. The capsulated form is commonly encountered which can be diagnosed on an India ink preparation or antigen detection. However, the noncapsulated forms are very rare and require a high index of suspicion for correct diagnosis. Herein, we present a case of cryptococcal meningitis due to a noncapsulated strain in an apparently immunocompetent patient with no proven immunodeficiencies along with review of world literature. Such cases are a diagnostic challenge for the clinician as well as microbiologist.

**Keywords:** Antigen, capsule deficient, Cryptococcus, culture, diagnosis

## Introduction

Cryptococcal meningitis (CM) is a common opportunistic fungal infection. *Cryptococcus* is human pathogenic yeast causing subacute and chronic meningitis, with the potential for complications and significant mortality. *Cryptococcus neoformans* species can also cause localized or disseminated infection in both immunocompromised and immunocompetent patients.[1] Its incidence has escalated in the past four decades due to HIV epidemic.[2,3]

The capsulated form is commonly encountered which can be diagnosed on an India ink preparation, antigen detection, and by a special stain. However, the noncapsulated forms are very rare and require a high index of suspicion support of molecular tests for correct diagnosis. Herein, we present a case of CM due to a noncapsulated strain in an immunocompetent patient.

## Case Report

A 69-year-male patient presented to the emergency department of our hospital with fever, progressively worsening headaches, and altered sensorium for 2 days. The patient had a history of similar complaints 4 months back during which private practitioner started ATT and steroids on the basis of computed tomography (CT) brain, which showed postinfective mild hydrocephalus with dilatation of all four ventricals with no apparent cause. Contrast-enhanced computed tomography chest showed borderline enlarged paratracheal lymph node of 9-mm size. Cerebrospinal fluid (CSF) laboratory parameters indicated a picture of chronic meningitis. Appearance of the CSF was clear, the CSF pressure was elevated, the protein and glucose levels were increased along with an increased lymphocytes count.

On first-time admission to our hospital, similar findings were present. Acid-fast bacillus (AFB) staining and polymerase chain reaction in CSF for *Mycobacterium tuberculosis* were done to rule out tuberculosis (TB). Both Gram stain and India ink performed on CSF sample were inconclusive. Latex agglutination for cryptococcal antigen was weak positive (1:2). Therefore, according to the manufacturer (CALAS), it was reported as negative. On 8$^{th}$ day of CSF culture, two dry-looking yeast-like colonies were observed. Subculture of this isolate

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on birdseed agar showed brown color colonies. Urease test was also positive. The isolate was identified as *Cryptococcus neoformans*. This was further confirmed by matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF). The serum was negative for anti-HIV antibodies. Bacterial culture was sterile. Herpes simplex virus-1 was negative. GeneXpert was performed. MTB was not detected, thus ruling out MDR and XDR TB also. However, the patient got discharged before the final culture result was available.

The patient was informed about the same and was readmitted to our hospital. On readmission, the body temperature was 36.6°C, heart rate was 84/min, respiration rate was 20/min, and blood pressure was 100/80 mmHg. On physical examination, the patient had shuffling gait, motor power right 4+ and left 5, plantar ↓↓, and tone mild cogwheeling, and cerebellar signs were negative.

The patient was started on amphotericin B and fluconazole as susceptibility report was yet not available. In addition, clonazepam and dexamethasone were also given. Because of hypokalemia and renal toxicity with amphotericin B, it had been given irregularly. The isolate was later found to be sensitive to amphotericin B and flucytosine but fluconazole resistant. Unfortunately, the patient succumbed to his illness after 1 month of his present admission.

## Discussion

*C. neoformans* is an encapsulated yeast which causes opportunistic infections in humans. The infection is acquired through inhalation of the respiratory droplets resulting in initial involvement of the lungs followed by hematogenous dissemination which then can lead to infection of the central nervous system. The degree of host's immune response influences the clinical presentation. In immunocompromised hosts, especially in patients with depleted cell-mediated immunity, *C. neoformans* can cause serious and fatal meningoencephalitis.

*C. neoformans* is a narrow-based budding, spherical-to-oval (4–10 μm) capsulated yeast. The presence of the capsule, ability to synthesize melanin, presence of urease enzyme and phospholipid secretion, and survival in host body temperature are the important virulence factors of the organism. The polysaccharide capsule surrounding the yeast is the major virulence factor and triggers complement activation and antibody production in the host. Glucuronoxylomannan present in the capsule can also help the yeast in evading complement-mediated phagocytosis.

The capsule of *C. neoformans* has been known to exhibit morphological and phenotypic variations by changing its structure and size. Giant cell/titan cells and other microforms have also been described by variation in the total size of the yeast.[4] Such variations can largely influence the host–pathogen interaction dynamics. There are few documented case reports of noncapsulated *Cryptococcus* causing CNS and pulmonary infections in literature [Table 1].

The capsule is not only the major virulence factor but also the most commonly demonstrated part of the yeast over which most of the diagnostic modalities focus. The capsule can be detected by negative staining methods such as India ink or by mucicarmine staining which stains the mucin-rich capsule or by targeting the presence of the cryptococcal antigen by latex agglutination assay, enzyme immunoassay, and lateral flow assay. Point-of-care tests such as lateral immunoassays are rapid and reliable in diagnosing CM. Immunoassays such as latex agglutination assay carry both diagnostic and prognostic values by estimating titers and hence can be also useful in monitoring treatment responses. While false-positive results might occur due to cross-reactivity with certain fungal species such as *Trichosporon*, false-negative results, though rare, have also been reported. Infection due to capsule-deficient forms can also give such results. We also report a case of noncapsuled *C. neoformans* causing chronic meningitis infection that occurred in an immunocompetent host where the latex agglutination for cryptococcal antigen was repeatedly weak positive (1:2). In cases with capsule-deficient forms, capsule demonstration methodologies may fail making diagnosis becomes difficult and one has to rely on culture for diagnosis.

Being the outermost structure of the yeast, capsule is one of the most important and responsible factors for the colony characteristics. The colonies of *C. neoformans* are generally smooth and mucoid morphology on standard growth agar. In case of infection due to noncapsulated form, even the growth on culture may not show the typical morphology due to variable capsule expression. In this case also, the culture revealed dry looking in contrast to the smooth creamy mucoid colonies of *Cryptococcus*. This was further identified both conventionally and by MALDI-TOF. Hence, conventional identification also becomes tricky as such infections may not always yield straightforward typical results, especially on culture.

Table 1 summarizes the published case reports/series of capsule deficient or noncapsulated cryptococcosis. Out of these 33 cases, none showed India ink or CSF cryptococcal antigen positivity and for most others of them reports were not available as the capsule-deficient *Cryptococcus* was either reported as a chance finding on histopathology or it was recovered from culture. Serum cryptococcal antigen was positive in six case reports, and the titer was given in only four that was 1:32 in two, 1:50 and 1:4056 in one each. Culture positivity was seen in eight case reports. Out of these case reports, the maximum were of pulmonary cryptococcosis followed by CNS and others were septic arthritis, cutaneous, prostatic, hepatic, axillary lymphadenopathy, and disseminated. Most of the cases were recovered after treatment and in seven case reports, the patient succumbed with the illness including our case report.

The formation of capsule is dependent upon various factors including carbon dioxide, glucose, amino acids, pH, and temperature. The presence of an active infection, type of host immune response, and type of tissue infected can also result in variability of the thickness of capsule. It is still unclear whether these poorly capsulated forms are a result of the host factors or are typical to the strain infecting the host. In a study

**Table 1: Review of important published reports of infections due to capsule-deficient *Cryptococcus***

| Year | Age/sex | Predisposing factor | CD4 cou-nt | Presentation | India ink | Cr Ag serum | Cr Ag CSF | Culture | Histopathology | | Treatment | Outcome | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | MMS | FMS | | | |
| 1973 | 54/female | | - | Dyspnoea | | - | - | - | Positive | - | AMB | - | Farmer and Komorowski[5] |
| 1974 | 38/female | Sarcoidosis and trauma | - | Septic arthritis | NA | Positive | Negative | - | - | - | AMB and 5FC | Recovered | Levinson *et al.*[6] |
| 1975 | | | - | - | - | - | - | - | Negative | - | - | - | Gutierrez *et al.*[7] |
| 1979 | 21/male | Exposure to pigeon droppings | - | Pneumonia | - | 1:32 | Negative | Positive | Negative | Negative | AMB | Recovered | Harding *et al.*[8] |
| 1983 | 23/male | Farmer | - | Disseminated | - | - | - | - | - | - | - | - | Attal *et al.*[9] |
| 1985 | - | - | - | - | - | - | - | - | - | - | - | - | Bottone and Wormser[10] |
| 1985 | - | - | - | - | - | - | - | - | - | - | - | - | Mackenzie and Hay[11] |
| 1987 | - | - | - | Pulmonary infection | - | - | - | - | Negative | Positive | - | - | Ro *et al.*[12] |
| 1989 | 83/female | SIADH | - | Sepsis and CNS | - | - | - | Positive | - | - | Miconazole | Died | Mukae *et al.*[13] |
| 1990 | 59/male | | - | Prostatitis | - | Positive | - | - | Negative | Positive | Ketoconazole | Recovered | Milchgrub *et al.*[14] |
| 1993 | - | - | - | Meningoencephalitis | - | - | - | - | - | - | AMB + 5-FU | | Lacaz *et al.*[15] |
| 1998 | 58/female | Cirrhosis pancytopenia | - | Pulmonary nodules | - | Negative | NA | NA | Negative | Positive | - | Died | Kimura *et al.*[16] |
| 1998 | 17/male | HIV | 125 | CNS | - | - | - | POS | - | - | AMB + itraconazole + fluconazole | Recovered | Laurenson *et al.*[17] |
| 2005 | 42/male | Farmer | - | CNS | - | - | ± | Negative | - | - | AMB + flucyto + fluconazole | Died | Sugiura *et al.*[18] |
| 2005 | - | - | - | - | - | - | - | - | - | - | - | - | Torres *et al.*[19] |
| 2006 | 25/female | - | - | Pneumonia | NA | - | NA | - | Negative | Positive | Fluconazole | Recovered | Cheon *et al.*[20] |
| 2008 | - | - | - | - | - | - | - | - | - | - | - | - | Kanazawa *et al.*[21] |
| 2008 | 42/female | - | - | Pneumonia | - | Negative | NA | NA | Negative | Positive | - | - | Gazzoni *et al.*[22] |
| 2010 | 67/female | - | 818 | Pneumonia | - | 1:32 | Negative | Negative | Negative | Positive | AMB, fluconazole | Recovered | Bavishi and McGarry[23] |
| 2010 | 40/female | - | - | Pneumonia | - | Negative | NA | NA | Negative | Positive | - | - | Gazzoni *et al.*[24] |
| 2010 | 59/male | Lung transplant | - | Pneumonia | - | Negative | NA | NA | Negative | Positive | - | - | |
| 2010 | 60/male | Lymphoma | - | Pneumonia | - | NA | NA | Positive | Negative | | - | - | |
| 2010 | 40/male | Renal Transplant | - | Cutaneous | - | Negative | NA | NA | Negative | Positive | - | - | |
| 2010 | 42/male | Renal Transplant | - | Pulmonary | - | Negative | NA | NA | Negative | Positive | - | - | |
| 2010 | 23/male | AIDS | - | Hepatic | - | Negative | NA | NA | Negative | Positive | - | - | |
| 2010 | 10/male | AIDS | - | Axillary LAP and CNS | - | 1:4056 | NA | NA | Negative | Positive | - | - | |
| 2011 | 7/- | AIDS | - | - | - | - | - | - | - | - | - | - | Ramdial *et al.*[25] |

*Contd...*

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| Year | Age/sex | Predisposing factor | CD4 cou-nt | Presentation | India ink | Cr Ag serum | Cr Ag CSF | Culture | Histopathology | | Treatment | Outcome | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | MMS | FMS | | | |
| 2012 | 27/female | - | - | CNS | Negative | Negative | Negative | *C. neoformans* | - | - | AMB + flucyto + fluconazole | Recovered | Garber and Penar[26] |
| 2015 | 78/male | - | - | Cutaneous | | Negative | NA | NA | Negative | NA | Itraconazole | Recovered | Herring *et al.*[27] |
| 2015 | 58/male | - | - | CNS | - | POS 1:50 | NA | NA | Negative | Positive | AMB + flucyto + fluconazole | Recovered | Garcia-Santibanez *et al.*[28] |
| 2016 | 63/male | - | - | CNS | Negative | Negative | Negative | Positive | - | - | AMB + flucyto | Died | Mahajan *et al.*[29] |
| 2019 | 36/male | - | - | CNS | Negative | N/A | Negative | Positive | - | - | AMB | Died | Birkhead *et al.*[30] |
| 2019 | 51/female | - | 119 cells/ul | CNS | Negative | | Negative | Positive | - | - | AMB + fluconazole | Died | Birkhead *et al.*[30] |

SIADH: Syndrome of inappropriate antidiuretic hormone secretion, Cr Ag: Cryptococcal antigen, MMS: Ma FMS: Fontana-Masson stain, NA: Not available, CNS: Central nervous system, LAP: Lymphadenopathy, AMB: Amphotericin B

by Mahajan *et al*., repeat subculturing of the isolate yielded mucoid colonies that indicated capsule re-expression.[29] In another study by Sugiura *et al*., thick capsulated forms were recovered only after intraperitoneal inoculation of the capsule-deficient strain into murine peritoneal cavity.[18]

## Conclusion

This case demonstrates a rare example of an immunocompetent patient who was found to have meningitis due to a noncapsulated strain of *C. neoformans*. The current case emphasizes the importance of conventional identification approaches such as CSF fungal culture which aided in the diagnosis after repeated antigen testing showing 1:2 dilution weak positivity. Such cases can pose a diagnostic challenge for the clinician as well as microbiologist and routine fungal antigen testing does not always reveal the offending organism hence resulting in a delay in diagnosis and treatment. Hence, the possibility of infection with noncapsulated *Cryptococcus* should be kept in mind if patients are not responding to antimicrobial treatment with high clinical suspicion of *Cryptococcus* meningitis is there.

### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that his name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

### Research quality and ethics statement

The authors followed applicable EQUATOR Network guidelines, notably the CARE guideline, during the conduct of this report.

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### Conflicts of interest

There are no conflicts of interest.

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