Perspectives on non-neoformans cryptococcal opportunistic infections

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ABSTRACT

Non-neoformans Cryptococcus species, including C. laurentii and C. albidus, have historically been classified as exclusively saprophytic. However, recent studies have increasingly implicated these organisms as the causative agent of opportunistic infections in humans. Herein, the case is presented of C. laurentii meningitis in a critically ill patient receiving corticosteroids. C. laurentii has been implicated in an additional 18 cases of opportunistic infection, predominantly of the skin, bloodstream, and central nervous system. The most clinically significant risk factors for non-neoformans cryptococcal infections include: impaired cell-mediated immunity, recent corticosteroid use, and invasive catheter placement. This article provides a comprehensive review of the clinical relevance, pathogenesis, risk factors, and treatment of non-neoformans Cryptococcus species.

1. Introduction

Non-neoformans Cryptococcus species, including C. laurentii and C. albidus, have historically been considered saprophytic and non-pathogenic. However, a literature review demonstrates an increasing prevalence in opportunistic infections [1–3]. The Cryptococcus genus consists of >70 species of ana-morphic, basidiomycetous, encapsulated yeast [4]. C. neoformans, which includes the C. gattii and C. neoformans varieties, remains a common cause of opportunistic infections in immunocompromised states and is classified as an AIDS-defining illness [1]. Non-neoformans Cryptococcus species include C. laurentii, C. albidus, C. curvatus, C. humicolus, and C. uniguttulatus. These species were traditionally believed to be non-virulent to humans. However, there has been an increased incidence in recent decades of opportunistic infections involving the skin, lungs, bloodstream, and central nervous system (CNS). It has been theorized that this increasing incidence may be secondary to a growing number of at-risk or immunocompromised patients, improved awareness, or advancements in laboratory technology [3]. Within the non-neoformans cryptococcal species, C. laurentii and C. albidus account for 80% of pathogenic infections [1–5]. Understanding of the epidemiology and pathogenesis of these species will allow for prompt recognition of non-neoformans cryptococcal infections and improved patient outcomes. Additionally, understanding the common patterns of resistance will prevent further treatment failure.

2. Case description

A 48-year-old male with no significant past medical history presented with non-productive cough, fevers, chills, abdominal pain, and diarrhea. He was initially diagnosed with H1N1 Influenza A (2009 pandemic strain) and was started on oseltamivir, as well as empiric community acquired pneumonia coverage (ceftriaxone and azithromycin). The patient developed worsening respiratory status secondary to acute respiratory distress syndrome (ARDS), requiring intubation on day 2. Intravenous methylprednisolone was started at an initial dose of 60 mg intravenously (IV) q6h, per the ARDS Network Late Steroid Rescue Study (LaSRS) Steinberg. The patient underwent a prolonged 3-week steroid taper. Despite appropriate ARDs management, he was unable to be weaned from the ventilator and required tracheostomy placement. His hospital course was further complicated by atrial fibrillation with rapid ventricular rate and acute renal failure, which required continuous renal replacement therapy.

A notable mental decline was noted over the next 21 days. Although the patient opened eyes to commands, he did not withdraw when noxious stimuli...
were applied to the extremities, and he had marked hyporeflexia. Neurology was consulted, and an electroencephalogram demonstrated diffuse background slowing without epileptiform discharges. Magnetic resonance imaging (MRI) of the brain demonstrated multiple abnormalities, most significantly increased Fluid-Attenuated Inversion Recovery signal of the subcortical white matter, suggestive of acute hemorrhagic leukoencephalopathy (AHL). Given the H1N1-related AHL, the patient’s prognosis was considered poor. The initial differential diagnosis included cerebritis and acute disseminated encephalomyelitis (ADEM). Steroid dosing was increased to 500 mg of methylprednisolone twice a day for 5 days and was eventually decreased to 250 mg twice a day. After a few days of high-dose steroids, he demonstrated mild neurologic improvement, with the ability to maintain intermittent basic communication through blinking.

The patient developed a gastric perforation and displaced gastrotomy tube requiring exploratory laparoscopy. Further antibiotic coverage was provided with cefepime and metronidazole for the intra-abdominal infection. He developed worsening pneumoperitoneum with complex free fluid, and consideration was given for a repeat exploratory laparoscopy/laparotomy.

Blood cultures became positive for Candida tropicalis on day 8 of hospitalization, which was treated successfully with micafungin. All invasive lines, including central venous access and hemodialysis catheters, were removed to prevent colonization or further infection. Blood cultures from the central venous catheter grew Staphylococcus aureus on subsequent days, and the patient received a course of vancomycin for 3 weeks. Subsequent blood cultures showed resolution of Candida fungemia. Repeat blood cultures performed on day 19 for persistent fevers and leukocytosis demonstrated C. laurertii, despite concurrent micafungin therapy. After speciation was notable for Cryptococcus, the patient was started on IV amphotericin B and flucytosine.

The patient’s new diagnosis of C. lauertii fungemia and MRI findings were felt to represent a CNS infection. However, he remained too unstable to perform further evaluation with lumbar puncture. Although human immunodeficiency virus (HIV) antibody and antigen testing was negative, T- and B-cell enumeration demonstrated impaired cell-mediated immunity, with a CD4 count of 187. This was felt to be secondary to high-dose intravenous corticosteroid use as treatment of ARDs and ADEM. No abnormalities were noted on bronchoscopy, and no new infiltrate on chest imaging were noted to suggest a pulmonary source. As the patient showed no neurologic improvement, his family elected to pursue comfort measures, and he succumbed to his illness.

3. Discussion

A literature review identified 44 cases of non-neofor mans species causing infections in humans, with approximately 18 cases due to C. lauertii. C. lauertii is found worldwide, although its natural habitat remains largely unknown [2,3]. It is the most common yeast inhabiting the soil of traditionally hostile environments, including tundra, the Antarctic, the Himalayas, the Caribbean, and the prairies. This survivability may be attributed to its psychrophilic abilities, with an optimal culture temperature of 15.0°C, and poor growth at temperatures >30.0°C [3,6]. Historically, it has been used as a biological pesticide to prevent the decay of fruits and has been demonstrated as a contaminant in the fermentation process of wines and beer [1,7]. Additionally, C. lauertii has been isolated in cases of bovine mastitis [5]. There have been approximately 18 reported cases of C. lauertii infections in the literature, most commonly disseminated, pulmonary, and cutaneous forms [3].

In a systematic review of 38 articles by Khawcharoenporn et al., non-neofor mans cryptococcal infections typically presented as fungemia (39%) or CNS infection (32%), as well as pulmonary, gastrointestinal, ocular, or dermatological infections. HIV is associated with a higher risk of CNS infections (57% vs. 27%; p = 0.05). CNS infections most likely present with meningeal signs (50%), but may also present with encephalopathy, gait instability, nausea, vomiting, paresthesia, or flaccid/spastic paralysis. Pulmonary non-neofor mans infections predominantly follow a course of chronic, indolent disease [3].

Transmission of C. lauertii is primarily through inhalation of infective particles by close contact with pigeons or contaminated soil [1–3,6]. In 1998, Johnson et al. initially proposed that nosocomial spread was the primary means of transmission. However nosocomial transmission appears to be a rare cause of disease spread. In a systematic review by Khawcharoenporn et al., only two cases of such transmission were reported, and it was theorized that disease spread in the hospital setting was perpetuated through infected respiratory supplies or via direct inhalation from airborne yeast. Disseminated infections are thought to be the result of hematogenous spread from pulmonary infections or via indwelling catheters. Even purely asymptomatic pulmonary infections can progress to widespread disseminated disease. A number of unique virulence factors expressed by cryptococcal species aid in dissemination, including melanin deposition, use of laccase enzyme, and outer capsule. Melanin deposition alters cell-wall integrity, allowing for evasion of the host immune system, and reduces the sensitivity to antifungal therapies. The capsule is composed of polysaccharides and participates in the evasion of phagocytosis [3].
The primary risk factor for development of non-
C. neoformans cryptococcal infections is impaired cell-
mediated immunity, which is implicated in 48% of
such infections. Impaired immunity is often second-
ary to neutropenia, malignancy, lymphoproliferative
disorders, immunosuppressant use, or prior organ
transplant. Other common risk factors include HIV
infection with a CD4 count of <100 (associated with
16% of non-C. neoformans infections), exposure to
azoles, and the use of an invasive catheter device
(specifically in C. laurentii infections; see Table 1).
Khawcharoenporn et al. highlighted the significant
differences between cases of C. laurentii and C. albi-
dus infections, including the fact that C. laurentii
infections tend to involve younger patients
\( p = 0.01 \) and carry a higher likelihood of survival
\( p = 0.01 \). Prior research into the predictors of mor-
tality in non-C. neoformans infections have lacked sta-
tistical power to make conclusions due to the low
prevalence of disease. However, prior research has
suggested an association between age >45 years,
CNS infection, and mortality [3,7].

Standard cryptococcal antigen testing demon-
strates reduced sensitivities to the non-C. neoformans
species compared to C. neoformans (25% vs. 99%).
It is proposed that structural differences in the yeast
antigen, lower disease burden in non-C. neoformans
infections, or inherent limitations of the assay are
likely contributors [3,4]. Further differentiation can
be assisted with the use of birdseed agar, in which
non-C. neoformans species do not form the typical
brown/black colonies of C. neoformans species [4].
Additionally, traditional fungal testing using \( \beta-1-3-
D \)-glucan assays are insufficient due to low levels of
\( \beta-1-3-D \)-glucan in the cell wall in comparison to
other fungal species (e.g. Candida). Although all
cryptococcal species contain laccase, non-C. neoformans
typically exhibit a lower level of laccase activity,
which may aid in differentiation [3]. Speciation of
Cryptococcus colonies requires genomic/DNA
sequencing of non-coding DNA regions known as
Internal Transcribed Spacer (ITS), specifically regions
D1 and D2 of the 26S rDNA. Matrix-assisted laser
desorption/ionization is a new diagnostic test that
uses mass spectroscopy to analyze biopolymers that
shows promising utility in the speciation of
Cryptococcus [4,7].

Although echinocandins are commonly used in
the treatment of fungal infections due to their activity
against the \( \beta-1-3-D \)-glucan cell wall, Cryptococcus
species are intrinsically resistant to this class of med-
ications without a known etiology [4,8]. C. laurentii
has added resistance due to biofilm formation, which
prevents adequate antibiotic penetration [9]. Despite
the lack of validated standardized treatment for non-
C. neoformans cryptococcal infections, case reports have
shown adequate treatment with the traditional neo-
formans treatment regimens, amphotericin B and flu-
cytosine [2,3]. Prior in vitro studies indicated poor
activity of fluconazole and fluvcytosine for non-C. neoformans
Cryptococcus. However, more recent in vivo
data demonstrate adequate susceptibilities.

Nevertheless, there has been documented resistance
to fluconazole and fluvcytosine in some cases, which
warrants the need for susceptibility testing. Increased
risk for fluconazole resistance is conferred with prior
azole exposure (83% vs. 50%) [4]. Per the 2014
European Congress of Clinical Microbiology and
Infectious Diseases and European Confederation of
Medical Mycology guidelines on management of rare
invasive yeast infections, amphotericin B with or
without fluvcytosine is recommended as induction
therapy, followed by fluconazole as maintenance in
severe C. laurentii infections or CNS infections; Level
of Evidence Class C-III [4]. Non-C. neoformans may
also demonstrate higher intrinsic minimum inhibi-
tory concentration than traditional C. neoformans
infections [1,3,10]. Duration of therapy depends on the
clinical scenario, but most commonly an induction
therapy of 14 days followed by approximately 28 days
for the maintenance regimen is sufficient for treat-
ment [3]. Additionally, Khawcharoenporn et al. sug-
ject that preventative measures for C. laurentii
infection are similar to typical cryptococcal infec-
tions, including avoiding exposure to contaminated
environments, the use of antifungal prophylaxis in
appropriate immunocompromised patient, and
implementing measures to improve native host
defenses (including the use of antiretroviral therapy
in HIV patients). There have been no guidelines for
prophylaxis of non-C. neoformans species in immuno-
compromised patients, but it has been suggested to
follow the guidelines for Cryptococcus neoformans
prophylaxis. Per the IDSA 2010 guidelines, primary
prophylaxis is not routinely recommended for immu-
no compromised patients in the USA or the UK
(Table 2). Although azole therapy has been shown
to reduce the frequency of cryptococcal disease in
patients with a CD4 count of <50, prophylaxis has
not been shown to improve survival and may
increase drug resistance as well as risk drug–drug
interaction. The exception to this would be areas with

**Table 1. Risk factors for Cryptococcus laurentii infection**

| C. laurentii risk factors |
|---------------------------|
| Immune suppression         |
| AIDS                      |
| hämoglobinopathy          |
| Hodgkin lymphoma          |
| Malignancy                |
| Immunodeficiency           |
| Prior infections           |
| Infection with azoles     |
| HIV infection (CD4 count <100) |
| Invasive catheter device  |

HIV = human immunodeficiency virus.
high prevalence of cryptococcal infections, increased antiretroviral drug resistance, and lack of access to antiretroviral therapy. In such scenarios, medication prophylaxis against Cryptococcus may be appropriate and should be considered on an individual basis [11].

This case illustrates a rare opportunistic infection in a critically ill patient. The patient underwent an extended hospital course during which he required high-dose steroids, likely contributing to the development of fungemia due to C. laurentii. Other risk factors in this patient included immunocompromised state (low CD4 count), prior exposure to azoles, and invasive central catheter placement. He was treated with amphotericin B and flucytosine, but due to the severity of his illness and multiple comorbid conditions, the patient succumbed to his illness.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Table 2. Antifungal treatment regimens for non-neoformans cryptococcal infections

| Infection severity | Treatment Level of recommendation |
|--------------------|----------------------------------|
| CNS/severe induction therapy | Induction: amphotericin ± flucytosine B-III |
| CNS/severe consolidation therapy | Consolidation: fluconazole ≥400 mg/day (if susceptible in vitro) B-III |
| Non-CNS/mild to moderate Preferred therapy: amphotericin B C-III |
| Prophylaxis | Primary: none Select high-risk populations may be treated with azole therapy per clinical judgment* B- I |

*Areas of high prevalence, increased antiretroviral resistance, or lack of access to antiretroviral therapy. CNS = central nervous system.