Case report

First report of Cryptococcus laurentii meningitis and a fatal case of Cryptococcus albidus cryptococcaemia in AIDS patients


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We report the first case of Cryptococcus laurentii meningitis and a rare case of Cryptococcus albidus cryptococcaemia in AIDS patients. Both infections were treated with amphotericin B and flucytosine. The C. laurentii meningitis was controlled after 2 weeks of treatment with no evidence of infection 20 months later. The patient with C. albidus cryptococcaemia, despite the amphotericin B/flucytosine combination therapy, died on the 14th day of treatment. The minimum inhibitory concentrations (MICs) for C. laurentii, as determined by Etest on RPMI 1640 agar, were 0.25 μg ml⁻¹ of amphotericin B, 1.25 μg ml⁻¹ flucytosine, 4 μg ml⁻¹ fluconazole, 0.5 μg ml⁻¹ itraconazole and 1.0 μg ml⁻¹ of ketoconazole. The MIC of amphotericin B for C. albidus was 0.5 μg ml⁻¹, flucytosine 1.25 μg ml⁻¹, fluconazole 4 μg ml⁻¹, itraconazole 0.5 μg ml⁻¹ and ketoconazole 0.25 μg ml⁻¹. The agreement of the amphotericin B MIC values obtained in antibiotic medium 3 by the broth microdilution method, with those obtained on casitone medium by Etest, was within a two-dilution range for both isolates. C. laurentii may cause meningitis and may also involve the lungs in AIDS patients.

Keywords AIDS, Cryptococcus albidus, Cryptococcus laurentii, cryptococcaemia, meningitis

Introduction

Cryptococcosis is a serious infection often occurring in immunocompromised hosts and is frequently used synonymously with infection caused by Cryptococcus neoformans [1]. Recent reports have indicated an increasing incidence of the infection among patients with the acquired immunodeficiency syndrome (AIDS) [2, 3]. Despite the recognition of several species of the genus, the non-C. neoformans species are generally regarded as non-pathogenic saprophytes. There are only a few previously reported putative cases [4–8] of cryptococcoses due to C. albidus in immunocompromised patients, and one case of septicemia in an AIDS patient reported recently [9]. Although six cases of C. albidus meningitis, some more adequately documented than others, have been published we were able to find only three previous reports on C. laurentii extrameningeal infection: one in an immunocompromised patient [10], the second in a patient with chronic uveitis where C. laurentii was the aetiological agent of cryptococcal endophthalmitis [11] and the third in a bone marrow transplant recipient [12]. The former two cases have been the subject of dispute due mainly to the questionable mycological data concerning the identification of the organism [13,14].
We describe two rare manifestations of cryptococcosis due to non-\textit{C. neoformans} species in AIDS patients. To our knowledge, there are no published reports on the isolation of \textit{C. laurentii} from AIDS patients, and therefore it is believed that \textit{C. laurentii} meningitis is the first reported case.

**Case history 1**

A 34-year-old Caucasian heterosexual male, from the island of Lesbos (East of the Aegean sea), a drinker usually of home-made wine, a non-smoker, whose HIV infection had been diagnosed in 1990, had a CD4 count at that time of $427 \times 10^3 \text{cells} \text{mm}^{-3}$.

He attended a local clinic and was diagnosed with onychomycosis attributable to \textit{Trichophyton rubrum}, for which he was prescribed terbinafine; 1-5 months later he developed facial varicella-zoster infection and treated with acyclovir. The patient presented in July 1996 with a 20-day history of dyspnoea on exertion, dry cough, fever (38–39 °C), anorexia and concomitant weight loss (65 kg), frontal headache, dizziness and bilateral diplopia. Physical examination revealed an acutely ill, lethargic, feverish (40.5 °C) individual, with signs of crusting facial varicella-zoster, persisting tinea unguium infection due to \textit{T. rubrum} and Kaposi’s sarcoma of the left nostril.

His blood pressure was 95/65 mm Hg and pulse 115 min^{-1}. The patient’s haematocrit was 40.9\%, haemoglobin was 13.1 g dl^{-1}, a leucocyte count of $4.7 \times 10^9 \text{cells} \text{mm}^{-3}$ with a differential of 65\% neutrophils, 25\% lymphocytes and 10\% monocytes; the platelet count was 360 $\times 10^9 \text{cells} \text{mm}^{-3}$ and the CD4 count 99 $\times 10^6 \text{cells} \text{mm}^{-3}$. Au-
nalysis revealed bilateral interstitial infiltrates. The patient had signs of meningeal involvement and a lumbar puncture was performed. The cerebrospinal fluid (CSF) was clear, the leucocyte count was $2 \times 10^3 \text{cells} \text{mm}^{-3}$, protein = 23 mg dl^{-1} and glucose = 0.51 g\%. India ink preparations revealed the presence of encapsulated ovoid yeast cells, while five sets of blood cultures were negative. He was immediately started on intravenous amphotericin B (40 mg day^{-1}) and fluycytosine (10 g day^{-1}), while treatment with zido-
vudine (ZDV) and didanosine (ddI) was initiated.

After 14 days of therapy fever and headache subsided, his diplopia improved, he started gaining weight and his chest roentgenogram showed marked clearing. He was discharged 3 weeks later, on fluconazole maintenance treatment (200 mg day^{-1}). At the time of the most recent follow-up, March 1998, his roentgenogram remained stable, and on physical examination no evidence of cryptococcal infection was detected. He is currently on triple antiretroviral therapy (Indinavir, lamivudine, ddC).

**Mycology**

Cerebrospinal fluid (CSF) cultures on Sabouraud glu-
cose agar (SGA) agar at 37 °C revealed the presence of slow-growing hyaline colonies consisting of en-
capsulated ovoid yeast cells. Five different single colonies were selected from the original plate, streaked onto five different SGA-containing plates and incubated at 30 °C for 48 h. Colonies from each of the five different sub-
cultures were selected for further physiological and bio-
chemical studies. After 3 days at 25 °C in malt extract (OXOID, Basingstoke, UK), cells from each of the five subcultures were identical. They appeared ovoid in short chains of three or four cells, 2.5–3.5–7.8 μm, with capsules ranging between 0.3 and 0.5 μm in thickness. The streak culture after 1 month on malt extract agar (OXOID) was pink to orange, pasty with lobate border.

No pseudomycelium was produced on slide culture on potato agar. Assimilation tests were performed using the API ID 32 yeast identification system. The species profile was compatible with that of \textit{C. laurentii} in the API APILAB database (bioMerieux, Marcy d’Etoil, France). Nitrate assimilation was negative and the strain was capable of producing starch. All tests were subsequently run simultaneously with the NCPF 3760 standards (NCCLS) guidelines for drug concentrations and inoculum size [15] in antibiotic medium 3 (OXOID) supplemented with 2% glucose, revealed an am-
photericin B (Squibb & Sons, UK) MIC of 0.15 μg ml^{-1}. The quality control strains ATCC 90028 and 90018 MICs were 1.0 μg ml^{-1} and 1.5 μg ml^{-1}, respectively.

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Case history 2

A 47-year-old Caucasian heterosexual female with AIDS from the North West of Greece, whose HIV infection had been diagnosed in 1992 and whose CD4 count at that time was $220 \times 10^3 \text{ cells}^{-1}$, was receiving zidovudine treatment, to which ddl was added in January 1995. Her clinical course was complicated by central nervous system toxoplasmosis (August 1995), wasting syndrome, fever of unknown aetiology and myelodysplastic syndrome (January 1996). In March 1996 she presented with a 20-day history of high fever (41 °C). Her blood pressure at presentation was 90/70 mmHg, pulse 112 min$^{-1}$, with signs of meningeal involvement. On examination she was acutely ill and marasmic and weighed 32 kg. The haematocrit was 22% and glucose and the microscopic examination of CSF such as in the clinical specimens were excluded by repeatedly testing individual colonies from subsequent subcultures of the tested colonies had the same physiological characteristics and carbon assimilation profiles compatible with presence of the saprobic non-capsulated yeast C. albidus. After 1 month at 20 °C, the streak culture on malt extract agar was tan, smooth, glossy and slimy with the ability of the isolate to assimilate a variety of carbohydrates was determined using the API ID 32 yeast identification system as described [6,14]. Both C. laurentii and C. albidus are pathogenic fungi but the pathogenicity of the former is extremely rare [3–11] and poorly substantiated [13,14]. The broth microdilution method [15] revealed an amphotericin B MIC of 0.31 μg ml$^{-1}$ in antibiotic medium 2 supplemented with 2% glucose.

Discussion

Fungal infections caused by C. neoformans are most commonly seen in patients with defective T-lymphocyte function. In the setting of AIDS many uncommon fungal diseases are frequently reported [3]. However, reports of infection due to other species of Cryptococcus such as C. albidus, C. laurentii and C. luteolus are extremely rare [3–11] and poorly substantiated [13,14]. In the present study evaluation of clinical data suggested that C. laurentii lung involvement could be associated with meningeal infection. This observation has been reported for the first time in an AIDS patient.

It must be noted, however, that C. albidus is usually isolated from the skin of healthy individuals and from indoor and outdoor air. In addition, C. laurentii is a saprobic organism and a known contaminant during the fermentation process of wine and beer [16]. Both these species have very rarely been reported to cause disease [10,11]. Despite the confirmed exclusive growth of C. laurentii and C. albidus, precautions were taken in each case to exclude the possibility of contamination of clinical material through repeated isolation of the organisms from the clinical specimens. The likelihood of the involvement of C. neoformans and the transient presence of the saprobic non-C. neoformans species in the clinical specimens were excluded by repeatedly testing individual colonies from subsequent subcultures of the original isolates. All individual colonies demonstrated the same morphological and biochemical characters conforming to those of C. laurentii and C. albidus, respectively.

Unlike previously reported cases of fungaemia where non-specific symptoms were described and laboratory diagnosis was based on a single positive blood culture [6,14] C. albidus fungaemia in this case was characterized by typical symptoms and the organism was isolated from two consecutive blood cultures. In previously reported cases no Cryptococcus-like cells were...
found in CSF [5,10]. In the present case of C. laurentii meningitis typical encapsulated yeast cells were detected upon microscopic examination of CSF with India ink. Moreover, both our isolates grew at 37 °C upon incubation of the clinical samples. This is compatible with that recorded for most strains of C. albidus and C. laurentii that can grow at 37 °C [16]. It is noteworthy, however, that after several subcultures both isolates grew poorly at 37 °C and profusely at 28–30 °C.

Despite the repeated isolation of both non-C. neoformans species from clinical material and their identification with physiological and biochemical tests, it was necessary to obtain further confirmation of identification of the isolates by comparison of our isolates with reference strains. Both isolates were deposited to the National Collection of Pathogenic Fungi, at the PHLS, Bristol, UK, and were assigned the following numbers: for C. laurentii NCPF 8225 and for C. albidus NCPF 8249. Although C. albidus has been reported to share many biochemical characteristics and some capsular antigens with C. neoformans, cryptococcal antigen latex agglutination testing has been reported to yield negative results in cases of C. albidus infection [8]. In both cases presented here the in vitro latex agglutination tests for cryptococcal antigen were negative.

The treatment of choice for the more common meningeal and extrameningeal cryptococcal infections due to C. neoformans is amphotericin B and flucytosine. This combination therapy has been reported to control 90% of patients, including those with AIDS, whose CSF was sterile after 2 weeks of treatment [15–21].

Treatment regimens comprising amphotericin B combined with fluconazole have been found to be effective and well tolerated in first-line therapy in patients with AIDS-associated meningeal cryptococcosis [22].

Both our patients were treated with the conventional combination therapy of amphotericin B and flucytosine. Thus far the combination of fluconazole and flucytosine has been employed in treating C. neoformans infections. In all these cases clinical data were supported by in vitro studies on a large number of C. neoformans strains [23–25].

Different treatment schemes have not been employed on infections attributable to any other Cryptococcus species, except in a previously reported case of cutaneous mycosis attributed to C. laurentii, where fluconazole monotherapy was sufficient to treat the infection [11].

The C. laurentii meningeal infection we report here corresponded well to the combination therapy of amphotericin B and flucytosine within 14 days, and no relapse was detected until March 1998. The patient is still on low-dose fluconazole maintenance treatment of 200 mg day⁻¹. In contrast our patient, who was infected with C. albidus, was at the latest stage of AIDS and the C. albidus septicaemia was the terminal event.

This is the first time that non-C. neoformans clinical isolates have been evaluated as to their susceptibility to antifungals. Interestingly, the Etest MICs of amphotericin B on RPMI 1640 agar were higher, 0.25 µg ml⁻¹ and 0.5 µg ml⁻¹ for C. laurentii and C. albidus, respectively, that those recorded on Casitone medium (0.19 µg ml⁻¹ and 0.25 µg ml⁻¹) for each Cryptococcus species, respectively. The microdilution method performed in antibiotic medium 3 revealed MIC values closer to those obtained by the Etest on Casitone medium, 0.15 µg ml⁻¹ and 0.37 µg ml⁻¹ for C. laurentii and C. albidus, respectively. This indicates that the agreement between the MIC values of amphotericin B obtained on casitone medium by Etest and in antibiotic medium 3 by the microdilution method are in better agreement than those obtained by the Etest on RPMI 1640.

Cryptococcosis attributable to non-C. neoformans species requires extensive investigation in order to be substantiated. Provided the possibility of contamination of clinical specimens with such species is excluded, the potential of saprobic cryptococcal species to infect AIDS patients cannot be eliminated.

The epidemic of AIDS has set new challenges to the clinician and the mycologist by broadening and intensifying studies on the emerging opportunistic infections. It has been estimated that for every million AIDS patients 50 000–100 000 will contract cryptococcosis [2]. The unusual emerging cryptococcal, as well as other, fungal infections [26] will require novel strategies in the surveillance of patients at risk, in preventive measures and in basic and applied research.

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References