Specific antibody response in a patient with *Candida dubliniensis* fungemia

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Summary

We report a case of fungemia caused by *Candida dubliniensis* in a non-HIV infected patient. Multiple cultures of blood performed over a period of 13 days were positive for this recently described yeast species. The *C. dubliniensis* isolates recovered were susceptible to fluconazole *in vitro* and the patient responded to intravenous therapy with this antifungal agent. It was possible to differentiate the fungemia caused by *C. dubliniensis* in this patient from that caused by *C. albicans* in other patients on the basis of the analysis of the antibody response since the *C. dubliniensis*-infected patient exhibited a characteristic and specific antibody response against a cell wall component of 160-170 kDa.

Key words

Candida dubliniensis, Fungemia, Antigens, Antibodies, Serology

Respuesta de anticuerpos específica en un paciente con fungemia por *Candida dubliniensis*

Resumen

Se describe un caso de fungemia causada por *Candida dubliniensis* en un paciente sin infección por el Virus de la Inmunodeficiencia Humana. El paciente presentó múltiples hemocultivos positivos para *C. dubliniensis* durante un periodo de 13 días. Los aislamientos de *C. dubliniensis* fueron susceptibles al fluconazol *in vitro* y el paciente respondió al tratamiento intravenoso con este antifúngico. El caso demuestra que, a pesar de las estrechas relaciones fenotípicas y genotípicas que presentan *C. dubliniensis* y *Candida albicans*, es posible difereciar la fungemia causada por *C. dubliniensis* de la producida por *C. albicans* en base al análisis de la respuesta de anticuerpos, ya que el paciente infectado por *C. dubliniensis* presentó una respuesta de anticuerpos característica y específica frente un componente de la pared celular de 160-170 kDa.

Palabras clave

Candida dubliniensis, Fungemia, Antígenos, Anticuerpos, Serología

Candida fungemia is a common complication in hematological patients and abdominal surgery patients. The main etiological agent is *Candida albicans* but other species are being isolated with increasing frequency [1]. *Candida dubliniensis* is a recently described species which shows a worldwide distribution [2] and a close phenotypic and genotypic relationship with *C. albicans* [3]. Although it seems to be primarily associated with recurrent oral infections in HIV-infected individuals, more recently it has also been isolated from healthy persons as well as cases of superficial and systemic disease in non-HIV infected patients [4-8]. We present here a case of fungemia caused by *C. dubliniensis* in an HIV-negative patient which resolved following therapy with fluconazole. Serological tests were performed to determine if it was possible to differentiate infection by *C. dubliniensis* from that by *C. albicans* on the basis of the detection of the antibody response.

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CASE REPORT

A 35-year-old woman user of intravenous cocaine was admitted to the Marqués de Valdecilla Hospital (Santander, Spain) on 8 October 1999 with a clinical picture of low grade fever, malaise and chills. Three months before she had a tricuspid endocarditis, pulmonary septic emboli and bacteriemia caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which were successfully treated with cloxacillin and gentamicin. On admission, the patient was febrile (axillary temperature, 37.6°C). Physical examination revealed a marked pallor, septic mouth, laterocervical adenopathy and signs of venepuncture and thrombophlebitis. Laboratory tests included a hemoglobin level of 6.2 g/dl and hematocrit of 18%. The white blood cell count was 7,600/mm^3, with 76% polymorphonuclear leukocytes, 18% band forms, and 5% lymphocytes. Serological tests for HBV and HCV infection were positive. HIV serology was negative. A chest roentgenography showed nodular lesions in the right upper lobe and a lower left lobe pleural effusion. An hepatosplenomegaly was detected by an abdominal ultrasonogram and a vegetation in the tricuspid valve (2.1 x 1.2 cm) was demonstrated by cardiac ultrasonography. Cerebral computed tomography examination was normal. A blood culture taken on admission yielded *C. dubliniensis*. The same yeast was isolated from blood cultures taken on days 1, 3 and 12 post-admission. Intravenous antifungal treatment with fluconazole (200 mg/12 h) was instituted on the first day of hospitalization for a total of 37 days. Antibiotic therapy included cloxacillin, piperacillin-tazobactam and ciprofloxacin. The patient condition improved and she was discharged on November 16th on methadone maintenance therapy.

Mycological investigation. The identification of the isolates was initially performed by their assimilation profile using the API ID 32 C yeast identification system (bioMérieux, Marcy l'Etoile, France). They were identified with a 99.9% probability as *C. dubliniensis* and the code obtained was 7142100015. Several tests were performed to confirm this identification. All isolates produced germ tubes on horse serum and chlamydospores on cornmeal agar. The isolates failed to grow at 45˚C and the colonies showed a dark green color on the chromogenic medium CHROMagar Candida after 48 h of incubation. Finally, all isolates were strongly positive by indirect immunofluorescence using an anti-*C. dubliniensis* specific antiserum [9]. Identification was confirmed by Dr. D. Coleman at the School of Dental Science, University of Dublin, Republic of Ireland, using PCR with *C. dubliniensis*-specific primers [10]. One of the isolates has been deposited in the Colección Española de Cultivos Tipo (CECT n˚ 11473).

A serum specimen taken on day 15 post-admission was used to study the anti-*Candida* antibody response by indirect immunofluorescence and Western blotting. Sera from two patients with fungemia caused by *C. albicans* were used as controls. The *C. albicans* isolates from these patients were compared to *C. albicans* reference strains NCPF 3153 and 3156 by indirect immunofluorescence and immunoblotting using anti-*C. dubliniensis* and anti-*C. albicans* antisera [9], and assimilation profiles using the API ID 32 C yeast identification system in order to ensure that they were *C. albicans* and not *C. dubliniensis*. Indirect immunofluorescence assay (IFA) was carried out by a modification of a previously described method [9]. Briefly, blastospores from *C. dubliniensis* (NCPF 3949) grown on Sabouraud agar plates for 48 h at 37˚C, were resuspended in PBS at a cell density of 1 x 10^6 cells/ml

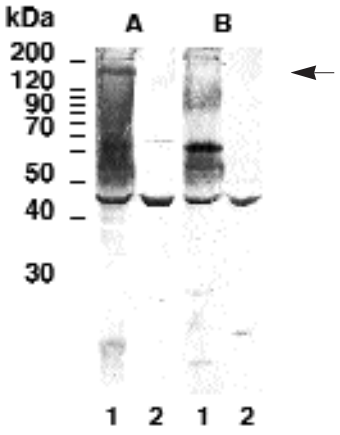

Figure 1. Immunoblot analysis of 12.5% (w/v) slab gels loaded with extracts from *C. dubliniensis* NCPF3949 (lane 1) and *C. albicans* NCPF3153 (lane 2), stained with the serum from the patient with fungemia caused by *C. dubliniensis* (A) and the serum from a patient with fungemia caused by *C. albicans* (B). Molecular weights of standard proteins (in kDa) are listed to the left of the gel. The arrow points to the cell wall component of 160-170 kDa.

and placed on Teflon coated immunofluorescence slides. The slides were incubated with serial dilutions of the patient's sera, which had been previously adsorbed three times with *C. albicans* formalin-killed blastospores, washed and the reacting antibodies were revealed by incubation with FITC-conjugated anti-human polyvalent immunoglobulins (Nordic Immunology, Tilburg, The Netherlands).

To obtain cell wall antigenic extracts, *C. dubliniensis* (NCPF 3949 and CD33) [3] and *C. albicans* (NCPF 3153 and NCPF 3156) reference strains were grown in medium 199 (Sigma Chemical Co., St. Louis, Mo.) at 37˚C and the cell walls were extracted in the presence of dithiothreitol (DTT; Sigma) as reported previously [11]. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of yeast cell wall extracts was performed as previously described [9]. Proteins contained in gels were electrophoretically transferred to PDVF membranes (Westran, Schleicher & Schuell, Dassel, Germany) using a fast blot system (Biometra, Göttingen, Germany). After transfer, the membranes were blocked in 8% (w/v) nonfat dry milk in Tris-buffered saline, incubated with the patient's sera adsorbed three times with *C. albicans* formalin-killed blastospores [9], and finally incubated again with peroxidase-conjugated anti-human polyvalent antibodies (Sigma). Immunoreactive bands were visualized with 4-chloro-1-naphthol following standard procedures.

Small differences in antibody titers were observed when the adsorbed sera were studied by IFA. The serum from the *C. dubliniensis* infected patient showed an anti-*C. dubliniensis* antibody titer of 32 and an anti-*C. albicans* antibody titer of 8. However, the sera from the *C. albicans*- infected patients showed anti-*C. dubliniensis* and anti-*C. albicans* antibody titers of 8. By Western blotting, the serum from the *C. dubliniensis*-infected patient showed a higher reactivity with the antigenic extracts from *C. dubliniensis* strains than with those from *C. albicans* strains (Figure 1a). In both *C. dubliniensis* extracts it was possible to identify an immunodominant antigen of 160-170 kDa not observed in the extracts from *C. albicans*. The sera from patients with fungemia caused by *C. albicans* showed no reactivity with the component of 160-170 kDa from *C. dubliniensis* extracts (Figure 1b).

In vitro susceptibility of the *C. dubliniensis* isolates to fluconazole, amphotericin B, flucytosine, ketoconazole and itraconazole was performed by the Sensititre YeastOne Colorimetric Antifungal Panel (AccuMed International, Westlake, Ohio). *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were included as quality control strains. The *C. dubliniensis* isolates were susceptible to all antifungals tested. The MICs for the isolates were ≤0.0125 µg/ml for fluconazole, 0.25 µg/ml for amphotericin B, ≤0.03 µg/ml for flucytosine, ≤0.008 µg/ml for ketoconazole and 0.03 µg/ml for itraconazole.

DISCUSSION

Due to the recent description of *C. dubliniensis* and its close relationship with *C. albicans* there is very little information about the pathogenic potential of this yeast species. *C. dubliniensis* has been mainly associated with oral candidasis in HIV-infected patients but it can also cause oral or systemic disease in non-HIV-infected individuals. Meis and colleagues [5] have reported three cases of candidemia by *C. dubliniensis* from Europe in HIV-negative patients with chemotherapy-induced immunosuppression and bone marrow transplantation. In two of the cases *C. dubliniensis* was also isolated from stool, suggesting that the source of the infection may have been the gastrointestinal tract. Brandt *et al.* [6] have recently reported four cases of *C. dubliniensis* fungemia in the United States, three of them in HIV-negative patients with chronic lymphocytic leukemia or end-stage liver disesase. The candidemia reported in our case has little in common with those of the cases described by Meis *et al.* [5] since our patient was not neutropenic. Although the portal of entry for the fungemia was unknown, it may be related to the intravenous injection of cocaine. Interestingly, two of the patients reported by Brandt *et al.* [6] had a history of intravenous drug abuse and six *C. dubliniensis* isolates, initialy identified as *C. albicans*, have also been isolated from intravenous drug abusers in Spain [12].

The *C. dubliniensis* isolates were susceptible to fluconazole *in vitro* and the patient responded well to fluconazole therapy. This susceptibility is in agreement with that observed by Meis *et al.* [5] in *C. dubliniensis* isolated from blood cultures. However, resistance to fluconazole has been observed in some studies [13].

Identification of *C. dubliniensis* is often difficult because it shares many phenotypic characteristics with *C. albicans* and it may require the use of molecular-based techniques which may be difficult to perform in all clinical laboratories. The recent inclusion of many specific *C. dubliniensis* carbohydrate assimilation profiles in the database of the API ID 32C was an important aid in the initial identification of our isolates and it will facilitate the identification of this species in the future.

As in some *C. albicans* systemic infections [14], the isolation of *C. dubliniensis* from blood in patients with invasive disease may not always be possible. In those cases, a specific antibody response may be the basis for the diagnosis of the infection. This study provides evidence, for the first time, that it is possible to differentiate candidemia caused by *C. dubliniensis* from that caused by *C. albicans* since the *C. dubliniensis*-infected patient exhibited a characteristic and specific antibody response against a cell wall component of 160-170 kDa. If these results are confirmed in other invasive infections by *C. dubliniensis*, the anti-*C. dubliniensis* antibody response may be useful in the diagnosis of this infection when blood cultures are negative. Further studies are needed to confirm these preliminary results.

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