Could *Candida dubliniensis* be involved in lung fungal balls?

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Summary We describe a case of cavitary pneumonia due to *Candida dubliniensis* along with fungemia due to *Candida kefyr* in a leukemic patient. This is the first case of *C. dubliniensis* isolated in our laboratory. The identification was performed by phenotypic and molecular methods such as thermotolerance test, carbohydrate fermentation and polymerase chain reaction.

Key words *Candida dubliniensis*, *Candida kefyr*, Pneumonia, Fungus ball, Fungemia

¿Candida dubliniensis puede producir bola fúngica pulmonar?

Resumen Se presenta un caso de neumonía cavitaria con bola fúngica, probablemente producida por *Candida dubliniensis,* en un joven paciente leucémico que además sufría una fungemia por *Candida kefyr*. Este fue el primer aislamiento de *C. dubliniensis* en nuestro laboratorio. La identificación de esta especie fúngica fue realizada usando métodos fenotípicos y de biología molecular, tales como termotolerancia, fermentación de carbohidratos y reacción en cadena de la polimerasa.

Palabras clave *Candida dubliniensis*, *Candida kefyr*, Neumonía, Bola fúngica, Fungemia

An 18-year old male with malaise, weakness generalized pain was admitted to the hospital on 7 May 2003. His symptoms had begun two weeks before he was hospitalized. An acute lymphoblastic leukemia was diagnosed. Radiographs of the chest were normal. He received chemotherapy consisting of vincristine (2 mg/day iv, pulse) daunorubicin (45 mg/m^2 x 3 days), and prednisone (45 mg/day). The patient developed fever and a blood culture specimen was taken (BACTEC 9240 fluorescent series instruments; Becton Dickenson Diagnostic Instrument Systems, Cockeysvielle, Md). On 26 May 2003, growth was detected in blood culture vials and yeast cells were observed by microscopic examination. The yeast was identified as *Candida kefyr* by using conventional methods such as germ tube test, morphology on corn meal agar plates, and carbohydrate assimilation profile using ID32 C commercial kit (bioMerieux, France). Liposomal amphotericin B was started. One week later, chest X-ray revealed a right paracardiac, nodular lesion (Figure 1). Highresolution computerized tomography (HRCT) showed a 3,5x5 cm sized area of parenchymal consolidation with rough counters resembling a fungus ball on the right posterobasal segment of the lung (Figure 2). A bronchoalveolar lavage (BAL) fluid was collected with probable diagnosis of invasive fungal infection. BAL fluid samples were tested for possible causative microbial agents including *Mycobacterium tuberculosis*, all fungi including *Aspergillus* species and *Cytomegalovirus* (CMV), by microscopically, by culture and/or polymerase chain reaction (PCR) assay. Yeast cells were seen in the direct examination of the BAL specimen. Culture for *M. tuberculosis*, *Aspergillus,* other fungi and PCR results for *M. tuberculosis*, *Aspergillus* and CMV were negative. Yeast colonies were seen on Sabouraud dextrose agar plates. No other bacterial or fungal growth was observed. Yeast colonies were identified as *C. dubliniensis* by using conventional methods such as germ tube test, chlamydospore production, carbohydrate assimilation profile using ID32 C commercial kit (bioMerieux, France), and thermotolerance test at 42 °C and 45 °C, respectively. The identification of *C. dubliniensis* was confirmed by PCR using the previously designed primer pair, DUBF (5'-GTATTTGTCGTTCCCCTTTC-3') and DUBR (5'-GTGTTGTGTGCACTAACGTC-3') (Metabion GmbH, Martinsried, Germany), which yields *C. dubliniensis*-specific 288 bp fragment. Serological tests were performed with two consequently obtained blood

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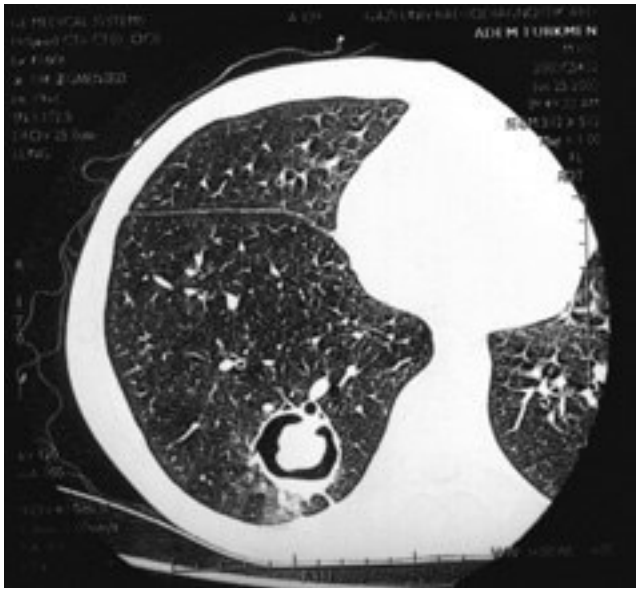

Figure 1. High resolution computerized tomography shows cavitated nodular lesion at the lower lobe of the right lung of the patient.

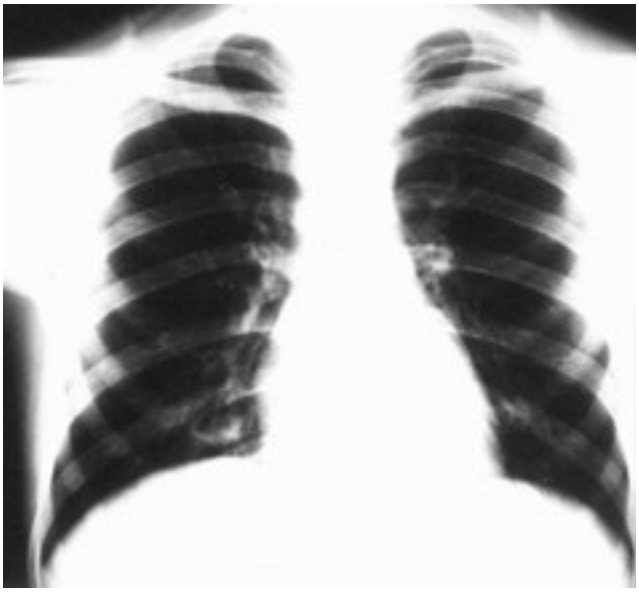

Figure 2. Chest X Ray reveals a cavitary image located on the paracardiac region on the right lung base.

specimens for *Aspergillus* galactomannan antigen by using ELISA (Platelia Aspergillus, BIO-RAD, France). The results were found to be negative. The follow-up chest radiographs and CT scan revealed a decrease in size of the lesion with prominent cavitation resembling fungus ball detected before. Therapeutic lobectomy was not indicated in this case due to the thrombocytopenia. The two *Candida* strains isolated from the patient were tested in vitro for fluconazole, itraconazole, and amphotericin B susceptibility according to NCCLS M27-A document. For *C. dubliniensis* amphotericin B, itraconazole and fluconazole MICs were found to be 0.03 µg/ml, 8 µg/ml, 128 µg/ml, respectively. For *C. kefyr* amphotericin B, itraconazole and fluconazole MICs were found to be 0.25 µg/ml, 0.062 µg/ml and 0.125 µg/ml, respectively. After a month of the antifungal treatment, second course of chemotherapy was started but the patient died on 4 july 2003 with intracranial hemorrhage related to trombocytopenia. Postmortem examination was not performed due to the refusal of the family.

This case is interesting due to the double infection of *C. kefyr* and *C. dubliniensis* of the same patient. The isolation of *C. kefyr* from blood culture was defined as proven invasive fungal infection with the presence of host factors and clinical signs according to EORTC/MSG criteria [1]. Recently, the European Organisation for Research and Treatment of Cancer (EORTC) Invasive Fungal Infections Cooperative Group and the Mycoses Study Group (MSG) of the National Institute of Allergy and Infectious Diseases (NIAID) published consensus definitions for defining opportunistic invasive fungal infections (IFI) based on a combination of host factors, clinical manifestations, and mycological results [1].

Recent studies described isolates of *C. dubliniensis* from different geographic areas, including Turkey [4,6,7]. There are limited number of pneumonia cases caused by *C. dubliniensis* [8]. Bonchoscopy and HRCT scans are mutually complementary diagnostic tools and should be performed as early as possible in the course of pneumonia in patients at high risk of fungal diseases [9] since they directly correlated to death in 84% of the patients [3]. There is no clear radiologic pattern indicative of candidal pneumonia. Miliary-nodular pattern and localized or diffuse bronchopneumonia can be seen [2]. However nodular pattern and cavitary masses have also been described [10]. Ball-like mass of lung cavity is termed "fungus ball" which is mostly specific for invasive pulmonary aspergillosis. However, our results of culture, galactomanan antigen and PCR studies for *Aspergillus* species were found to be negative. Although the lung biopsy can provide more diagnosis, it could not be performed due to the patient's poor general health status. In the present case, the chest radiograph showed a fungus ball, such finding has not been described previously. Although there was no histological confirmation from the cavity, the isolation of *C. dubliniensis* from the BAL strongly suggested the cause of the pneumonia. BAL is a good diagnostic predictor in leukemic patients with *Candida* pneumonia [5].

The diagnosis of the pulmonary pathogen was based only on BAL culture. Thus in the absence of a lung biopsy or autopsy, our diagnosis of *C. dubliniensis* pneumonia was classified under probable invasive fungal infections according to EORTC/MSG criteria.

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