Note

Isolation of *Aspergillus lentulus* in Spain from a critically ill patient with chronic obstructive pulmonary disease

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Summary

Aspergillus lentulus was first described in the year 2005, and since it cannot be phenotypically distinguished from *Aspergillus fumigatus,* it is conceivable that earlier descriptions (before 2005) could be attributed to this new species. Currently invasive infections caused by *A. lentulus* are rare and very few cases have been previously published in neutropenic patients, all of them with fatal outcome. Here we report a critically ill non neutropenic patient with chronic obstructive pulmonary disease (COPD) who was admitted to the medical intensive care unit with an exacerbation of COPD and who had been treated with long term corticosteroids. *A. fumigatus* was cultured from two bronchial aspirates and in a third bronchial aspirate both *A. lentulus* and *A. fumigatus* were isolated. On two consecutive days detection of galactomannan in serum was negative whilst detection of (1-3) beta-D glucan was positive (> 518 pg/ml). Minimal inhibitory concentrations (MIC) for itraconazole, voriconazole, caspofungin and amphotericin B were high for this strain of *A. lentulus*. Given the high MIC values of *A. lentulus* to available antifungals, the accurate identification of this new species is warranted. To our knowledge, this is the first report of the isolation of *A. lentulus* in a non-neutropenic critically ill patient, although we note that since it was isolated only once from respiratory specimens, its implication as an etiologic agent of infection for this patient remains to be established.

Key words *Aspergillus lentulus, Aspergillus fumigatus*, Chronic obstructive pulmonary disease, COPD, Corticosteroids, Spain, (1-3) beta-D glucan, Antifungals

Aislamiento de *Aspergillus lentulus* en España en un paciente crítico con enfermedad pulmonar obstructiva crónica

Resumen

Aspergillus lentulus es un hongo de reciente descripción (año 2005) y practicamente idéntico a *Aspergillus fumigatus*; posibles aislamientos de *A. lentulus* anteriores a 2005 eran identificados como *A. fumigatus*. Actualmente se han publicado muy pocos casos de infecciones invasoras causadas por *A. lentulus*, todos ellos con evolución fatal, en pacientes neutropénicos. Comunicamos el caso de un paciente crítico no neutropénico con enfermedad pulmonar obstructiva crónica (EPOC) admitido en la unidad de cuidados intensivos médicos con una exacerbación de EPOC y que había sido sometido a un tratamiento prolongado con corticosteroides.

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Se aisló *A. fumigatus* en dos aspirados bronquiales, y *A. lentulus* y *A. fumigatus* de un tercero. En dos días consecutivos la detección de galactomanano en suero fue negativa, mientras que la detección de (1-3) beta-D glucano fue positiva (>518 pg/ml). Los valores de concentración mínima inhibitoria (CMI) obtenidos con el itraconazol, el voriconazol, la caspofungina y la anfotericina B fueron altos para *A. lentulus*. Dado lo elevado de estos valores en *A. lentulus*, es necesaria la identificación precisa de esta nueva especie en aislamientos clínicos. A nuestro entender, este es el primer aislamiento de *A. lentulus* en un paciente crítico no-neutropénico, aunque como su aislamiento se realizó una sola vez de secreciones respiratorias (dada la dificultad de la obtención de biopsias en este enfermo por su situación comprometida), su implicación como agente etiológico de infección es dudosa en este enfermo.

Palabras clave *Aspergillus lentulus, Aspergillus fumigatus*, Enfermedad pulmonar obstructiva crónica, EPOC, Corticosteroides, España, (1-3) beta-D glucano, Antifúngicos

The epidemiology of invasive fungal infections (IFI) in the setting of critically ill nonneutropenic patients is changing. Although *Candida* spp. remain the cause of over 80% of IFI's [12], *Aspergillus* is now the second most important fungal agent, and *Aspergillus* infections are currently recognized with increasing frequency [7,10,11].

Aspergillus fumigatus can be morphologically variable and several new species have been proposed on the basis of phenotypic characteristics [2,3,8,18]. Balajee et al. [2] using phylogenetic methods described a new *Aspergillus* species, *Aspergillus lentulus*, as a cause of invasive aspergillosis in four hematopoietic stem cell transplant recipients. Since the initial description, only a small group of isolates have been described [3,18]. This new species showed decreased in vitro susceptibilities to available antifungals and all four patients described by Balajee et al. [2] had a fatal outcome. We herein report the isolation of *A. lentulus* in a patient with an *A. fumigatus* probable invasive pulmonary aspergillosis admitted to a medical intensive care unit (MICU) with an exacerbation of chronic obstructive pulmonary disease (COPD) who had been treated with corticosteroids.

Case report

A 68-year-old male smoker with an 8-year history of COPD with long term inhaled and systemic corticosteroids when acute exacerbations of COPD occurred, was admitted to our hospital in April 10th, 2006 due to respiratory infection. The patient presented widespread osteoporosis with wedged lesions in vertebras (D11, D12, L2, L3 and L4) and type II diabetes *mellitus*. The patient was treated with iv ceftriaxone and later due to atrial fibrillation and heart congestive failure with digoxin and diuretics. In April 25th, 2006 the patient was admitted in the MICU due to acute respiratory failure secondary to severe bronchospasm. For five days the patient needed non invasive mechanical ventilation but due to deterioration of the pulmonary function with bilateral images of lung condensation on chest radiography, the patient required mechanical ventilation which was maintained thereafter. There were no remarkable pathogens in the microbiological study of respiratory samples. Due to the compromised clinical situation of the patient, computed tomography of the chest was not done. On day 13 of MICU stay, a pure culture of typical *A. fumigatus* colonies in a bronchial aspirate was obtained and iv voriconazole treatment was started. On day 16, on a second bronchial aspirate a pure culture of *A. fumigatus* was again obtained. On day 17 the bronchial aspirate yielded a mixed fungal culture with typical colonies of *A. fumigatus* and white colonies of a mycelial fungus of slow sporulation later identified as *A. lentulus*. The patient's condition deteriorated and he died due to multiorganic failure on day 17. The mean SAPS II score was 34 and APACHE II score was 20 and the length of ICU stay was 17 days. Permission for necropsy was denied. The patient was included in a study that had been approved by the ethics committee of the Hospital 12 de Octubre and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The patient gave his informed consent prior to his inclusion in the study.

Mycological studies

Growth characteristics of the two fungal isolates yielded by the bronchial aspirate were studied on potato dextrose agar (Becton Dickinson, Sparks, MD), and Sabouraud dextrose agar (Difco; Becton, Dickinson and Co., France) at 25, 37, 45 and 48 °C for 5 days. One of the fungal isolates showed the typical characteristics of *A. fumigatus* isolates, including green colonies, abundant sporulation and profuse growth at all temperatures tested (Figure). However, the other isolate resembled *A. lentulus* in that it sporulated poorly, it did not grow at all at 48 °C and had smaller conidial heads with diminutive vesicles compared to *A. fumigatus* (Figure). For the confirmation of this presumptive identification as *A. lentulus* and to exclude other possible identities, the ß-tubulin (benA-F, 5-AAT TGG TGC CGC TTT CTG G-3, and R, 5-AGT TGT CGG GAC GGA ATA G-3) and rodlet A (rodA-F, 5-GCT GGC AAT GGT GTT GGC AA-3, and R, 5-AGG GCA ATG CAA GGA AGA CC-3) regions of the DNA obtained from the atypical isolate were sequenced as described by Balajee et al. [2]. The sequences obtained were compared with that of the GenBank database, showing 99% homology with the type strain of *A. lentulus* (FH5).

Antifungal susceptibility was tested using the Etest (AB BIODISK, Sweden).

Multiple antifungal drugs showed variable in vitro susceptibilities in the case of *A. fumigatus:* amphotericin B (MIC 0.75 µg/ml), itraconazole (MIC 1 µg/ml), voriconazole (MIC 0.125 µg/ml), and caspofungin (MIC > 32 µg/ml), whilst MIC values for amphotericin B (MIC 4 µg/ml),

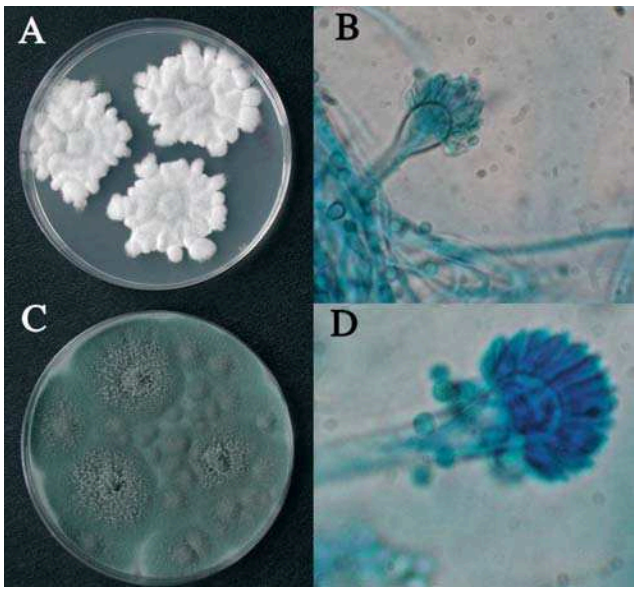

Figure. Colony morphology of *A. lentulus* (A) and *A. fumigatus* (C) isolates. Microscopy images of lactophenol cotton blue-stained conidial heads of *A. lentulus* (B) and *A. fumigatus* (D) isolates. Magnification, x200.

itraconazole (MIC 3 µg/ml), voriconazole (MIC 1.5 µg/ml), and caspofungin (MIC > 32 µg/ml) were high for the *A. lentulus* isolate.

On day 16 galactomannan (GM) (Platelia *Aspergillus,* Bio-Rad, France) detection in serum was negative (index 0.166) but detection of (1-3) beta-D glucan (BG) (Fungitell, Associates of Cape Cod, USA) was positive (> 518 pg/ml). On day 17 of MICU stay GM index was 0.061 and BG assay in serum continued positive (> 518 pg/ml).

Discussion

Currently the in vivo diagnosis of invasive aspergillosis is a clinical challenge. In the setting of critically ill patients the diagnosis is even more difficult, since the clinical picture is non-specific, fever is usually absent and other morbidities are usually present. In addition, these patients may not have the classical predisposing factors. It appears that the prevalence of invasive aspergillosis in MICU patients is increasing in patients without leukemia or cancer [7,13] and the most prevalent species is *A. fumigatus* [11].

In a retrospective study of 1,850 patients in a MICU, Meersseman et al. [10] reported that in a group of 89 patients without hematologic malignancy there were 67 patients with proven (30) and probable (37) invasive aspergillosis. Interestingly 31 of these patients had COPD treated with corticosteroids. The rate of proven and probable invasive aspergillosis was 3.7% in this group of 1,850 patients [10]. When corticosteroids are administered continuously at low doses as in our patient, the development of invasive aspergillosis is facilitated, as an early report of Rello et al. [15] has shown. This fact has been widely reported [6,7,10,11,13]. The administration of corticosteroids for the management of septic shock with adrenal hyporesponse is widely used in the setting of MICU patients [7,9]. Corticosteroids impair the neutrophil and macrophage function and also increase the growth rate of *A. fumigatus* and *A. flavus* in vitro [14].

The gold standard for the microbiological diagnosis of invasive aspergillosis requires demonstration of hyphae in tissue and/or the culture of *Aspergillus* species from sterile specimens [1]. In MICU patients requiring artificial or non-invasive ventilation, biopsies or needle aspiration is not feasible. The definitions of Ascioglu et al. [1] are suitable for patients with hematologic malignancies or recipients of stem cell transplants but these criteria are not evidenced based through large prospective studies with well-designed multivariate analysis as Vandewoude and Vogalaers comment in a recent seminal editorial article [17]. It is important to point out that invasive pulmonary aspergillosis may occur in other different populations in which risk groups and clinical-radiological data are currently poorly defined as is the case in critical ill patients in which invasive pulmonary aspergillosis is emerging as an important problem [6,7,10,11].

According to the definitions of Ascioglu et al. [1], the case described in this report can be classified as a probable invasive pulmonary aspergillosis since three bronchial aspirates were positive for *A. fumigatus* and one of them also for *A. lentulus.* In addition to this, BG detection was positive on two consecutive days. In critically ill non-neutropenic patients exposed to corticosteroids, as the patient reported herein, GM would appear to achieve a low diagnostic yield possibly due to the fact of lower angioinvasion [4,5,11].

In 2005, Balajee et al. [2] reported four fatal invasive pulmonary aspergillosis infections in four hematopoietic stem cell recipients at the Fred Hutchinson Cancer Research Center, Seattle, WA (USA) caused by a new *Aspergillus* species designated *A. lentulus* following phylogenetic methods based on multilocus sequence typing of five genes. This new species has been isolated in soil and air samples [16], it does not survive at 48ºC and has in vitro high MIC values to available antifungal agents, although the clinical significance of this fact is currently poorly understood. Our isolate shares with those described by Balajee et al. [2] higher MIC for itraconazole, voriconazole, caspofungin and amphotericin B when compared with those of *A. fumigatus*.

The prevalence of *A. lentulus* is unknown, but the fact that this species has been isolated in different countries suggests that it is ubiquitous [2,3,18]. The isolation of *A. lentulus* only in the last bronchial aspirate in association with *A. fumigatus* makes difficult to ascertain the clinical relevance of *A. lentulus* in this patient. However, the possibility of a contamination of the specimen is unlikely. It is possible that *A. lentulus* was selected due to its poor susceptibility to voriconazole.

To our knowledge this is the first isolation of *A. lentulus* in a non-neutropenic critically ill patient with an exacerbation of COPD treated with corticosteroids.

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