Case Reports

Aspergillus viridinutans: an agent of adult chronic invasive aspergillosis

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In contrast with the common hematogenous dissemination of invasive aspergillosis (IA), we present case with a protracted course through anatomical planes in an immunocompromised adult male. The unusual clinical features and laboratory findings led to fungal genotyping and identification of the mold as Aspergillus viridinutans. It appears to be the first described case of IA caused by this agent in an adult patient.

Keywords Aspergillus viridinutans, adult patient, invasive aspergillosis, fungal genotyping, antifungal susceptibility

Introduction

The incidence of invasive aspergillosis (IA) has been increasing steadily for the past few decades [1,2].

The most common etiological agent of IA is Aspergillus fumigatus, followed by Aspergillus niger, Aspergillus flavus, Aspergillus terreus [3,4] and other members of Aspergillus section Fumigati [5].

Aspergillus viridinutans, a mold of Aspergillus section Fumigati, was originally isolated from rabbit dung in Australia and from soil samples collected from around the world [6,7]. It has subsequently been identified during retrospective analyses of Aspergillus species in cultures [8–10] but its clinical importance has not yet been defined [11].

Here we describe the clinical case of a 56-year-old immunocompromised man who died from IA which exhibited distinct clinical manifestations compared to infections caused by A. fumigatus. We also present the morphological characteristics, genotype-based identification and the antifungal susceptibility of the isolate. To the best of our knowledge, the case described here is the first in an adult patient and the third in the literature, following two cases reported in children due to A. viridinutans [11].

Case report

A 56-year-old Caucasian male with diabetes mellitus type II and rheumatoid arthritis, had been treated since 2003 with: prednisolone, 17.5 mg/day; etarnecept, 50 mg/day; methotrexate, 2.5 mg 3 ×/week; salazopirine, 1,500 mg/day; and metformine, 1,700 mg/day. Due to the observation in March 2007 of a single pulmonary nodule in a routine chest X-ray, a computed tomography (CT) was ordered which revealed a nodular, 26 mm in diameter, hypodense, irregular, spiculated lesion, suggestive of a neoplastic process, located in the posterior segment of the upper lobe of the right lung. The remaining upper lobe exhibited a micro-reticulo-nodular pattern. Trans-thoracic needle aspiration biopsy and cytological examination of the aspirate revealed septate hyphae suggestive of Aspergillus but the search for malignant cells was negative. At the microbiology laboratory, septate hyphae were found on direct exam (Fig. 1) but cultures of the material, although incubated for 1 month, were negative. In the following weeks, the patient was afebrile, had right-sided chest pain of moderate intensity, without interference with daily-life activities, and did not have other associated complaints.

In April 2007 he started outpatient treatment with itraconazole PO 100 mg/day. One month later the lesion was
found to be identical to that seen on the initial X-ray and he again underwent trans-thoracic needle aspiration biopsy. Septate hyphae were once more observed in direct exam, but on this occasion a filamentous fungus, morphologically identified as *Aspergillus* spp., probably *A. fumigatus* was recovered in culture.

At this point the patient was started on posaconazole therapy 400 mg/day, continuously for 5 months, with positive clinical and radiological response.

In October 2007, following unrelated abdominal surgery, posaconazole was discontinued for a month. Due to worsening of pulmonary imaging, a bronchoscopy was performed but no endobronchial lesions were identified. Direct examination of bronchial and bronchoalveolar lavage revealed hyphae and cultures were again positive for *Aspergillus* spp. Upon discharge from hospital in November, the patient was maintained on voriconazole PO 200 mg/day and continued with his usual medication, including methotrexate and prednisolone, which he took regularly during the course of the disease.

In early July 2008, a right cervical, deeply adherent inflammatory swelling, with a maximum diameter of 10 cm, was observed. Aspiration needle biopsy and cytological exam of the aspirate revealed fungal structures suggestive of *Aspergillus*. Treatment with intravenous voriconazole resulted in clinical improvement and decrease in mass size. The patient was discharged with oral voriconazole. However, at the end of the second month of treatment, there was a resurgence of the right cervical swelling and the appearance of a second one with similar characteristics in the thoracic region at the right anterior axillary line, 6th and 7th rib. This time there was a clear clinical worsening, including fever of 38–40°C, deterioration of general condition, swelling enlargement and chest X-ray changes. In late August 2008, he was admitted and observed for the first time in the Infectious Diseases Service.

Medical treatment was optimized with the combination of posaconazole, 200 mg four times a day and caspofungin, 50 mg daily and empirical therapy with amoxicillin and clavulanic acid. While blood cultures were negative for bacteria, mycobacteria and fungi, an *Aspergillus* spp. isolate was recovered from bronchial secretions.

The CT scan revealed multiple lymph adenopathies and a right cervical conglomerate beginning in the supraclavicular region down to the 5th rib, where a 3.5 cm nodule with central necrosis was seen. A right pleural effusion with pleural thickening and a nodular lesion with spiculated margins of 5 cm, located in the posterior aspect of the right upper lobe, in contact with the pulmonary hilum, were also found (Fig. 2).

The fever and lesions persisted despite spontaneous and surgical drainage of both swellings. Cancer and infection by other fungi, mycobacteria, or bacteria were excluded. The clinical condition of the patient progressively worsened with respiratory failure and oliguric irreversible renal failure, and he died after 39 days of hospitalization. No post mortem study was performed.

All isolates sent to the microbiology laboratory between May 2007 and September 2008 presented the same morphological and microscopic characteristics and were identified as *Aspergillus* spp., probably *A. fumigatus*. They
showed the usual features of members of the genus *Aspergillus* including: ‘foot cells’; upright, usually nonseptate, conidiophores, with swollen vesicles at the tips; vesicles covered entirely or in part by the phialides [12]. However, in comparison to *A. fumigatus*, colonies of the isolates grew slower in Sabouraud-gentamicin-chloramphenicol culture medium at 25°C, were initially white (Fig. 3) and required additional incubation time to develop their green color due to conidial formation. On microscopical exam of a lactophenol cotton-blue tease preparation, the vesicles were smaller than those seen with *A. fumigatus*, i.e., their diameters ranged from 17–31 μm (average 23.8 μm) whereas those of *A. fumigatus* measured from 28–51 μm (average 38.6 μm). In addition, a lower proportion of vesicles exhibited phialides (39%, compared to 46% on *Aspergillus fumigatus*), which, when present, appeared in smaller numbers (Fig. 4).

The mold was genotypically identified as *A. viridinutans* at Centro Nacional de Microbiologia Instituto de Salud Carlos III, Madrid, Spain, according to a previously described procedure based on partial sequencing of the β-tubulin and rodlet A genes [8]. Susceptibility testing was performed according to the recommendations of the Clinical and Laboratory Standard Institute (CLSI), document M38-A2 [13]. The minimal inhibitory concentrations (MIC) were 1.0, 4.0, 0.25, >16 μg/ml for amphotericin B, voriconazole, posaconazole and itraconazole respectively. Minimal effective concentrations for caspofungin and anidulafungin were ≤0.016 μg/ml for both. Susceptibility results were similar for the first and last isolate tested. Serum levels of administered antifungal drugs were not measured.

**Discussion**

The case reported here shows a form of aspergillosis with a clinical course different from that usually observed in neutropenic and transplanted patients. Invasive aspergillosis typically presents as an acute, rapidly progressive disease with predilection for angioinvasion and hematogenous dissemination. In contrast, this case and the two others previously reported in the literature [11] showed chronic evolution and progressive spread across anatomical planes.

This third case strengthens the previous assertion that *A. viridinutans* is able to cause a distinct form of aspergillosis [11]. In fact, the patients were immunocompromised in some manner and the disease started in the lungs, likely after inhalation of spores (the most frequent portal of entry in IA), before disseminating to adjacent areas. Moreover, the infection developed over several months in all cases (3 months in the 14-year-old boy, 7 months in the 8-year-old boy and almost 18 months in this 56-year-old man), and did not respond to medical and surgical therapy, emphasizing the indolent, albeit progressive, features of this form of aspergillosis. An interesting observation is that all three cases involved male patients, although the limited number does not allow for conclusions on gender susceptibility (there is no such predilection in usual forms of aspergillosis).

This case further evidences the need for genotypically-based identification of isolates of members of *Aspergillus*, section *Fumigati* [8–10]. Indeed, the mold isolated in the present case was found to belong to that group, according to morphological and microscopic characteristics, but its features did not fully correspond to those of *A. fumigatus* (for example, differences in growth patterns), which prompted the use of molecular biology tools to obtain definite species identification.

The earlier therapeutic decisions deserve attention. When the patient presented with a solitary nodule, he was treated with 100 mg of itraconazole each day. Due to the lack of a positive response, posaconazole PO 400 mg/day was prescribed and, later, after surgery for unrelated reasons, he was medicated with voriconazole PO 200 mg/day. The under dose schedule employed for the three antifungal drugs did not meet fully the Infectious Diseases Society of America recommendations [14]. Although they included the oral route for administration, it may not have been adequate in this setting.

Finally, *in vitro* susceptibility tests of *A. viridinutans* revealed peculiar findings when compared to the antifungal susceptibility of other *Aspergillus* spp. The MIC of

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**Fig. 3** Image of *Aspergillus viridinutans* culture on Sabouraud-gentamicin-chloramphenicol medium at 25°C at 5 days (A) and 13 days (B).
voriconazole against *A. viridinutans* was consistently higher when compared to *A. fumigatus* isolates [15–19], a finding in agreement with the refractory nature of the infection to voriconazole, observed in the patient for almost 1½ years. *A. viridinutans* does appear to be generally susceptible to posaconazole and the echinocandins, according to our results and those described in the two previous reports [8,11]. However, it should be pointed out that MIC criteria for susceptibility may differ. In fact, the MIC we found for posaconazole indicates susceptibility according to CLSI guidelines, but, according to European Committee for Antimicrobial Susceptibility Testing [20], it is at the uppermost limit of susceptibility and is probably intermediate.

Therefore, this case emphasizes the peculiar clinical features of an unusual *Aspergillus* species infection caused by *A. viridinutans*. It further highlights the importance of employing specific diagnostic tools to achieve the correct identification of molds and the need to perform antifungal susceptibility testing, using reference procedures and breakpoints established by expert committees [13,20]. This is particularly important in those clinical isolates whose microbiological examination presents unexpected features and the empirical therapy proves to be ineffective. Further epidemiological and antifungal susceptibility data are necessary before definitive practice recommendations may be generated to guide the clinical management of IA by *A. viridinutans*, other *A. fumigatus*-like molds, or rare and emerging species.

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**References**

1. McLennan EI, Tucker SC, Thrower LB. New soil fungi from Austral-
ian heathland: Aspergillus, Penicillium, Spegazzinia. Austral J Bot

within the Aspergillus viridinutans species. Folia Microbiol (Praha)
2000; 45: 423–428.

3. Alcazar-Fuoli L, Mellado E, Alastrauey-Izquierdo A, Cuenca-Estrella
M, Rodriguez-Tudela JL. Aspergillus section Fumigati: antifungal
susceptibility patterns and sequence-based identification. Antimic-

4. Katz ME, Dougall AM, Weeks K, Cheetham BF. Multiple genetically
distinct groups revealed among clinical isolates identified as atypical

5. Yaguchi T, Horie Y, Tanaka R, et al. Molecular phylogenetics of mul-
tiple genes on Aspergillus section Fumigati isolated from clinical

6. Vinh DC, Shea YR, Jones PA, et al. Chronic invasive aspergillosis caused

7. Sigler L, Kennedy MJ. Aspergillus, Fusarium, and other opportunis-
tic moniliaceous fungi. In: Murray PR, Baron EJ, Pfaller MA, Ten-
over FC, Yolken RH (eds.) Manual of Clinical Microbiology, 7th edn.

Broth Dilution Antifungal Susceptibility Testing of Filamentous
Fungi; approved standard. Document M38-A2, 2nd edn. Wayne, PA:
CLSI, 2008.

This paper was first published online on Early Online on 11 February
2011.

9. Walsh TJ, Anaissie EJ, Denning DW, et al. Treatment of aspergil-
losis: clinical practice guidelines of the Infectious Diseases Society of

cross-resistance among more than 700 clinical isolates of Aspergillus

11. Espinel-Ingroff A, Johnson E, Hockey H, Troke P. Activities of vori-
conazole, itraconazole and amphotericin B in vitro against 590 moulds
from 323 patients in the voriconazole phase III clinical studies. J An-

comparison of the activities of currently available antifungal agents
against 3,378 Spanish clinical isolates of yeasts and filamentous fungi.

fluconazole, itraconazole, voriconazole, and amphotericin B against a
large collection of clinically important moulds and yeasts. Antimic-

against yeasts and filamentous fungi: assessment according to the meth-
ology of the European Committee on Antimicrobial Susceptibility

15. European Committee for Antimicrobial Susceptibility Testing. Meth-
od for the determination of broth dilution MICs of antifungal agents

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