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Note

Refractory disseminated fusariosis by *Fusarium verticillioides* in a patient with acute myeloid leukaemia relapsed after allogeneic hematopoietic stem cell transplantation: A case report and literature review

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ABSTRACT

Background: *Fusarium* species are among the leading fungal pathogens to cause invasive mould infections in patients with hematopoietic malignancy. The *Fusarium* species most frequently involved in human infections are *Fusarium solani*, *Fusarium oxysporum* and *Fusarium verticillioides*. However, identification is a cumbersome and time-consuming task. *Fusarium* is resistant in vitro to many of the antifungal agents and the management of fusariosis is not well defined.
Objectives: To emphasise the difficulty of identifying *Fusarium* spp. by conventional methods and the need of new rapid molecular tests to achieve earlier diagnosis and appropriate therapy.
Methods: A disseminated *Fusarium* infection due to *F. verticillioides* was documented in a neutropenic refractory patient with acute myeloid leukaemia, relapsed after allogeneic hematopoietic stem cell transplantation.
Results: The patient died despite liposomal amphotericin B and voriconazole combination and "in vitro" susceptibility of agents employed. Morphological and molecular identification of *F. verticillioides* was obtained only after the death of the patient.
Conclusions: This case highlights the poor outcome of an invasive fungal disease caused by *Fusarium* in aplastic patients. Identification of members of *Fusarium* genus remains restricted to selected laboratories and should be introduced into routine mycological diagnostics. In immunocompromised patients, diagnosis of fusariosis is directly related to prompt diagnosis and to patient's status. Current diagnosis methods and therapeutic options are discussed.

Fusariosis diseminada refractaria debida a *Fusarium verticillioides* en un paciente con leucemia mieloide aguda que experimentó recidiva después de un alotrasplante de células progenitoras hematopoyéticas: informe del caso y revisión de los estudios publicados

Palabras clave:
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RESUMEN

Fundamento: *Fusarium* es uno de los principales patógenos fúngicos que provoca infecciones invasoras en pacientes portadores de neoplasias malignas hematopoyéticas. Las especies del género *Fusarium* implicadas habitualmente en las infecciones del ser humano son *Fusarium solani*, *Fusarium oxysporum* y *Fusarium verticillioides*. No obstante, la identificación es una tarea lenta y que consume mucho tiempo. *Fusarium* spp. es resistente *in vitro* a numerosos fármacos antimicóticos y el tratamiento de la fusariosis no está bien definido.
Objetivos: Destacar las dificultades en la identificación de *Fusarium* spp. por los métodos convencionales y la necesidad de disponer de nuevas técnicas moleculares rápidas para obtener un diagnóstico más precoz y un tratamiento apropiado.
Métodos: En un paciente portador de una leucemia mieloide aguda con neutropenia refractaria, que experimentó recidiva tras alotrasplante de células progenitoras hematopoyéticas se documentó una infección diseminada por *Fusarium* debida a *Fusarium verticillioides*.

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Resultados: A pesar de recibir un tratamiento combinado con anfotericina B y voriconazol liposómicos y de la sensibilidad *in vitro* de los preparados administrados, el paciente falleció. Sólo después de su muerte se obtuvo la identificación morfológica y molecular de *Fusarium verticillioides*.
Conclusiones: El caso descrito en el presente informe destaca el desenlace desfavorable de las micosis invasivas debidas a *Fusarium* en pacientes con aplasia de médula ósea. La identificación de los miembros del género *Fusarium* sigue limitándose a laboratorios seleccionados y debe introducirse en el diagnóstico micológico sistemático. En el huésped inmunocomprometido el diagnóstico de fusariosis se relaciona directamente con el estado del paciente. Se describen los métodos diagnósticos y las opciones terapéuticas actuales.

Fusarium species cause severe infections in patients with hematologic malignancies. The *Fusarium* species most frequently involved in human infections are *Fusarium solani*, *Fusarium oxysporum* and *Fusarium verticillioides*.[12,19,27] *Fusarium* spp. are very resistant to single antifungal agents, and new treatment strategies, such as combination therapy, can be considered life-saving for immunocompromised patients.[16,27] We report a fatal disseminated *F. verticillioides* infection in a refractory patient with acute myeloid leukaemia (AML), unresponsive to all antifungal agents, including liposomal amphotericin B and voriconazole combination.

Case report

A 40-year-old Caucasian female was admitted to the haematology unit in January 2010 for AML, M2 FAB subtype, normal karyotype, FLT3 ITD+, NPM1 negative.

After standard induction-chemotherapy and consolidation, in April 2010 the patient underwent a peripheral allogeneic HSCT from HLA-matched family donors, without any serious infectious complications. In August 2010 the patient relapsed and a course of reinduction therapy with Clofarabine and Citarabine was started. Anti-infective prophylaxis included levofloxacin (500 mg PO/day), acyclovir (500 mg, IV every 12 h), and itraconazole (400 mg PO/day). Surveillance cultures were taken weekly; *Aspergillus* galactomannan antigenemia test (GM) was taken 2/times/weekly. No febrile episode was documented during neutropenia, but the treatment failed to achieve a complete remission.[5] A new salvage-regimen with MEC (mitoxantrone, etoposide, and cytarabine) plus cyclosporine was started, continuing the same anti-infective prophylaxis. On day +4 post chemotherapy, while neutropenic, the patient experienced fever refractory to broad spectrum antibiotics. Blood cultures, taken from both the peripheral vein and the CVC, chest X-ray and GM were negative. On day 4 of persistent fever, empiric antifungal therapy with liposomal amphotericin B (L-Amb), 3 mg/kg/day was added. After 8 days the patient, still febrile, developed multiple skin lesions which had necrotic centres surrounded by spreading erythema; a new chest X-ray, pulmonary CT scan, cultures and GM were negative. It was not possible to obtain cultures through skin biopsy. After 13 days of fever, all new repeated blood cultures tested positive for fungi; L-Amb was raised to a dose of 5 mg/kg/day and combined with voriconazole (loading dose 6 mg/kg/day, followed by 4 mg/kg/day IV every 12 h). Five days later the fungus was recognised as a *Fusarium* species and combination therapy was continued on the basis of data literature susceptibility. A new X-ray and pulmonary CT scan, documented lung involvement with nonspecific findings, although the patient remained asymptomatic for dyspnea, cough and hemoptysis. A new bone marrow aspirate revealed persistence of AML and after 25 days of aplasia and fever the patient died. Six days after the death, following the method and the key proposed by Guarro and Gené,[11] the isolated etiological agent was morphologically identified as *F. verticillioides*.

The confirmation of this species was obtained 20 days later by molecular methods. Fungal DNA was extracted by the Bowman method[22] and 5 ng of fungal template DNA were amplified in a PCR assay using *F. verticillioides* specific primers (VER1: 5′-CTTCCTGCGATGTTTCTCC-3′ and VER2: 5′-AATTGGCCATTGGTATTATATATCTA-3′) according to Mulé et al.[18] PCR products, consisting of a single band of about 580 bp specific for *F. verticillioides*, confirmed the morphological identification.

For the antifungal susceptibility, the broth microdilution assay was performed according to the Clinical and Laboratory Standards Institute (CLSI).[6] The concentrations of amphotericin B, itraconazole, and voriconazole assayed ranged from 64 to 0.06 mg/l. The strain was tested in duplicate and the control strains *Candida parapsilosis* ATCC 22019 and *Candida albicans* ATCC 90028 were included. The MIC of the strain studied was 8 mg/l to voriconazole and 2 mg/l to amphotericin B, while it was resistant to the itraconazole maximum tested concentration (64 mg/l).

Discussion

Disseminated fusariosis is a serious invasive mould infection in hematologic patients. The most commonly found pathogens are *F. solani* and *F. oxysporum*, although other species such as *F. verticilloides*, have been frequently reported as aetiological agents of human infections.[17,28] This fungus in neutropenic patients may affect multiple organs and frequently also the skin as the primary or the metastatic site. The skin is often the single source of diagnosis and cutaneous lesions may precede positive blood cultures for up 5 days.[4,9]

Currently, the identification to species level of *Fusarium* is based on the production of macroconidia. However, recognition may be difficult when the macroconidia are not produced in culture; in this case the isolates can be confused with other genera such as *Acremonium* and *Verticillium*. In order to solve this issue, new rapid molecular methods are developed. The majority of molecular methods are PCR-based techniques that ensure high sensitivity and specificity and are fully discriminative even for closely related species.[2,14] However identification is a time-consuming task, only reserved to trained mycologists.

In our case, recognition of the *Fusarium* species was obtained only after the death of the patient, while microbiological cultures were still awaiting.

The high mortality caused by *Fusarium* is attributed to high resistance to many antifungal agents.[1] Amphotericin B is the most effective of the antifungal drugs. Fluconazole, itraconazole and flucytosine have no activity against *Fusarium* spp., and ketoconazole, miconazole, terbinafine and echinocandins have limited activity. *F. solani* and *F. verticillioides* are usually resistant to azoles and exhibit higher amphotericin B MICs than other *Fusarium* species[21]. The new triazole agents, voriconazole, posaconazole and ravuconazole, exhibit activity against these *Fusarium* species and

have been reported as a successful treatment in oncohematologic patients or in refractory fungal infections.[7,15,25]

Our patient developed a disseminated fusariosis, after receiving itraconazole prophylaxis. On the other hand, breakthrough fusariosis is not an unexpected event in the immunocompromised patients and may occur also employing agents as voriconazole or posaconazole.[3,8]

The management of fusariosis is not well defined. There are case reports [10] where the early described *F. verticillioides* (moniliforme) infections resulted in the death of the patients, and more recently successful cases with patients, treated with the combination of amphotericin B and other agents as caspofungin, voriconazole, terbinafine or posaconazole,[13,15,24,26,27,29] suggesting a promising role for this approach, even in the case of late identification to the species level. At present, treatment with voriconazole + amphotericin B is the main alternative.

However patients with severe and prolonged immunosuppression are at high risk for refractory disseminated fusariosis; in fact our patient died after a persistent fungemia, despite early aggressive combination therapy and at least "in vitro" susceptibility of the agents employed.

The failure of antifungal therapy in neutropenic patients with invasive fusariosis has been reported before in a multicentre study.[20] Despite the overall relative efficacy, all antifungal agents, including new azoles as posaconazole, were effective only in the case of recovery from myelosuppression.[23]

In conclusion, morphological and molecular identification of *Fusarium* spp. remains cumbersome and restricted to selected laboratories; further rapid molecular methods should be introduced into routine mycological diagnostics to achieve earlier diagnosis and appropriate therapy.

In the immunocompromised patients, prognosis of fusariosis remains directly related to prompt diagnosis and to patient's status.

Conflict of interest

The authors declare no conflict of interest.

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