Rev Iberoam Micol. 39 (2022) 21–24

Revista Iberoamericana de Micología

www.elsevier.es/reviberoammicol

Note

Lethal destructive sinusopathy due to amphotericin B-resistant *Aspergillus flavus*: A case report

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ARTICLE INFO

Article history:
Received 18 March 2021
Accepted 13 January 2022
Available online 4 March 2022

Keywords:
Aspergillosis
Pansinusopathy
Antifungal resistance

ABSTRACT

Background: The prevalence of pulmonary aspergillosis and the importance of its early diagnosis are recognized. However, non-pulmonary involvement, including the sinuses region, is not frequently reported, and an infection in this area can affect all paranasal sinuses (pansinusopathy), being a rare pathology that affects immunocompromised hosts. Recent studies have highlighted the occurrence of *Aspergillus flavus* resistant to antifungal therapy. Therefore, a nasal sinus infection by resistant *Aspergillus* strains in immunocompromised patients may be linked to a high risk of lethality.
Case report: We are reporting a resistant *A. flavus* infection in an allogeneic hematopoietic stem cell transplant recipient with episodes of febrile neutropenia, and prolonged use of various antibacterial drugs and antifungal prophylaxis. The patient underwent brain magnetic resonance, which showed the presence of pansinusopathy, and presented necrosis in the left nasal region. Direct microscopic examination of a sample taken from the nasal mucosa revealed the presence of septate hyphae and conidiophores resembling those of *A. flavus*, that species being the identification achieved with MALDI-TOF MS. Antifungigram was performed by microdilution in broth (EUCAST-E.DEF. 9.3.2) and E-test, and resistance to amphotericin B was shown in both tests. The patient died after septic shock and hemorrhage.
Conclusions: Invasive fungal infections due to amphotericin-B resistant *A. flavus* may lead to the death of the patient due to an ineffective therapeutic management. Therefore, antifungal susceptibility testing are of utmost importance for administering the proper treatment.

Sinusopatía destructiva y letal por *Aspergillus flavus* resistente a anfotericina B: a propósito de un caso

Palabras clave:
Aspergilosis
Pansinusopatía
Resistencia antifúngica

RESUMEN

Antecedentes: La prevalencia de la aspergilosis pulmonar y la importancia de su diagnóstico precoz son ampliamente conocidos; sin embargo, la afectación extrapulmonar no se informa con frecuencia y son pocos los casos documentados de infección de los senos nasales. Una infección en esta área puede alcanzar todos los senos paranasales (pansinusopatía) cuando afecta a pacientes inmunodeprimidos, lo que agrava la enfermedad preexistente. Estudios recientes han destacado la aparición de cepas que muestran resistencia al tratamiento con fármacos antimicóticos. En el paciente inmunodeprimido una infección de los senos nasales por una cepa de *Aspergillus* resistente implica un alto riesgo de letalidad.

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https://doi.org/10.1016/j.riam.2022.01.001
1130-1406/

Caso clínico: Presentamos un caso de infección por *Aspergillus flavus* resistente en un receptor de trasplante alogénico de células madre hematopoyéticas, con episodios de neutropenia febril y uso prolongado de diversos fármacos antibacterianos, además de profilaxis antifúngica. Se realizó una resonancia magnética cerebral que reveló la existencia de pansinusopatía con necrosis en la región nasal izquierda. En el examen microscópico directo de la mucosa nasal se observaron hifas tabicadas y la presencia de conidioforos compatibles con *Aspergillus flavus*; la identificación con el método MALDI-TOF MS arrojó la especie mencionada. El antifungigrama fue realizado por el método de microdilución en caldo (EUCAST-E.DEF. 9.3.2) y E-test; con ambas técnicas el aislamiento mostró resistencia a la anfotericina B. El paciente falleció tras un shock séptico y hemorragia.
Conclusiones: Las infecciones fúngicas invasivas por cepas de *Aspergillus flavus* resistentes a la anfotericina B puede derivar en la muerte del paciente debido a la ineficacia del tratamiento antifúngico. Es por ello que las pruebas de sensibilidad a los antifúngicos son de suma importancia para establecer así el tratamiento correcto.

Fungal infection of the respiratory tract is often caused by *Aspergillus fumigatus*.[1] Nevertheless, *Aspergillus flavus* is the predominant pathogen in tropical and dry regions due to its unique ability to survive at higher temperatures.[3] Moreover, the larger size of *A. flavus* conidia eases their accumulation on the upper respiratory tract mucous, which makes this species the main agent among fungal etiologies of sinusitis.[6] Amphotericin B (AMB), an antifungal agent widely used in these cases and considered the gold standard treatment for invasive fungal infections in patients with slow or unsatisfactory response to azole drugs, is one of the main therapeutic options.[2] However, recent studies highlights the occurrence of strains of this species exhibiting high MIC values for AMB (>8 μg/mL)[2] associated with unknown mechanisms of resistance.[4]

This work reports a case of a 10-year-old male with a history of rhabdomyosarcoma in the eye socket treated over five years. In 2018, he started a new follow-up in pediatric oncology due to pancytopenia, with a neutrophil count lower than 1000 cells/μL, and received a new diagnosis of myelodysplastic syndrome. Less than a year after the onset of this condition, the patient underwent an allogeneic hematopoietic stem cell transplantation (allo-HSCT) from isogroup A+, and a conditioning regimen with busulfan and fludarabine, along with prophylaxis with acyclovir, bactrim, levofloxacin and voriconazole, was started.

In the pre-transplant period, the patient, who suffered from febrile neutropenia episodes, had a prolonged administration of multiple antibacterial drugs (cefepime, meropenem and vancomycin), as well as antifungal prophylaxis with voriconazole plus liposomal-AMB over 30 days. There were hemorrhagic complications, such as hematuria and alveolar hemorrhage, but to greater degree episodes of epistaxis that were managed with nasal tampons. From the beginning of the conditioning regimen, white blood cells remained below 1000 cells/μL, and dropped to fewer than 100 cells/μL after HSCT. The first day after allo-HSCT a *Klebsiella pneumoniae* carbapenemase-producing colonization was diagnosed. Despite the absence of fever throughout post-HSCT, the patient showed respiratory distress and a convulsive episode that required intubation and mechanical ventilation to protect the airways. At this time, the antibiotic prescription was extended. The result of the blood culture was negative and the cranial tomography displayed a small hemorrhagic focus in the right parietal lobe. During this episode, the patient received liposomal-AMB for five days, returning to prophylactic voriconazole thereafter. An injury in the left nostril, explained by the local manipulation of dressings at the beginning, was also observed.

Twenty days after the allo-HSCT, the patient underwent a magnetic resonance imaging of the brain that showed pansinusopathy (Fig. 1A); the day after, necrosis in the left nasal region was observed (Fig. 1B). At this point, voriconazole-based prophylaxis was switched to liposomal amphotericin B and micafungin, and a debridement and a biopsy of the necrotic tissue was carried out the same day by an otorhinolaryngologist. However, the patient died shortly after due to septic shock and hemorrhage in several body sites.

The direct microscopic examination of a sample taken from the nasal mucosa by swabbing showed septate hyphae and long conidiophores, mostly rough in the distal part, with elongated vesicle and globose, smooth-walled conidia, typical of *Aspergillus* (Fig. 1C). It is noteworthy that the last laboratory finding from the biological sample is unusual. Postmortem microbiological culture from the nasal fragment yielded an *Aspergillus* isolate, and the histopathology showed the presence of septate branching hyphae, evidenced by the Grocott silver stain. The *Aspergillus* isolate was seeded on Sabouraud dextrose agar (SDA) and incubated at 28 °C in order to be identified by MALDI-TOF mass spectrometry (MALDI Autoflex III, Bruker Daltonics, Bremen, Germany), and in order to perform an antifungigram through the European Committee on Antimicrobial Susceptibility Testing (EUCAST) method and E-test. The isolate was identified as *A. flavus* with a score value of 2.00 (Biotyper system, 3.1 version, Bruker Daltonics, USA/Germany). The antifungal susceptibility testing by the methods aforementioned exhibited sensitivity to itraconazole and voriconazole. However, they showed resistance to amphotericin B (Fig. 1D).

Regarding misidentification of *Aspergillus nomius* and *Aspergillus tamarii* as *A. flavus* by MALDI-TOF MS, as pointed out by Tam et al.,[5] macro and micromorphological examinations were carried out after 5 days at 28 °C on SDA, which showed a velutinous, white-yellowish colony (Fig. 2A), that looked yellowish on the reverse (Fig. 2B). Conidial heads were biseriate, but some heads had phialides borne directly on the vesicle (uniseriate), producing brownish-yellow, globose to subglobose conidia (Fig. 2C). However, we did not use molecular techniques (e.g. beta-tubulin and calmodulin gene sequencing) to confirm the species identification.

Undoubtedly, the underlying condition of the patient contributed to the onset and severity of the destructive sinusopathy. In addition, any invasive fungal infection due to amphotericin B-resistant *Aspergillus* can lead to patient's death due to an ineffective therapeutic management. Our case highlights the high mortality rates related to complications in HSCT-patients. Measuring voriconazole serum concentration, with the purpose of reducing toxicity, as well as avoiding any failure due to sub-optimal antifungal concentration, must be followed. Since our infection control service could not provide that checking we opted for administering rescue therapy.

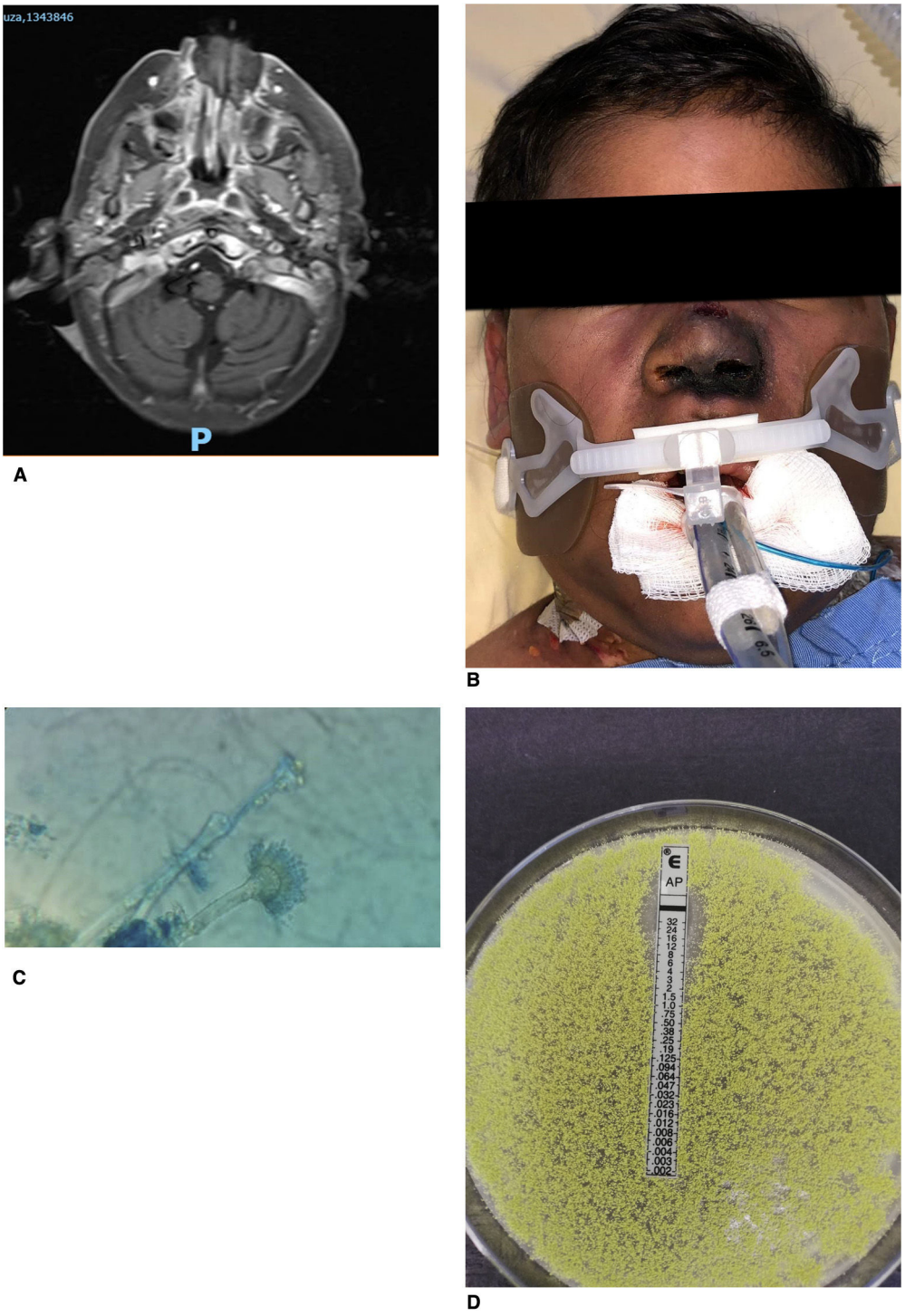

Fig. 1. A) Hypointense and hypocaptive mass involving the nasolabial area, with erosion of the nasal cartilages, extending into the cavity, and eroding the nasal septum, palate and anterior part of the left jaw. B) Extensive necrosis of the left wing of the nose, extending to the philtrum, septum and right nostril, in addition to ulceration on the nasal dorsum. C) Direct microscopic examination stained with lactophenol cotton blue showing a conidial head of Aspergillus sp. Magnification 400x. D) Antifungal susceptibility testing with E-test showing resistance against amphotericin B.

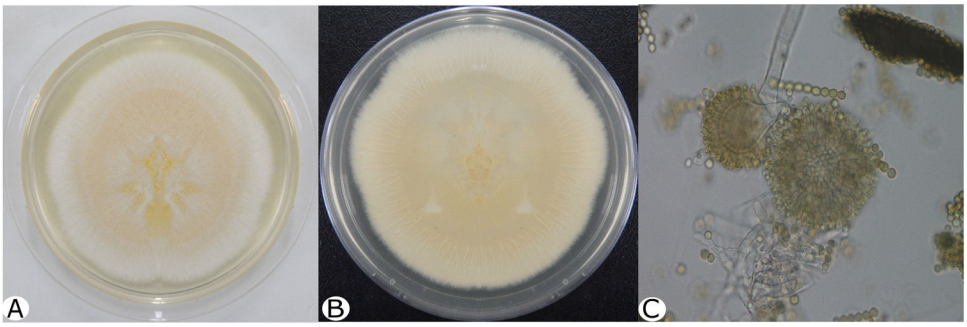

Fig. 2. Macroscopic features of the *Aspergillus flavus* clinical isolate after five days on Sabouraud dextrose agar at 28 °C. (A) White-yellowish colony with grainy appearance and radial grooves color. (B) Yellowish color on the reverse. (C) Long and large conidiophores with mostly biseriate heads and some others uniseriate. Long chains of oblong and mustard conidia.

Financial support

The authors wish to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (*CAPES, Ministry of Education, Brazil*) for the scholarship of Eduardo Marques de Araújo [process 88887.500775/2020-00]. The study was partially supported by CAPES-COFECUB agreement 59/2014. RG Lima-Neto is a researcher fellow of CNPq/Ministry of Science and Technology (Grant 310822/2018-1).

Conflict of interests

The authors declare that they do not have any conflict of interest with respect to this manuscript.

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