First case of fungal rhinosinusitis due to *Aspergillus nomius* in a child with aplastic anaemia

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Summary

Recently, infections caused by *Aspergillus* species have increased dramatically. Invasive aspergillosis (IA) is one of the most important causes of morbidity and mortality in immunocompromised patients, such as those with haematological malignancies who undergo chemotherapy, bone marrow and solid organ transplant recipients, and patients with other immunodeficiency. The most common species causing invasive infections include *Aspergillus fumigatus,* followed by *Aspergillus flavus*. *Aspergillus nomius* is an anamorphic species belonging to *Aspergillus* section *Flavi*, which currently include 22 species that can be grouped into seven clades (*Aspergillus flavus, Aspergillus tamarii, Aspergillus nomius, Petromyces alliaceus, Aspergillus togoensis, Aspergillus leporis* and *Aspergillus* avenaceus) based on morphological characters, sequence data, and extrolite profiles. These species may also produce toxic and carcinogenic aflatoxins. However, *Aspergillus nomius* is an emerging pathogen as a cause of IA; we found only two reported cases of invasive infection caused by this fungus in literature up till now. We reported a case of fungal rhinosinusitis caused by *Aspergillus nomius* in a child with aplastic anaemia and to our knowledge, it is the first case as an agent of rhinosinusitis. The isolate was identified by sequencing based methods.

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Key words: *Aspergillus nomius*, case, rhinosinusitis.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: none.

Received for publication: 2 March 2019.
Revision received: 5 April 2019.
Accepted for publication: 3 July 2019.

Licensee PAGEPress, Italy
Microbiologia Medica 2019; 34:8148
doi:10.4081/mm.2019.8148

Introduction

In recent years, the importance of *Aspergillus* species has increased dramatically. Invasive aspergillosis (IA) is one of the most important causes of morbidity and mortality in immunocompromised patients, such as those with haematological malignancies who undergo chemotherapy, bone marrow and solid organ transplant recipients, and patients with other immunodeficiency (1-4). The most common species causing invasive infections include *Aspergillus fumigatus,* followed by *Aspergillus flavus*. *Aspergillus nomius (A.nomius)* is a heterothallic and also sexually reproducing species belonging to *Aspergillus* section *Flavi*, which currently include 27 species that can be grouped into seven clades (*A. flavus*, *A. tamarii*, *A. nomius*, *Petromyces alliaceus*, *A. togoensis, A. leporis*, and *A. avenaceus*) based on morphological characters, sequence data, and extrolite profiles (5). Several of these species are known to produce highly toxic and carcinogenic aflatoxins (6). *A. nomius* is an emerging pathogen as a cause of IA; we found only two reported cases of invasive infection due to *A. nomius* in literature up till now. We reported a case of fungal rhinosinusitis caused by *A. nomius* in a child with aplastic anaemia; to our knowledge, it is the first case as an etiological agent of rhinosinusitis.

Case Report

An 11-year-old girl was hospitalized for 22 days with aplastic anaemia and febrile neutropenia. She was receiving corticosteroid and vancomycin, amikacin, imipenem empirically for persistent fever. Microbiological examination of blood, urine, stool and throat swab specimens was performed for three times during her hospitalization. A consolidation area of lung parenchyma was detected on routine chest X-ray and high-resolution computed tomography (HRCT) revealed a single nodule with ground glass sign and atelectasis suggestive of a fungal infection. Intravenous voriconazole was started in addition to empirical antibacterial treatment.

On day 28, pain, hyperaemia and oedema appeared in the left wing of her nose, progressing to a necrotic lesion. On day 29, nasal and sinus biopsies were obtained and sent to Clinical Microbiology

Laboratory for microbiological evaluations. Patient persisted as febrile on day 34, when paranasal computed tomography examination consistent with a fungal infection was performed. Considering being mucormycosis, voriconazole treatment switched to liposomal amphotericin B. On the 46th day of hospitalization, posaconazole was added to antifungal treatment because neither radiological nor clinical regression was subsequently detected with amphotericin B therapy. After another 20 days, cranial Magnetic Resonance Imaging (MRI) and HRCT examination showed the fungal infection progression with left maxillary and infraorbital oedema, bone destruction and mucosal thickening. Imipenem was stopped and the therapy switched to piperacillin-tazobactam, amikacin and metronidazole on the 70th day. Furthermore, caspofungin was added to posaconazole on the 72th day. Diffuse fungal progression was detected in cranial, orbital and paranasal MRI on 79th day, and surgical debridement was recommended for maxillary sinus but this latter could not be performed due to the patient's general condition. Nasal and maxillary lesions spread to her hard palate and upper mouth mucosa. *Stenotrophomonas maltophilia* was isolated from blood culture the 92th day. She died for septic emboli in spite of broad spectrum antibacterial and antifungal therapy the 97th day of the hospitalization.

Mycological studies

Nasal and maxillary specimens were used for microscopic examination, that revealed hyaline and septate hyphae, and for microbiological investigation.

The samples were plated on sheep blood agar, McConkey agar, chocolate agar and three Sabouraud dextrose agar (SDA) plates. The SDA plates were incubated at three different temperatures (26, 30 and 37 °C) for a period of 7 days. After 48 hours of incubation, filamentous colonies began to grow on all plates. Initially pale yellow-green, the colonies then became velvety to floccose consisting in light orange-brown vegetative mycelium. At maturity, colonies had green, yellow-green granule like loose mycelial mesh in central, yellow-orange colour in periphery and the colony reverse was light yellow-orange on SDA. Microscopically, conidial heads were uniseriate or biseriate, radiate; vesicles were spherical to subspherical. The conidiophores were variable in length, hyaline and echinulate; conidia were spherical to subspherical, echinulate, and variable in size (Figures 1 and 2). Based on macroscopic and microscopic features, the fungal isolate was identified as *Aspergillus flavus* (7).

All bacterial cultures were negative.

Precise identification of this fungus was made by PCR amplification of D1-D2 region of 28S rRNA gene, and sequencing of the resulting amplicons. The amplification of genomic DNA was carried out by semi-nested PCR. We used broad-range primer pairs ITS1 (5' TCC GTA GGT GAA CCT GCG G) in the first reaction followed by D1 (5' GCA TAT CAA TAA GCG GAG GA) in the second reaction; D2R (5' TTG GTC CGT GTT TCA AGA CG) was used as the reverse primer in both reactions. Also, a segment of the ß-tubulin gene was amplified using primers bT-F (5' CAACTCCTGACCGCTTCTCC 3') and bT-R (5' GACATGACAGCAGAGACCAG3') and a segment of the calmodulin gene was amplified using primers cmd-F (5' TCGTAAGTAGTTATCGTCGT 3') cmd-R (5' ATCATCTCATCAACTTCGTC 3'). DNA sequence was determined using a BigDye Terminator ver5.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and an ABI 3130 DNA sequencer. Sequence analysis was carried out by BLASTN similarity search at the website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST). A sequence of 534 bp was detected, exhibiting 100% identity with *Aspergillus nomius* isolates present in the publicly available GenBank sequence database of NCBI. After identification, viable cultures were deposited at the *Westerdijk Fungal Biodiversity Institute*, Utrecht, The Netherlands (CBS 142749).

The 35th day, antifungal susceptibility testing of the maxillary and nasal fungal isolate was performed to determine the minimum inhibitory concentrations (MICs) by using Clinical and Laboratory Standards Institute document M38-A2 (8). MICs of amphotericin B, voriconazole, posaconazole, and minimum effective concentrations (MECs) of caspofungin, and anidulafungin were as follows: 4 µg/mL, 0.25 µg/mL, 0.25 µg/mL, 0.12 µg/mL, and 0.03 µg/mL, respectively.

Discussion

IA remains one of the major clinical problems in haematological patients, and rapid discrimination among the etiologic agents is very important for definitive diagnosis and patient's treatment strategies. The long-term use of antibiotics and steroids, chronic bacterial sinusitis, underlying diseases such as diabetes mellitus, haematological malignancies, bone marrow or solid organ transplantation and subsequent chemotherapy, and aplastic anaemia constitute high risk factors for invasive fungal infections. Invasive fungal rhinosinusitis (IFRS) is a rare infection characterized by an infiltration by fungal pathogen of the nasal cavity mucosa and paranasal sinuses. It may metastasize to the orbit, cavernous sinus and cerebral parenchyma via the vascular invasion in immunosuppressed subjects, presenting a progressive course. The mortality of IFRS in immunocompromised patients ranges from 50% to 80% since early physical findings are non-specific and ambiguous (*i.e.*, nasal obstruction, purulent discharge, and epistaxis) (1-4). In fact, most patients do not have the classic findings in the early phase of infection: the girl of our case presented facial pain, oedema and necrotic lesions on the nose in late stage.

IFRS is largely attributed to *Aspergillus* spp. and Zygomycetes in patients with stem cell transplantation and haematological disease (3-4). The genus *Aspergillus* is divided into several sections or complexes that provide 340 species, of which approximately 40 have been reported in human aspergillosis (9). Therefore, the identification of the agent to species level may be clinically important, since *A. flavus*-like species can show variable antifungal susceptibility, and treatment management may be challenged by drug resistance (10,11). However, many *Aspergillus* species have similar morphological features, and their identification is quite difficult according to phenotypic features (12). In this regard, comparative sequence analysis of one or several gene regions is required for species-level identification within *Aspergillus* complexes.

A. nomius, described by Kurtzman *et al.* in 1987, could be more common than expected because it is very difficult to morphologically distinguish it from *A. flavus,* the second important cause of invasive aspergillosis. Classification of an isolate in the *Aspergillus* genus can be based on morphological features but the identification of closely related and highly variable species is difficult by conventional criteria (9,13). *A. nomius* isolates can produce variously sized sclerotia including the originally described indeterminate ones considered characteristic of this species, as well as S-type sclerotia (13). As an aflatoxigenic member of *Aspergillus* section *Flavi*, *A. nomius* can produce both B and G aflatoxins, similar to *Aspergillus parasiticus* which cause serious problems worldwide in agricultural commodities (14). In addition, this species is widespread in different countries in the world such as India, Iran, Thailand, Brazil and it has been reported as foodborne or environmental origin (14-17). However, *A. nomius* was recently isolated from keratitis, onychomycosis and pneumonia in

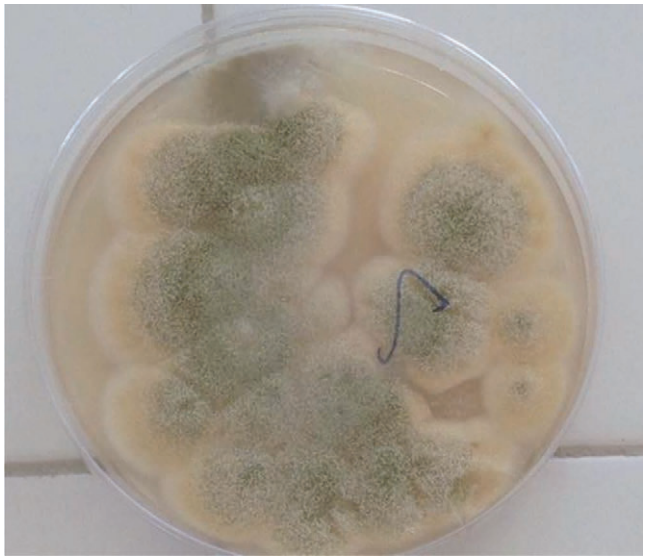

Figure 1. *Aspergillus nomius* on Sabouraud Dextrose Agar.

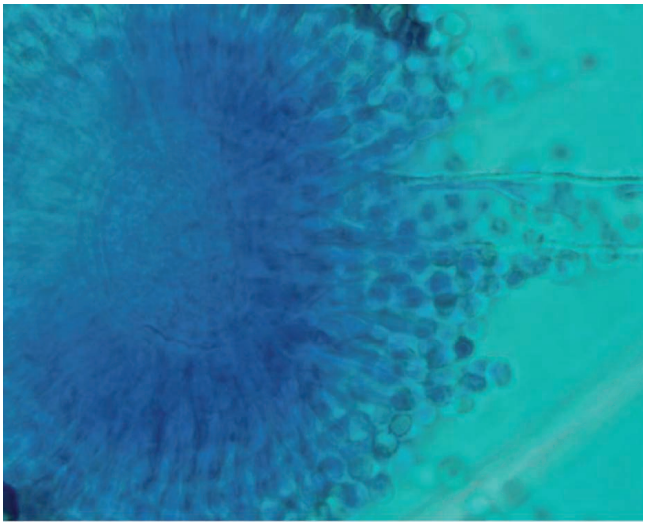

Figure 2. Microscopic appearance of *Aspergillus nomius* conidial head.

humans (18-20). To our knowledge, our patient is the first fungal rhinosinusitis case caused by *A. nomius* in Turkey. Initially this isolate was identified as *A. flavus* based on morphologic characteristics because of the high similarity between these sister species. The final identification was carried out by molecular methods. It is possible that other *A. nomius* isolates have been misidentified as *A. flavus* in our laboratory until now. The differentiation of *A. nomius* from other members of *Aspergillus* section *Flavi* can be made by only molecular techniques, including calmoduline, beta tubuline and ITS sequence analysis as it was done in this study (13,21). It should be noted that molecular methods are not applicable in most routine clinical microbiology laboratory.

The present case shows that clinicians should be aware of a possible *Aspergillus* species when the manifestations of sinusitis appeared in immunocompromised patients, and invasive fungal rhinosinusitis must be taken into consideration. Although *A. fumigatus* is the most common agent of invasive aspergillosis, rare species such as *A. nomius* may also cause an invasive infection. However, correct identification of uncommon fungal pathogens is difficult for most of clinical microbiology laboratories. Accurate identification of these rare pathogens is important for both epidemiological and management of treatment.

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