

SHORT COMMUNICATION

Mycetoma Caused by *Aspergillus nidulans*Stefano Veraldi¹, Anna Grancini², Luigia Venegoni¹, Valentina Merlo¹, Elena Guanziroli¹, Claudia Menicanti¹, Gianluca Nazaro¹ and Annamaria Tortorano³¹Department of Pathophysiology and Transplantation, Università degli Studi di Milano, IRCCS Foundation, Cà Granda Ospedale Maggiore Policlinico, IT-20122 Milan, ²UOS Microbiologia, Laboratorio Centrale di Analisi Chimico Cliniche e Microbiologia, IRCCS Foundation, Cà Granda Ospedale Maggiore Policlinico, Milan, ³Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy. *E-mail: stefano.veraldi@unimi.it
Accepted May 11, 2015; Epub ahead of print May 27, 2015

Aspergillus nidulans is a common soil saprophyte with a worldwide distribution. It was first isolated and described in 1906 by Nicolle & Pinoy in a Tunisian patient with mycetoma. Since then, only 4 cases of mycetoma have been published (1–5). We describe here a case of *A. nidulans* mycetoma in which, for the first time, mycological and histopathological examinations were confirmed by molecular identification.

CASE REPORT

A 59-year-old man, from Senegal, was admitted to the Department of Pathophysiology and Transplantation of the University of Milan, Italy, in September 2012 because of a giant nodular lesion on the left ankle and dorsum of the foot. His medical history revealed type I diabetes (diagnosed in 1996), peripheral diabetic neuropathy, amputation of the 2nd, 3rd, 4th and 5th left toes because of peripheral arterial occlusive disease and/or osteomyelitis (in 2007), amputation of the 1st left toe because of osteomyelitis of the metatarsal head (in 2010), and bilateral proliferating diabetic retinopathy treated with argon laser (in 2012). At admission, no medication other than insulin was used by the patient.

The patient reported that the nodular lesion had appeared in 1996. A biopsy had been made at another centre with a diagnosis of eumycetoma, although no fungi were observed either by histopathological or mycological examination. The patient had been treated, although unsuccessfully, with oral terbinafine (unknown daily dosage and duration of therapy). From 1996, the patient had been followed up at different centres in Italy and Senegal. In August 2011, several sinuses appeared with purulent and bloody discharge. In October 2011, therapy with oral itraconazole (200 mg/day) was started. In September 2012, the patient was admitted to our department. Dermatological examination revealed a nodular lesion, approximately 13 cm long, on the left ankle and dorsum of the foot. The lesion contained several sinuses, from which a discharge of pus and blood was visible (Fig. 1). The consistency of the lesion was parenchymatous-hard. The patient reported experiencing pain.

Laboratory examination revealed hyperglycaemia. X-rays of the left leg, ankle and foot showed several areas of osteoporosis.

A new biopsy was performed. Histopathological examination revealed hyperplasia of the epidermis and a dermal infiltrate. The latter was made up of histiocytes, neutrophils and plasma cells, surrounding some polylobated eosinophilic structures, filled with mycelial hyphae and spores (Fig. S1¹). A fragment of skin biopsy was cultured on brain heart infusion agar plus 5% sheep blood, Sabouraud-dextrose agar (SDA) and Tryptone Soya Broth (Oxoid Limited, Basingstoke, UK). Wrinkled, glabrous, white-buff fungal colonies grew after 30 days of



Fig. 1. Nodular lesion, with several sinuses, on the ankle and dorsum of the left foot.

incubation at 32°C. The colonies were transferred onto SDA, Czapek-Dox (HiMedia Laboratories Pvt, Mumbai, India) and potato dextrose agar and incubated at 28°C and 32°C. Flat white colonies grew very slowly at 32°C. Microscopic examination showed septate and gnarled hyphae. Subsequent subcultures were performed. Four months later, colonies became buff to light yellow (Fig. S2a¹); the reverse was purplish. Microscopic features showed brown, short and smooth-walled conidiophores, hemispherical vesicles with small metulae and phialides on the upper portion. Conidia were globose and rough; Hülle cells were abundant (Fig. S2b¹). *A. nidulans* was suspected, and confirmed by growth at 37°C, performed in order to differentiate it from *A. versicolor*. The identification was confirmed by DNA sequencing. Genomic DNA was extracted from a sporulating colony using PrepMan Ultra Sample Preparation™ reagent (Applied Biosystems, Waltham, MA, USA). A portion of the beta-tubulin gene was amplified using the primers TUBF (TGACCCAGCAGATGTT) and TUBR (GTTGTTGGGAATC-CACTC). The PCR products (~340 bp) were visualized on 1.4% agarose gel stained with ethidium bromide and used as a template for DNA sequencing by means of Big Dye terminators (Applied Biosystems) in a 310 ABI PRISM sequencer (Applied Biosystems). Nucleotide sequences were analysed using Mobylyte portal (<http://mobylyte.pasteur.fr/>) and compared with the sequences present in GenBank. The sequence was deposited in Genbank with accession number KP233835.

In February 2013, the patient began therapy with oral fluconazole (400 mg/day). Four months later, no improvement was observed. We therefore decided to use liposomal amphotericin B. However, the patient was lost to follow-up.

DISCUSSION

The genus *Aspergillus* is classified into 7 subgenera. The identification of *A. nidulans* (teleomorph *Emeri-*

¹<https://doi.org/10.2340/00015555-2144>

cella nidulans) is usually based on macro- and microscopic morphology. Colonies on potato dextrose agar at 25°C are dark green, with an orange to yellow colour in areas of cleistothecial production. The reverse is purplish to olive in colour. The growth rate is slow, hyphae are septate and hyaline, conidial heads are columnar, and the conidiophores are brown, short and smooth-walled. The vesicles are hemispherical, small, with metulae and phialides on the upper portion. The conidia are globose and rough. *E. nidulans* produces brown-black globose cleistothecia, which are engulfed by Hülle cells. Ascospores are reddish-brown, lenticular, with 2 longitudinal crests in comparison with other clinically significant *Aspergillus* species.

No reports of mycetoma due to *A. nidulans* were published from 1906 until 1968, when this fungus was isolated twice from a farmer in Senegal with mycetoma of a knee (1, 2). In 1971, Mahgoub (3) described a farmer from Sudan affected by a painless swelling on a sole, with many sinuses and bloody discharge. Some white-yellowish grains, 1–2 mm in diameter, were observed. Histopathological examination revealed an abscess, with oval or round or bilobed grains and irregular oval or round pale spores. Specific antibodies against *A. nidulans* were detected. An additional case was reported by the same author (4): the patient was a Sudanese farmer with a swelling involving a thigh and knee, accompanied by severe pain. Sinuses discharging pus and white grains were observed. Histopathological examination revealed the presence of round, white grains, 1–2 mm in diameter, and highly segmented hyphae. Radiographs revealed fungal involvement of the heads of the tibia and fibula, cavitation in femur, patella periosteal bone deposition and diffuse osteoporosis. Finally, Joshi et al. (5) described an Indian farmer with a mycetoma of the neck characterized by many sinuses discharging blood. Round-to-oval, white-yellowish granules, 0.5–1 mm in diameter, were isolated. Mycological examinations showed pale spores, whitish filamentous and eosinophilic hyphae. No data were published on the therapy of all these patients. It is important to emphasize that, in all these cases, the diagnosis of mycetoma caused by *A. nidulans* was based only on the morphology of the colonies. Our case can therefore be considered as the first report of mycetoma caused by *A. nidulans* identified by means of a molecular study. The fact that *A. nidulans* can be a pathogenic agent is demonstrated by cases of osteomyelitis (6–10), pneumonia (6, 7, 9, 11, 13, 14) and skin abscess (14), reported especially in patients with chronic granulomatous disease (6–10, 12–15). In these cases, amphotericin B (7, 8, 12–14), caspofungin (13, 14), flucytosine (7), itraconazole (8) and voriconazole (13, 14) were used, although with conflicting clinical results.

A. nidulans must be considered as a potential pathogenic agent in mycetoma. The final mycological diagnosis must be based on molecular identification.

The authors declare no conflicts of interest.

REFERENCES

1. Baylet R, Camain R, Chabal J, Izarn R. Recent contribution to the study of mycetoma in Senegal. *Neotestudina rosatii*, *Pyrenochaeta romeroi*, *Aspergillus nidulans*. *Bull Soc Med Afr Noire Lang Fr* 1968; 13: 311–313.
2. Baylet R, Lacoste L, Camain R, Basset A, Chabal J, Izarn R. *Aspergillus nidulans*, agent de mycétomes. *Bull Soc Pathol Exot* 1968; 61: 359–365.
3. Mahgoub ES. Maduromycetoma caused by *Aspergillus nidulans*. *J Trop Med Hyg* 1971; 74: 60–61.
4. Mahgoub ES. Mycetomas caused by *Curvularia lunata*, *Madurella grisea*, *Aspergillus nidulans*, and *Nocardia brasiliensis* in Sudan. *Sabouraudia* 1973; 11: 179–182.
5. Joshi KR, Mathur DR, Sharma JC, Vyas MCR, Sanghvi A. Mycetoma caused by *Aspergillus nidulans* in India. *J Trop Med Hyg* 1985; 88: 41–44.
6. Bujak JS, Kwon-Chung KJ, Chusid MJ. Osteomyelitis and pneumonia in a boy with chronic granulomatous disease of childhood caused by a mutant strain of *Aspergillus nidulans*. *Am J Clin Pathol* 1974; 61: 361–367.
7. White CJ, Kwon-Chung KJ, Gallin JI. Chronic granulomatous disease of childhood. An unusual case of infection with *Aspergillus nidulans* var. *echinulatus*. *Am J Clin Pathol* 1988; 90: 312–316.
8. Dotis J, Panagopoulou P, Filioti J, Winn R, Toptsis C, Panteliadis C, et al. Femoral osteomyelitis due to *Aspergillus nidulans* in a patient with chronic granulomatous disease. *Infection* 2003; 31: 121–124.
9. Dotis J, Roilides E. Osteomyelitis due to *Aspergillus* spp. in patients with chronic granulomatous disease: comparison of *Aspergillus nidulans* and *Aspergillus fumigatus*. *Int J Infect Dis* 2004; 8: 103–110.
10. Dotis J, Roilides E. Osteomyelitis due to *Aspergillus* species in chronic granulomatous disease: an update of the literature. *Mycoses* 2011; 54: e686–696.
11. Mizuki M, Chikuba K, Tanaka K. A case of chronic necrotizing pulmonary aspergillosis due to *Aspergillus nidulans*. *Mycopathologia* 1994; 128: 75–79.
12. Segal BH, DeCarlo ES, Kwon-Chung KJ, Malech HL, Gallin JI, Holland SM. *Aspergillus nidulans* infection in chronic granulomatous disease. *Medicine (Baltimore)* 1998; 77: 345–354.
13. Rösen-Wolff A, Koch A, Friedrich W, Hahn G, Gahr M, Roesler J. Successful elimination of an invasive *Aspergillus nidulans* lung infection by voriconazole after failure of a combination of caspofungin and liposomal amphotericin B in a boy with chronic granulomatous disease. *Pediatr Infect Dis* 2004; 23: 584–586.
14. Dellepiane RM, Tortorano AM, Liotto N, Laicini E, Di Landro G, Carnelli V et al. Invasive *Aspergillus nidulans* infection in a patient with chronic granulomatous disease. *Mycoses* 2008; 51: 458–460.
15. Henriët SSV, Verweij PE, Warris A. *Aspergillus nidulans* and chronic granulomatous disease: a unique host-pathogen interaction. *J Infect Dis* 2012; 206: 1128–1137.