

Case Report

Mycetoma due to *Pseudallescheria boydii* and co-isolation of *Nocardia abscessus* in a patient injured in road accident

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We report the case of a patient who developed a mycetoma after experiencing a road accident. From surgical biopsies *Pseudallescheria boydii* was isolated. Subsequently, after the infection had been treated with itraconazole, a Gram-positive bacterium, identified as the newly described species *Nocardia abscessus*, was cultured from wound fluids.

Keywords mycetoma, *Pseudallescheria boydii*, *Nocardia abscessus*, human infection

Introduction

Mycetoma is a local, progressive, destructive infectious disease of subcutaneous tissue, muscles, fasciae and bones. It is caused by aerobic actinomycetes or fungi. The etiologic agents form grains or granules in tissue. These structures are organized mycelial aggregates or colonies formed *in vivo* and represent the diagnostic hallmarks of mycetoma. Lesions provoked by Gram-positive filamentous bacteria of the genera *Nocardia*, *Actinomyadura*, or *Streptomyces* are called actinomycetoma, while those caused by fungi are termed eumycetoma, referring to the eukaryotic nature of these organisms. The disorder is mainly observed in tropical regions, where the infections often follow local, subcutaneous inoculation of contaminated material. We describe a case of mycetoma after a road accident in Germany, in which the fungus *Pseudallescheria boydii* as well as the bacterium *Nocardia abscessus* were isolated.

Case history

A 24 year-old healthy male patient had a motorcycle accident near Bonn, Germany, in which a tractor drove over him. The polytraumatized patient received in-hospital treatment in the Department of Traumatology

of the University of Bonn. His left lower leg had been severely injured and contaminated with soil. Radiological examinations showed a fracture of his left tibia (Fig. 1). The following surgical treatment was performed:



Fig. 1 Radiogram of the bone fracture of the patient's left lower leg.

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necrotic areas were removed and bones stabilized with a fixateur externe. During the first two months, the patient received intensive-care treatment. Four months after the accident, the left lower leg showed edematous swellings and signs of infection. Further surgical treatment followed and samples of bones and muscles of the tibia were sent for pathological and microbiological examination. A mycetoma was diagnosed histopathologically and *P. boydii* was isolated as the potential agent. The patient was thus treated with itraconazole ($3 \times 200 \text{ mg d}^{-1}$) for six months. Sinus discharges were collected for microbiological examinations at two-week intervals during the following three months (6 samples taken altogether). The first two wound aspirates yielded growth of *P. boydii*. Six weeks after antifungal therapy had been initiated, cultures remained negative for *P. boydii*, but instead, Gram-positive bacteria were isolated repeatedly during the following six weeks. Although the patient received no specific antibiotic treatment, one month later two further aspirates showed no growth of fungi or bacteria. Six months later the patient was still in good health and his leg wounds had healed.

Pathology

Macroscopically, most of the aspirates and biopsied tissue samples were brownish-red in colour. Histopathologically, an edema of the bone marrow could be recognized. Multinucleated giant cells and epitheloid cells could be recognized, surrounded by a zone of histiocytes. With Gomori's methenamine-silver stain masses of hyphae and chlamyospore-like structures were demonstrated in the centre of the lesions. The histopathological findings confirmed the diagnosis of a eumycetoma (Fig. 2). With Fontana-Masson stain the hyphae remained hyaline. Therefore white grains caused

by primarily non-melanized fungi were diagnosed histopathologically. Neither Gram nor haematoxylin and eosin staining showed any hint of a concomitant bacterial infection.

Microbiology

The surgical biopsies and the aspirates were cultured on Columbia 5%-sheep-blood agar and McConkey agar at 37 °C, on Brain Heart dextrose and Sabouraud's glucose agar (SGA) at 30 °C. In addition, anaerobic cultures were performed using the Gas Pak-system (Becton Dickinson, Heidelberg, Germany). After three days on SGA, colonies 2 mm in diameter with white woolly aerial hyphae were seen. With aging, the mycelium became grey and the reverse of the culture turned brownish-black. Microscopically, hyaline septate hyphae with conidiophores were seen. The conidiophores produced one-celled, smooth-walled, subspherical conidia, which became brown and thick-walled after liberation. The identification as *P. boydii* was confirmed by rDNA ITS sequencing and the strain was deposited in the CBS culture collection (Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands) as CBS 100870. All remaining fungal strains isolated from the patient were shown to belong to the same species. Some small, white, wrinkled colonies were also isolated from three consecutive specimens obtained after six weeks of antifungal treatment. These colonies grew primarily on Columbia-5%-sheep blood agar and were visible after three days of incubation. Gram staining revealed Gram-positive filamentous as well as rod-shaped and coccoid bacteria. Further investigations (e.g. mycolic acid analyses, carbon assimilation test and the suspended solids hydrolysis test series) showed that the strain was identical with a particular subgroup within the *N.*

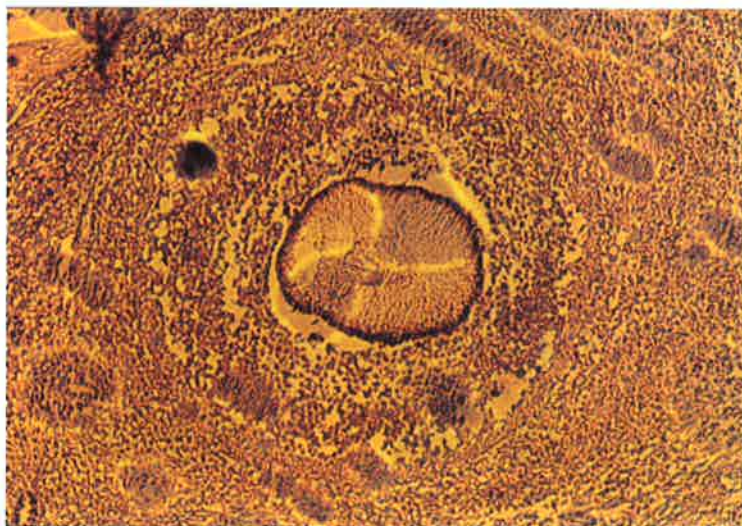


Fig. 2 Histopathological findings showing eumycetoma (Periodic acid-Schiff staining).

asteroides complex that has recently been recognized as *N. abscessus* [1].

Discussion

P. boydii (anamorph: *Scedosporium apiospermum*) and *Nocardia brasiliensis* are well known as causative agents of mycetoma [2,3,4,5]. Both form white granules and elicit a similar host reaction. In contrast, *N. abscessus* [1] until now has been isolated from localised purulent lesions but not from typical mycetoma. We were unable to clarify if *N. abscessus* was involved in causation of the patient's mycetoma, or if its presence was instead causing an abscess. Neither the Gram stain nor Gomori's methenamine-silver disclosed bacterial elements in the mycetoma. A modified Kinyoun stain was not performed, because in contrast to *N. farcinica*, *N. abscessus* is better stained with Gram and Gomori's methenamine-silver stains. All mycetoma grains observed were eumycotic. Nonetheless, it seems possible that *N. abscessus* contributed to the development and persistence of the infection primarily caused by *P. boydii*. *P. boydii* and probably also *N. abscessus* are otherwise saprotrophic, and can be isolated from soil. The development of mycetoma after wound contamination with soil has repeatedly been described [3]. In the present case, *N. abscessus* and *P. boydii* infections probably derived from the soil that contaminated the patient's wound.

The patient described here was primarily immunocompetent, but his injuries and the prolonged intensive-care treatment involving different antibiotic chemotherapies may have induced a transient immunosuppression that facilitated the establishment of the two opportunistic pathogens. The failure to isolate *N.*

abscessus from the patient during the first weeks of disease may have been due to the antibiotic therapy he received during intensive-care treatment. By the time treatment with itraconazole for *P. boydii* was initiated, antibiotic therapy had been discontinued and *N. abscessus* began to be isolated. The two organisms then, appeared to have coexisted in the wound from the time of the accident. The patient never received specific therapy against *N. abscessus* and appeared to be able to clear this infection spontaneously after his immune system was fully reconstituted during the later phase of his in-house treatment. The treatment of choice for members of the *N. asteroides* complex would have been imipenem/cilastatin (with a loading dose of 4–6 g d⁻¹) in combination with amikacin (doses according to serum-level monitoring) for four to six weeks [5]. Absence of treatment has a certain risk because reactivated infections may take a fatal outcome. Our patient remains free of complaints sixteen months after the accident.

References

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