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Severe Osteomyelitis Due to the Zygomycete *Apophysomyces elegans*

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We describe a previously healthy 69-year-old man presenting with osteomyelitis of the humerus due to the zygomycete *Apophysomyces elegans*. The infection was acquired in Aruba, The Netherlands Antilles. The skin provided the most likely portal of entry, although there was no history of a traumatic inoculation. The patient had no history of diabetes, and no underlying immune defects were found. Despite treatment with 7.9 g of amphotericin B, an interthoracoscapular amputation proved necessary to curtail the rapid spread of the fungus in this immunocompetent host.

Zygomycetes are filamentous saprophytic fungi that occur worldwide on decaying plants and in soil. The order *Mucorales* consists of several human pathogenic fungi causing zygomycosis. Members of the genera *Absidia*, *Mucor*, *Rhizomucor*, *Rhizopus*, *Cunninghamella*, *Saksenaea*, *Cokeromyces*, and *Syncephalastrum* produce invasive infections in patients with compromised immunity, although *Rhizopus* spp. and to a lesser extent *Mucor* spp. are the most commonly reported pathogens (2, 9, 15). *Apophysomyces elegans* is a recent addition to this order and was first isolated from soil in India in 1979 (11). This species has been rarely described as a pathogen and is apparently restricted to warmer climates. No particular risk factors have been identified, and most infected patients have been immunocompetent. We present a rather unusual case of osteomyelitic zygomycosis of the humerus which was associated with infection of the contiguous tissue, and we present a review of other cases of *A. elegans* infection reported in the literature.

Case report. The patient was a 69-year-old retired priest, who lived in Aruba, the Netherlands Antilles, for 25 years. He had had no significant illnesses before April 1993 when he complained of increasing pain in his left arm and shoulder. He was not ill, had no fever, and did not report recent trauma. X-ray examination of the shoulder and humerus showed no abnormalities and he was given injections of corticosteroid in his shoulder joint to treat what was thought to be painful arc syndrome. His arm became increasingly more painful, red, and swollen, and so he was readmitted to the local hospital in Aruba. Biopsies failed to provide any clues for diagnosis. Therefore he was referred to our hospital in June 1993 for evaluation of a tumor in his left humerus.

He was in good condition and had no fever. The left humerus was swollen, with a reddish ventral infiltrate measuring 7 by 10 cm. The erythrocyte sedimentation rate was 54 mm in the first hour, and the hemoglobin was 7.5 mmol/liter. Glucose, serum iron, and ferritin were normal.

A large, apparently malignant tumor was evident on X-ray, and there was a pathological subcapital fracture of the humerus. However, during surgery, a large abscess, filled with yellowish pus, was discovered in the muscles and in the adjoining bone, which also had an abnormal consistency. Microscopic examination showed many polymorphonuclear granulocytes but no bacteria. On fluorescent staining (Fungiqual; Ciba-Geigy, Basel, Switzerland), broad, irregular hyphae, consistent with a species of the zygomycetes, were seen and cultures yielded a fungus but no bacteria. The patient was discharged 2 days later but continued to drain purulent material from the wound. Treatment with amphotericin B (1 mg/kg/day) was therefore initiated and despite a search for other foci of infection, none was identified. Two months later, further debridement proved necessary to manage the copious discharge, but during the operation it became obvious that the shoulder joint had become involved and a substantial volume of pus was found in the marrow of the humerus. Hyphae were again demonstrated by fluorescent staining, and cultures yielded the same fungus. The medical course was complicated by diverticulitis with perforation of the sigmoid, for which a rectosigmoid resection was necessary. No hyphae were found in the resected colon.

Two weeks later the wound started to drain pus which yielded the same fungus. A bone scan showed new lesions in the humerus. By this time, the patient had received a total of 4.3 g of regular amphotericin B without any effect on the infection since the fungus persisted. Moreover, progressive nephrotoxicity had developed. Consequently the patient was treated with amphotericin B lipid complex (ABLC) (5 mg/kg/day; The Liposome Company Inc., Princeton, N.J.) which was well tolerated. However, dissemination of the fungus to the shoulder joint was evident, and after the patient had received 3.65 g of ABLC, a decision was made to perform a forequarter amputation. The wound was covered with a posterior shoulder flap. Culture of the lesions and the surgical margins yielded no growth, but broad, nonseptate hyphae were evident in the bone. The patient was discharged in stable condition 2 weeks after the amputation, and he continued to receive ABLC until a total dose of 9.7 g had been given. There was no evident underlying illness such as diabetes or iron overload, and no defects in granulocyte functions were identified. Ten months after the radical amputation, the patient was without any evidence of relapse of the zygomycotic infection.

Microbiological studies. Fungal cultures of infected tissue, muscle, bone, and wound drainage yielded a rapidly growing fungus within 2 days at 37°C on Sabouraud glucose (2%) agar and blood agar. Floccose aerial mycelium, initially white-grey, with no reverse pigment, covered the petri dish completely within a few days. The hyphae were broad, nonseptate, and typical of a zygomycete. The fungus grew readily at 25, 37, and 42°C but did not sporulate on the primary isolation medium or on subculture to Sabouraud glucose (0.2%), which we use routinely to stimulate sporulation in other molds. Sporulation

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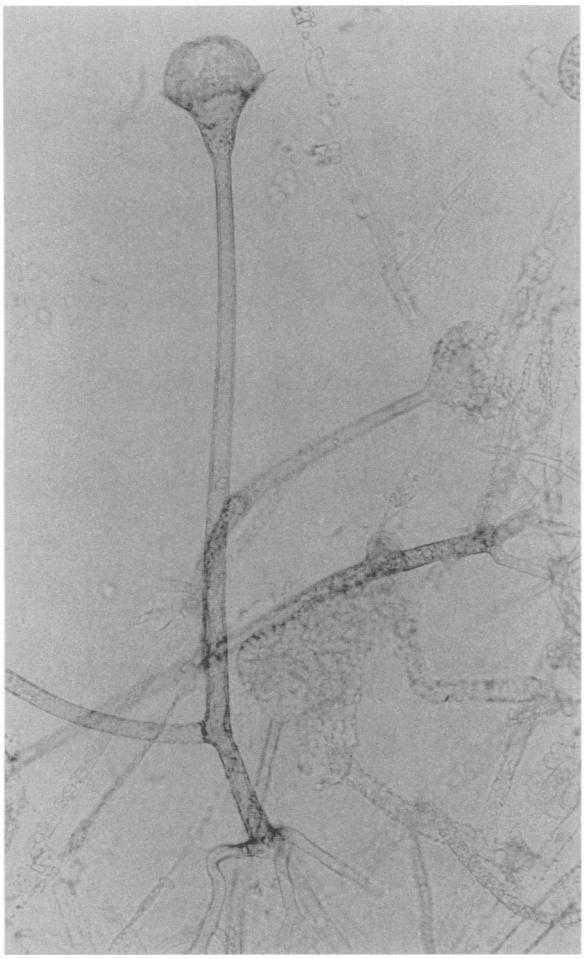

FIG. 1. Photomicrograph of water agar slide culture showing rhizoids, sporangiophore, apophysis, and sporangium of *A. elegans*. Lactophenol cotton blue stain. Magnification, ×400.

was subsequently induced by placing agar blocks (Sabouraud glucose 0.2%) with hyphae and aerial growth onto the surface of solidified 1% agar in distilled water (3, 13). After 2 weeks of incubation at 30°C, the culture demonstrated the sporangia characteristic of *A. elegans*. The sporangiophores had a diameter of about 4 μm and a length of 100 to 200 μm (200 to 300 μm in mature cultures). They arose at right angles and had a septate basal segment consistent with a foot cell. Sporangia with a diameter of 20 to 40 μm showed the distinct funnel-shaped apophysis, hemispherical columella, and dark subapical thickening of the sporangiophore below the apophysis (Fig. 1). In vitro antimycotic susceptibility was determined at the Royal Tropical Institute, Antwerp, Belgium (by C. de Vroey), by agar diffusion with Sensitabs (Rosco, Taastrup, Denmark). The strain was resistant to amphotericin B, ketoconazole, itraconazole, fluconazole, and 5-fluorocytosine but susceptible to terbinafine. Subcultures of the isolate have been deposited in the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands (CBS 658.93), and the American Type Culture Collection, Rockville, Md. (ATCC 90757).

Histopathological studies. The medullary humeral bone showed extensive coagulative necrosis involving the cancellous bone with bone marrow and, at one place, extension into the compact cortical bone. In the necrotic areas some neutrophilic granulocytes were seen together with focally ribbon-like broad nonseptate hyphae. These structures stained strongly with silver methenamine and were periodic acid-Schiff reaction positive, diastase resistant, and gram positive. No other microorganisms were observed. This picture was consistent with chronic active zygomycotic osteomyelitis. The soft tissue, bone surgical margins, lymph nodes, and thrombosed veins were all free of fungi.

Fungi seldom cause bone infection (16), and contiguously spread osteomyelitis due to zygomycetes is rare indeed (14). A total of nine cases of zygomycosis due to *A. elegans* (Table 1) have been reported in the English-language literature since the first description of this mold from soil in India in 1979 (11), of which seven came from the southern regions in the United States (5, 8, 19–21). The present report describes the third case encountered outside the United States, and the infection was probably contracted in the Caribbean. The other non-U.S. cases were a nosocomial infection in India (7) and a burn wound infection in the Northern Territories, Australia (1). This suggests that *A. elegans* is distributed worldwide in warmer climates. Only two patients had any underlying illness at the time of contracting the fungus infection. This contrasts markedly with data on other genera from the order *Mucorales*, which generally only become invasive in patients with compromised immunity as a result of hematological malignancies, immunosuppressive therapy, diabetes mellitus, or severe skin burns (2). In these patients, rhinocerebral invasion, pulmonary infection, and disseminated disease are mostly encountered whereas primary cutaneous and soft tissue infection are rarely seen. Nosocomial *Rhizopus* infections due to contaminated bandages (4) or intramuscular fluid (6) have been described, and traumatic wounds (17) can become infected with zygomycetes. But only in immunocompromized patients are these saprophytes able to induce deep-tissue invasion (9). In contrast, *A. elegans* was able to produce progressive tissue invasion in normal hosts, apparently beginning at the site of prior tissue injury such as trauma, burns, or invasive procedures (Table 1). However, none of these predisposing factors were apparent in the present case, and the corticosteroid injections in the shoulder did not appear to have any relationship with the development of osteomyelitis. Of the eight patients who have survived *A. elegans* infection, all required aggressive surgical debridement in addition to treatment with amphotericin B. Definitive cure was only achieved by resection of the extremity or organ involved, irrespective of the immunologic and physiologic status of the patient. A recently described patient with soft tissue and renal infection with *A. elegans* was cured with a combination of hyperbaric oxygen and gamma interferon therapy in addition to ABLC and surgical debridement (12). Accurate data on the effectiveness of medical treatment, the optimal duration, and total dose of amphotericin B for zygomycosis do not exist, although amphotericin B is regarded as the most effective single agent for this infection (22). The isolate was in vitro resistant to amphotericin B, but the patient appeared to respond when ABLC was given. In any event, in vitro resistance was not predictive of failure, as has been described in a case of rhinocerebral zygomycosis (10). Terbinafine has been used successfully in only one case of *Rhizopus* sp. wound infection in a renal transplant patient (18). Despite the in vitro susceptibility of *A. elegans* to terbinafine and its resistance to amphotericin B, we nonetheless decided to continue treatment with amphotericin B, albeit in another form, and we achieved mycological remission. However, the tissue damage was so severe that removal of the affected extremity was essential for complete healing.

TABLE 1. Clinical profile of patients with *A. elegans* infection[a]

Patient no., yr (reference)	Gender, age (yr), location	Underlying disease	Inoculation, portal of entry	Affected body site(s)	Treatment		Pathological findings	Outcome
					Surgical	Antifungal (total dose)		
1, 1982 (21)	M, ns, Tex.	None	Trauma	Extremity	Debridement	AmB (ns)	Necrosis	Survived
2, 1982 (21)	M, ns, Tex.	None	Trauma	Extremity	Debridement	AmB (ns)	Necrosis	Died
3, 1985 (20)	F, 49, Ariz.	Poorly controlled DM	Traumatized skin, left tibia	Left tibia	Debridement, above-knee amputation	AmB (790 mg)	Necrosis, angioinvasion	Survived
4, 1986 (8)	M, 56, Tex.	None	Unknown	Kidney, bladder, right tibia	Debridement, nephrectomy	AmB (2 g)	Necrosis, angioinvasion, hematogenous osteomyelitis	Survived
5, 1990 (1)	F, 45, Northern Territory, Australia	Extensive burns	Skin	Right ankle	Debridement, below-knee amputation	AmB (ns, 3 weeks)	Necrosis	Survived
6, 1992 (5)	M, 38, Tex.	None	Traumatized skin	Right arm, right leg	Debridement, arm and leg amputation	AmB (ns)	Necrosis, possible osteomyelitis	Survived
7, 1993 (7)	M, 27, India	None	Postinguinal herniorrhaphy	Necrotizing fasciitis lower abdomen, left inguinal area, and scrotum	Repeated debridement	AmB (ns) 5 days, 0.5 mg/kg	Necrosis, angioinvasion	Died
8, 1993 (19)	M, 59, Fla.	None	Insect (?) bite or sting, right prescapular area	Prescapular skin and scapula	Repeated debridement, scapulectomy	AmB (4 g)	Necrosis, angioinvasion, osteomyelitis	Survived
9, 1994 (12)	M, 29, Tex.	None	Traumatized skin	Right flank, left kidney	Debridement, nephrostomy	AmB (1 mg/kg/day, 6 days), ABLC (5 mg/kg/day, 8 weeks)	Necrosis, hematogenous spread to kidney	Survived
10, 1994 (PR)	M, 69, Caribbean	None	Unknown	Left humerus	Repeated debridement, radical forequarter amputation	AmB (4.3 g), ABLC (9.7 g)	Necrosis, osteomyelitis	Survived

[a] Abbreviations: M, male; F, female; ns, not stated; PR, present report; DM, diabetes mellitus; AmB, amphotericin B; ABLC, amphotericin B lipid complex.

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