Invasive Apophysomyces variabilis infection in a burn patient

Wilfred P. delaCruz, Tatjana P. Calvano, Matthew E. Griffith, Christopher E. White, Seung H. Kim, Deanna A. Sutton, Elizabeth H. Thompson, Jianmin Fu, Brian L. Wickes, Josep Guarro, and Duane R. Hospenthal

Department of Medicine, San Antonio Military Medical Center, and US Army Institute of Surgical Research, Fort Sam Houston, Texas; Fungus Testing Laboratory, Department of Pathology and Department of Microbiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas; and Mycology Unit, Medical School and IISPV, Universitat Rovira I Virgili, Reus, Spain

Key Words: Apophysomyces variabilis, trauma, burns, military

Running Title: Apophysomyces variabilis Infection

*Corresponding author: Duane R. Hospenthal, drhospenthal@gmail.com

Disclaimer: The views expressed herein are those of the authors and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the US Government. W.P.D., T.P.C., M.E.G., C.E.W., S.H.K., and D.R.H. are employees of the US government. This work was prepared as part of their official duties and, as such, there is no copyright to be transferred.
Abstract

*Apophysomyces variabilis* is an emerging fungal pathogen that can cause significant infection in immunocompetent patients. We report a case of *A. variabilis* invasive wound infection in a 21-year-old male after a self-inflicted burn injury.
Case Report

We report a case of a 21-year-old active duty US Marine with known psychiatric history who suffered 90% total body surface area (TBSA) burns after self-immolation with gasoline while stationed in Okinawa, Japan. After the flames were doused by immersion in freshwater, he was taken immediately to a local emergency department where he was intubated and underwent full body escharotomies. He was transferred to a military hospital in Hawaii where he underwent aggressive fluid resuscitation and received fresh frozen plasma for coagulopathy. Fiberoptic bronchoscopy was performed which showed no inhalational injury. He was then transferred to the US Army Institute of Surgical Research Burn Center in San Antonio Texas approximately four days after the burn. Upon arrival, he underwent fascial excision to bilateral lower and upper extremity with tangential excision of the anterior-posterior torso. A 4:1 mesh sandwich was applied to the anterior/posterior torso and allograft applied to 80% of TBSA. On post-burn day 8, he was found to have suspected fungal growth on the allograft on his lower back. Fascial excision was performed and histopathology of the excised tissue showed numerous large diameter aseptate hyphae, located mostly in the non-viable adipose tissue but with evidence of microinvasion to blood vessels (Figure 1). Due to clinical concern of fungal invasion beyond the burn wound the patient was initially empirically treated with voriconazole and liposomal amphotericin B. Deep wound cultures subsequently grew a fungus that was tentatively identified as a species within the order Mucorales. Blood cultures were negative for the fungus.

Amphotericin B was continued to cover for the mucoralean species while voriconazole was continued to empirically cover other possible fungal pathogens that may be infecting this patient. Despite aggressive debridement of infected tissue and systemic antifungal administration, fungal infection persisted. The patient eventually died due to extensive burn wounds and multi-organ
failure syndrome. The fungal isolate from lateral back tissue was referred to the Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio, where it was identified as *Apophysomyces variabilis* by phenotypic and genotypic testing.

**Fungal Identification**

The isolate submitted on Mycobiotic agar (Remel, Lenexa, KS) was accessioned into the Fungus Testing Laboratory collection as UTHSC 11-1354. Colonies were white and woolly, filling the agar slant tube, but failed to form any fruiting structures. Given the rapid growth and sterile nature of the isolate, the initial impression suggested either an *Apophysomyces* or a *Saksenaea* species. A Carnation leaf agar plate (CLA) (16) and a water agar culture (18), both prepared in-house, were inoculated and incubated at 25°C and 35°C, respectively, to induce sporangiospore formation. On Carnation leaf agar, the culture produced brown, unbranched sporangiophores, prominent apophyses, and pyriform sporangia similar to those observed in *A. ossiformis* and *A. trapeziformis* (2). However, a notable phenotypic difference of our isolate was the variable nature of the sporangiospores in size and shape (Figures 2A and 2B). A darkened area below the apophysis was also present – a feature described in other species within the genus (Figure 2C). Temperature studies on potato flakes agar slants (16), prepared in-house and incubated for 7 days, indicated 4+ growth at 37°C, 2+ growth at 40°C, and no growth at either 45°C or 50°C. Based on the above features, the isolate was identified as *Apophysomyces* sp. and molecular characterization was initiated.

Molecular identification of this isolate was performed as described previously (19). Briefly, genomic DNA was prepared from a 24-hour potato dextrose agar plate grown at 30°C using the Prepman Ultra reagent (Applied Biosystems, Foster City, CA). PCR reactions were
performed using the ITS1 and NL4 primers (19). The amplicon was purified using Qiagen PCR Purification Kit (Qiagen, Valencia, CA) and then sequenced on both strands at the UTHSCSA Advanced Nucleic Acids Core Facility (19). The sequences were assembled using MacVector software (MacVector, Inc, Cary, NC), and the individual ITS and D1/D2 regions were used to perform BLASTn searches of the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/).

Individually, the search results were considered significant at identity ≥ 97%. The three top hits for the ITS search were *Apophysomyces variabilis* (accession # FN556442.1, identity = 708/708 (100%), *Apophysomyces variabilis* (accession # FN813492.1, identity = 701/701 (100%), and *Apophysomyces variabilis* (accession # FN813491.1, identity = 701/701 (100%). Results for the D1/D2 search similar, with the top three hits consisting of *Apophysomyces variabilis* (accession # FN554255.1, identity = 679/680 (99%), *Apophysomyces variabilis* (accession # FN554254.1, identity = 655/656 (99%), and *Apophysomyces variabilis* (accession # FN554253.1, identity = 651/652 (99%). Thus, sequencing and phenotypic characterization confirmed the isolate as *A. variabilis*.

Antifungal susceptibility testing of the case isolate was performed with results as follows in µg/ml: amphotericin B < 0.03, posaconazole 0.06, voriconazole 8, itraconazole 0.125, anidulafungin >8, and caspofungin >8.

**Discussion**

*Apophysomyces* was first isolated in soil samples in India in 1979 (14). Morphologically, this fungus typically produces pyriform sporangia, conspicuous funnel and/or a bell-shaped apophyses, and clear, thin and smooth-walled sporangiospores that are mostly oblong with rounded ends. It is a thermotolerant fungus that grows rapidly between 26 and 42 ºC (5, 14, 20).
While most isolates have been reported from India, it also has been isolated in Australia, Southeast Asia, United States, and South America, suggesting broad distribution which covers tropical and subtropical climates. In the United States, *Apophysomyces* represents only 0.5% of all the clinical significant Mucorales (3).

Although *Apophysomyces* is typically an environmental mold, there have been an increasing number of human infections reported. Human infections by *Apophysomyces* involve a wide range of patients, the majority of which were immunocompetent (4). This is in contrast to infections caused by other members of Mucorales that tend to involve immunocompromised individuals, most commonly seen in poorly controlled diabetic patients. The most common mode of infection is by traumatic implantation of contaminated soil or water leading to cutaneous or subcutaneous infections, rhino-orbital infection after facial trauma (9, 10, 12), or osteomyelitis after breakdown of overlying skin (7, 13, 20). Renal infection by *Apophysomyces* has been reported, suggesting possible hematogenous mode of infection following traumatic implantation through the skin (17). Disseminated infection with *A. elegans* infection has also been reported after kidney transplantation from a donor who died of drowning (15). Recently, a cluster of cutaneous mucormycosis has been reported in the aftermath of Joplin, MS tornado in which 13 of the confirmed cases yielded *Apophysomyces trapeziformis* (8). In our patient, the mode of infection is unclear, but could have occurred by traumatic implantation from his immersion in water after the burn.

Until recently, it was believed that *Apophysomyces* comprised a single species, *A. elegans*. However, sequence analysis of several genes combined with physiological and morphological characteristics has led to the recognition of four distinct *Apophysomyces* species (*A. elegans, A. ossiformis, A. trapeziformis, and A. variabilis*) (2). The incidence of human
infection due to *A. variabilis* is unknown. Most reports of infections due to *Apophysomyces* have been attributed to *A. elegans* prior to the identification of the four distinct species of *Apophysomyces*. The first reported cases of *A. variabilis* human infection were identified as *A. elegans* morphologically and subsequently identified as *A. variabilis* by ITS sequencing (11). It is likely that some of the reported cases of *A. elegans* infection may have been due to *A. variabilis*, although the extent to which these infections are due to *A. variabilis* is uncertain. This also underscores the difficulty of identification by morphology and that definitive identification may require genetic analysis, although this approach may not be practical in routine laboratories. Interestingly, in the study where *A. elegans* was later identified to be a complex of at least four species, the isolates that were identified as *A. elegans* were obtained from non-clinical samples (2) which lends to the possibility that *A. variabilis* and possibly *A. ossiformis* and *A. trapeziformis* were the pathogenic species.

Most patients with *Apophysomyces* infection were treated with antifungal agents and aggressive debridement with variable results. Response was largely dependent on patient’s co-morbid conditions and immune status. Amphotericin is the recommended drug of choice for mucormycosis and has been used in most human infections incited by *Apophysomyces* species. Susceptibility studies have shown variable results. The study by Alvarez et al (2), showed that the four species of *Apophysomyces* were susceptible to amphotericin and posaconazole but not to voriconazole and caspofungin. A susceptibility study of Mucorales isolates by Dannaoui, et al (6) showed marginal activity of amphotericin against *Apophysomyces*. Another in vitro susceptibility study performed on *A. elegans*, also showed reasonable susceptibility of the fungus to posaconazole (1) and was used to successfully treat rhino-orbital *Apophysomyces* infection after failing treatment with amphotericin B (9). These reports suggest that amphotericin may still
be the drug of choice against Apophysomyces as well as other genera of Mucorales as an adjunct
to surgical debridement. Posaconazole may be used in patients not responding to amphotericin or
as an additional agent taking advantage of an alternative mechanism of antifungal activity.
Retrospective susceptibility testing of the isolate showed sensitivity to amphotericin and
posaconazole similar to results obtained by Alvarez (2).

Apophysomyces variabilis was previously thought to be an environmental fungus is
becoming an emerging pathogen that can cause serious infections in humans and has some
propensity to cause rare infections in immunocompetent patients. Infection is usually facilitated
by traumatic implantation via trauma or burn. The true extent of A. variabilis infection is
uncertain and requires genetic analysis of clinical isolates of Apophysomyces species. The
mainstay of treatment continues to be amphotericin while posaconazole may be used as an
alternative agent. To date, this is the first report to document infection by A. variabilis in a burn
patient following separation of this species within the A. elegans species complex.

Acknowledgements
Authors acknowledge Dora McCarthy for antifungal susceptibility testing of the isolate.

Accession numbers
Isolate has been deposited into the University of Alberta Microfungus Collection under the
accession number UAMH 11571. The sequences were deposited in Genbank under accession
numbers JN980700 for the ITS sequence and JN980699 for the D1/D2 sequence.
166 References


Figure legends

Figure 1. Histopathology (hematoxylin and eosin stained) of excised tissue from left lower back showing numerous large diameter aseptate hyphae, located mostly in non-viable adipose tissue with evidence of microinvasion of blood vessels.

Figure 2. Features of *A. variabilis* fruiting structures produced on carnation leaf agar after 7 days incubation at 25°C (Brightfield microscopy, lactophenol cotton blue mount): sporangiophore, apophysis, and variable sporangiospores (2A); sporangiospores of variable size and shape (2B); and fruiting structure showing darkened area below the apophysis (2C).