Tropical medicine rounds

Cutaneous phaeohyphomycosis caused by an Itraconazole and Amphoterecin B resistant strain of *Veronaea botryosa*

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**Background** Although the dematiaceous fungus *Veronaea botryosa* is rarely encountered clinically, it can be pathogenic.

**Methods** A patient with a history of diabetes mellitus, coronary artery disease, and Cushing’s syndrome had recurrent multifocal, crusted, brownish-red noduloplaques on the right forearm, left upper limb, and right knee. A skin biopsy was obtained for histopathology and fungal cultures.

**Results** The histopathology showed brownish hyphae and yeast-like cells scattered in granulomatous infiltrates. Slide cultures revealed erect and straight conidiophores with two-celled cylindrical conidia, which have round tops and truncated bases. The fungus was identified as *Veronaea botryosa*. The disease slowly progressed despite a 6-month itraconazole regimen (200 mg daily). Subsequent use of Amphoterecin B produced only mild clinical improvements. Susceptibility tests showed resistance to both agents.

**Conclusions** Cutaneous phaeohyphomycosis caused by *V. botryosa* is extremely rare. Antifungal susceptibility tests are important for choosing the appropriate drug and predicting the clinical outcome.

**Introduction**

Phaeohyphomycoses are rare, generally localized, subcutaneous, or intramuscular infections caused by certain dematiaceous fungi. The most common pathogens are *Bipolaris spicifera* and *Exophiala jeanselmei*. *Veronaea botryosa* also causes phaeohyphomycoses; however, only few cases have been reported in the literature to date. Here, we report a case of cutaneous phaeohyphomycosis caused by a strain of *V. botryosa* that was resistant to itraconazole and amphoterecin B. Clinical applications of antifungal susceptibility tests are also discussed.

**Case Report**

The patient was a 76-year-old male retired farmer with a history of diabetes mellitus, coronary artery disease, and Cushing’s syndrome (owing to his long-term use of oral corticosteroids). He visited our clinic complaining of nodules on his right forearm for more than 2 years. The nodules were asymptomatic, variable in size, erythematous to brownish, and crusted (Fig. 1).

A skin biopsy revealed epithelioid granuloma with multinucleated giant cells engulfing yeast-like cells. The latter stained positive with Grocott’s Methenamine Silver nitrate (GMS). Results from fungal and mycobacterial cultures were negative. Oral itraconazole (200 mg daily) was prescribed empirically. The patient took the medications regularly for 5 weeks and showed gradual improvement in his condition, but he failed to maintain a regular medication schedule and returned to our out-patient clinic 17 months later for further treatment of relapsing cutaneous lesions on the left upper extremity. At that examination, it was noted that the cutaneous nodules on the right forearm were subsiding.

Physical examination revealed multiple, erythematous, brownish, crusted nodules or plaques on the left forearm and left upper arm (Fig. 2). A skin biopsy was obtained for microscopic examination and fungal cultures. Granulomatous structures composed of mixed inflammatory cells were evident microscopically. Under H&E stain, brownish beaded hyphae and yeast-like cells measuring 3–5 μm in diameter were observed scattered in the stroma and within the multinucleated giant cells. The hyphae and yeast-like cells stained black and pink with GMS and PAS stains, respectively (Fig. 3). Also, they stained black with Masson-Fontana stain, indicating the presence of melanin (Fig. 3).

The specimen was incubated at room temperature in Sabouraud dextrose agar and potato media and yielded grayish-brown, velvety colonies after 2 weeks (Fig. 4). Slide cultures from the colonies showed erect and straight conidiophores with conidia born terminally and laterally on the apical part of the conidiophores. The conidia were pale brown, smooth-walled,

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two-celled, cylindrical, and with round tops and truncated bases (Fig. 4). The fungus was identified as *Veronaea botryosa*. According to the clinical manifestations and pathological findings, cutaneous phaeohyphomycosis caused by *V. botryosa* was diagnosed.

Itraconazole (orally, 200 mg daily) was prescribed to control the disease. Despite a 6-month treatment with itraconazole, more erythematous, crusted noduloplaques spread to the left upper arm, left dorsal hand, right forearm, and right knee (Fig. 2). Owing to the poor response to oral itraconazole, the therapeutic regimen was switched to intravenous amphotericin B, and drug susceptibility tests were performed.

Etest\(^8\) (AB BIODISK, Solna, Sweden) was used to determine the minimal inhibitory concentration (MIC) of antifungal agents. The conidial suspension was swabbed on standard agar media. Etest\(^8\) strips of amphotericin B and itraconazole were then placed on the agar’s surface. After incubating for 3 days, the fungus grew uninhibited on the agar. The MIC values of amphotericin B and itraconazole were greater than the highest concentrations on the strips: 32 µg/ml for amphotericin B and 256 µg/ml for itraconazole. The *V. botryosa* isolate was judged to be resistant to both drugs.

Despite amphotericin B resistance, the lesions seemed dryer and less erythematous following 17 days of treatment. The patient’s renal function had deteriorated, possibly owing to renal toxicity caused by amphotericin B. A lipid formulation of amphotericin B was considered because of decreased renal toxicity and increased efficacy. Unfortunately, the patient suffered an acute myocardial infarction with cardiogenic shock and was discharged from the hospital against the advice of physicians.

**Discussion**

*Veronaea botryosa* (= *Sympodina coprophila* = *Veronaeae coprophila*) is a dematiaceous fungus. Its epidemiology and ecology have not been well studied.\(^2\)\(^3\) Normally isolated from decaying stems, goat dung, ice of water reservoirs, and snow from gymnosperm trees, *V. botryosa* has only been documented in human lesions on few prior occasions.\(^2\)\(^3\)

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**Figure 1** Various-sized, erythematous to brownish, crusted nodules initially on the right forearm

**Figure 2** Upper: Closer view of the crusted nodules on the left forearm; Lower: More erythematous crusted nodules and plaques on the left upper limb and the right forearm
Identification of *V. botryosa* relies mainly on the morphological features. Phaeoid septate hyphae are 3–4.5 µm in diameter and produce abundant geniculate, generally unbranched, pale-brown conidiophores. Conidia are produced in sympodial sequences, typically bicellular with round tops and truncated bases. Occasionally, two-septate conidia are also found.\(^2,3,6\)

The present report adds to the previous reports of *V. botryosa* infection in humans. The first case was from China in 1989, and clinical manifestations included black verrucous nodules and cysts on the left dorsal hand, forearm, and both cheeks.\(^6,7\) In 1995, the second case was a 28-year-old immunocompetent Libyan woman who, for 3 years, simultaneously presented extensive nodular or ulceronodular lesions on her limbs and painless ulcerative patches of the nasal and oral mucosa.\(^8\) No information was provided regarding therapy and disease development. The third case, reported in 1999, was a sugarcane farmer living on La Reunion Island in the Indian Ocean who developed multiple painless coalescing nodules with pus on both feet and wrists after liver transplantation and chemotherapy.\(^6\) In 1999, the second case was a 28-year-old immunocompetent Libyan woman who, for 3 years, simultaneously presented extensive nodular or ulceronodular lesions on her limbs and painless ulcerative patches of the nasal and oral mucosa.\(^8\) No information was provided regarding therapy and disease development. The third case, reported in 1999, was a sugarcane farmer living on La Reunion Island in the Indian Ocean who developed multiple painless coalescing nodules with pus on both feet and wrists after liver transplantation and chemotherapy.\(^6\) All cutaneous lesions disappeared after an 8-month treatment course with itraconazole (300 mg daily). Foulet *et al.* also mentioned that *V. botryosa* was a saprophyte or a plant pathogen and probably had been surviving in the dermis without manifesting any clinical lesions. Only the post-transplantation immunodepressed status allowed the fungus to express pathogenic potential.\(^4\) The fourth case, reported in Taiwan in 2003, was an 81-year-old retired farmer who developed a dusky red, swelling plaque with pustules and sinus tracts over the left dorsal foot, accompanied with several fluctuating papulonodules spreading up along the lymphatics of the left lower leg. Partial surgical debridement and itraconazole (200 mg per day) resulted in gradual clinical improvements, and fungal cultures turned negative, although no definite end point of treatment was mentioned.\(^9\) Also in 2003, the fifth case presented as generalized crusted nodules and plaques on the face, upper limbs, legs, scrotum, and buttocks of a 12-year-old boy living in China. The patient failed to respond to treatments with terbinafine (125 mg/day) for 6 months followed by itraconazole (100 mg/day) for 6 months.\(^7\)

In the previously reported cases, the subcutaneous phaeohyphomycosis caused by *V. botryosa* manifested more commonly as a noduloplaque form or, less commonly, as a lymphocutaneous form. The mycosis usually develops over the extremities.\(^1,10\) As with other phaeohyphomycoses, *V. botryosa* is often transmitted by direct inoculation of the skin or, in rare cases, spread through the air to cause mucosal lesions.\(^1,10,11\) The infection develops in both immunocompetent and immunocompromised patients, and dissemination of cutaneous lesions may occur in an immunodeficient status through autoinoculations, hematogenous, or lymphatic spread.\(^1\)

Most cases of cutaneous phaeohyphomycoses caused by *V. botryosa* were farmers, consistent with the suggestion that the fungus may be a saprophyte or plant pathogen.\(^1,5\) In our case, the exclusivity of the lesions to the extremities is also consistent with direct entry of the fungus as the origin of infection. Our observations that the fungus grew on agar media at room temperature but not at 37°C, and that repeated blood cultures yielded negative results, suggest fungal tropism for lower temperature areas (such as the skin) and explain rare hematogenous spread.

The patient in our report initially developed some nodules on his right forearm, prompting treatment with itraconazole (200 mg daily) for 5 weeks, and the lesions resolved completely. However, he returned to our clinic 1 year later with extensive noduloplaques on the left upper limb, right knee, and right forearm. The new lesions on the right forearm were located at the same site as the previous lesions and had a similar clinical appearance and histopathologic findings. The
evidence suggests that these two events might be related to the same pathogen, *V. botryosa*, even though initial fungal cultures were negative.

There are two possible explanations for the resolution and recurrence of the lesions on the right forearm. One explanation is that the fungus responded well initially to itraconazole treatment but that a drug-resistant strain emerged owing to an insufficient treatment course. A 3-week course of itraconazole did not appear to be sufficient for treatment of the initial deep fungal infections on the right forearm, although the causative fungus was sensitive to this treatment. The other explanation is that the host immunity, instead of an exogenous drug, determines the remission and recurrence of the cutaneous lesions. In this scenario, the temporary resolution of the cutaneous lesions would be owing to transient success of the host immune defenses. This latter explanation, in agreement with the postulation of Foulet et al.," is that a decreased host immune response allows the fungus to express its pathogenic potential and manifest itself clinically.

Ideally, antifungal susceptibility tests should follow the guidelines proposed by the NCCLS (National Committee for Clinical Laboratory Standards). However, NCCLS standards and guidelines for antifungal susceptibility tests for *V. botryosa* do not exist. Given this limitation, the method presently employed was based on the overall NCCLS guidelines developed for the common filamentous fungi. Etest\(^{R}\) MIC values of amphotericin B and itraconazole exceeded 32 \(\mu g/ml\) and 256 \(\mu g/ml\), respectively. These drug concentrations are significantly higher than the effective concentration in the blood, indicating that the *V. botryosa* isolate is resistant to these two drugs.

As tools for choosing an adequate drug and predicting the clinical outcome, antifungal susceptibility tests play an important role in treating fungal infections. However, other factors also influence clinical outcome. Drug pharmacokinetics, drug interactions, host immunity, current underlying disease, proper patient management, virulence of the infecting organism, and the organism’s interaction with both the host and the therapeutic agent appear to be more valuable than the MIC as predictors of clinical outcome\(^{14}\). In short, successful treatment of fungal infections requires not only a sensitive pathogen, but also prolonged treatment with proper dosing and a cumulative dose, good patient compliance, prevention of drug interactions, adequate blood and lesion concentrations and, most importantly, adequate host immunity.\(^{14,15}\) In the present case, the fungus demonstrated resistance to amphoterecin B despite mild clinical improvements. Sufficient data were not available in order to make a conclusive statement about this condition. Host immunity may have been one of the factors influencing the outcome.

References