Primary subcutaneous Alternaria alternata infection of the hand in an immunocompromised host

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We describe a case of a progressive subcutaneous Alternaria alternata infection in the hand of a patient with chronic lymphocytic leukemia (CLL). The diagnosis was based upon the examination of tissue biopsy and isolation of the etiologic agent in culture. The identity of the isolate was determined by phenotypic characteristics and by sequencing the ITS and D1/D2 regions of the rDNA. Despite combination therapy with voriconazole and micafungin, the lesion continued to progress. Posaconazole therapy, along with surgical excision of the infected tissue, resulted in the eradication of infection. The limitations of the clinical management of invasive Alternaria infections are discussed.

Keywords Alternaria spp., fungal hand infections, immunocompromised, surgical management, antifungal therapy

Introduction

Opportunistic soft tissue fungal infections by Alternaria spp. are becoming increasingly common as immunosuppressive regimens are used more frequently, and the number of organ transplant recipients grows [1–5]. Other risk factors include hematologic malignancy, diabetes, and prolonged corticosteroid use [6–8]. In terms of chronic lymphocytic leukemia (CLL), patients are heavily immunocompromised not only due to prior therapies but also as a result of CLL itself. Even in the absence of therapy, CLL is associated with immune deficiency due to prominent deficits in both cell-mediated and humoral immunity [9]. Therefore, patients with CLL have an increased risk of opportunistic infections such as those caused by Alternaria. In fact, greater than half of CLL deaths are due to infectious causes [10]. Given the increasing frequency of Alternaria infections, it is important to review the clinical management, including the approach to refractory disease.

Case report

A 58-year-old male biologist and farmer with a history of treatment-refractory CLL was admitted with fever, rigors, and night sweats. A firm, non-tender 1 cm × 1 cm nodule was noted on the dorsum of his right hand 2 weeks prior to admission. The central area of the nodule was greenish blue and there was a peripheral area of induration. Given concern of an opportunistic infection due to the patient’s extensive prior chemotherapy and immunotherapy, the oncologist aspirated the nodule and sent the fluid to the microbiology laboratory.
A large number of fungal elements were seen on microscopic Gram stain examination suggesting a high burden of disease, and inoculation of a portion of the aspirate yielded *Alternaria* species in culture. Of interest was the observation that the nodule developed and progressed while the patient was on voriconazole therapy at a dose of 200 mg orally twice per day which was initially started 3 months prior to admission for possible pulmonary aspergillosis. The patient recalled a bee sting to the right hand in the area where the nodule developed.

On admission to the hospital for febrile neutropenia, the patient was found to have an elevated 1,3 beta-D-glucan level of 83 pg/ml in the peripheral blood (<60 pg/ml = negative, Viracor Laboratories, Lee’s Summit, MO), which had been negative at 41 pg/ml 3 months prior. Admissions testing also revealed a voriconazole trough that was subtherapeutic at <0.1 μg/ml. Review of the record found no therapeutic drug monitoring prior to this time.

Despite increasing the dose of voriconazole and adding micafungin as a second antifungal agent, the nodule continued to enlarge. Voriconazole was then changed to posaconazole at a dose of 400 mg orally twice per day taken with fatty meals. Posaconazole was chosen based on *in vitro* data in the literature demonstrating that it has the lowest MIC for all the azoles when tested against *Alternaria* species [11–13]. In addition, surgical resection was requested given the refractory nature of the lesion despite antifungal therapy, and the concern that the infection would worsen in the setting of the high dose systemic steroids needed for treating CLL.

Preoperative MRI demonstrated an 8 × 6 × 15 mm nodule invading the subcutaneous tissues over the radial aspect of the second metacarpal sparing the interosseous muscle. The nodule was mildly hyperintense on T2-weighted imaging and isointense on T1-weighted imaging with mild peripheral enhancement on post-contrast images. These findings are consistent with an infectious nodule (Fig. 1).

The patient was taken to the operating room for wide resection of the involved area, including removal of the involved fascia, subcutaneous tissues, and skin (Fig. 2). The resected tissue was sectioned and sent for pathological and microbiological analyses. The surgical bed was then irrigated using 1 liter of amphotericin B in normal saline (5 mg/1000 ml) for local drug delivery per our institutional protocol.

The tissue specimen was reviewed in pathology and showed a deep dermal and subcutaneous mixed inflammatory infiltrate with extensive necrosis and numerous open rounded bodies consistent with fungal elements. Special stains, including Gomori methenamine silver (GMS), periodic acid-Schiff (PAS) (Fig. 3A), and Fontana-Masson stain, highlighted numerous spores and hyphal forms. The Fontana-Masson stain confirmed the presence of melanin deposition found with phaeohyphomycosis, which is caused by a large group of dematiaceous, or darkly pigmented fungi to which the *Alternaria* species belong.

A portion of the excisional biopsy specimen was also sent to the microbiology laboratory where it was ground and inoculated onto three different media, i.e., Sabouraud agar (Emmons modification), Inhibitory Mold agar, and Brain Heart Infusion agar (all three from Remel Products, Lenexa, KS). The Sabouraud agar with Emmons modification differs from the original Sabouraud agar in that it has approximately neutral pH (near 6.9) and contains only 2% dextrose. These
cultures were incubated at 30°C in a non-CO₂ incubator and found to yield a rapidly growing dark mold. It also grew on the blood agar as used for routine bacteriology culture. The colony’s surface appearance was dark olivaceous brown and dark brown/black on the reverse side of the culture plate. A microscopic examination of a scotch tape prep in lactophenol cotton blue revealed septate hyphae with large dark muriform conidia with tapering apices (Fig. 3B) formed singly and in chains. These findings are consistent with the morphologic characteristics of an *Alternaria* species.

The isolate was sent to The Fungus Testing Laboratory at The University of Texas Health Science Center at San Antonio for corroboration of the initial identification, as well as susceptibility testing. Based on phenotypic characteristics, the laboratory confirmed that our isolate was an *Alternaria* species and molecular sequencing identified it as one of three species, i.e., *A. alternata*, *A. tenuissima*, or *A. longipes*. This was based on sequencing of the two phylogenetically variable regions, the internal transcriber spacer (ITS) and the longer D1/D2 region. The isolate was then re-reviewed morphologically in light of the molecular identification, which resulted in eliminating *A. tenuissima* and *A. longipes* on the basis of conidial features. The isolate was therefore identified as *A. alternata* and deposited in the University of Alberta Microfungus Collection and Herbarium and is available under accession number UAMH 11333.

Susceptibility testing by broth dilution method yielded the following MIC values: posaconazole, 0.25 μg/ml; itaconazole, 0.5 μg/ml; amphotericin B, 2 μg/ml; terbinafine, 2 μg/ml; and voriconazole 4 μg/ml. While there are no guidelines from the Clinical and Laboratory Standards Institute on how to interpret these *in vitro* susceptibility results, our MIC values are similar to those reported elsewhere [11–13]. In particular, posaconazole has the lowest MIC₅₀ (0.125 mg/ml) against all *Alternaria* species including *A. alternata* (0.125 mg/ml), whereas voriconazole has the highest MIC₅₀ of 1–2 mg/ml for all *Alternaria* species and against *A. alternata* (2–4 mg/ml). While there are no guidelines for interpretation, higher MIC₅₀ values correlate with poorer drug response.

At the time of discharge from the hospital, the patient was receiving posaconazole 400 mg orally twice daily as a single agent. His 1,3 beta-D-glucan dropped from 83 pg/ml to 36 pg/ml. He remained on posaconazole with no evidence of locally recurrent or disseminated disease at 3 months when he passed away from progression of his CLL.

**Discussion**

CLL treatment with nucleoside analogues such as fludarabine and pentostatin, the CD52-directed monoclonal antibody, alemtuzumab, and high-dose glucocorticoids is associated with marked impairment in lymphocyte number and function. This leads to increased risk of opportunistic infections, particularly in an already immunocompromised host. Therefore, infections in patients with advanced aggressively-treated CLL must prompt consideration of not only routine pathogens but also a wide array of opportunistic agents including fungi, CMV, *Pneumocystis jiroveci*, as well as progressive multifocal leukoencephalopathy among others. In fact, the patient in the present case was found to have CMV viremia in addition to his *Alternaria* fungal nodule.

In cases of soft tissue fungal infections where medical therapy appears to be failing, it is important to involve infectious disease specialists and surgeons. Some experts recommend surgical evaluation in combination with systemic antifungals for all local soft-tissue fungal infections in immunocompromised patients, regardless of whether the fungal infection is indolent or aggressive [14,15]. In one series of eight solid organ transplant recipients with
Alternaria infections, all patients presented with non-tender, erythematous or violaceous skin papules, nodules, or pustules in exposed areas of the extremities, without any visceral dissemination. All of these patients eventually required surgical excision of the lesion resulting in successful clinical outcomes [1].

Cutaneous infections with Alternaria usually occur on the extremities. Although the history of trauma may initially go unrecognized, a careful and detailed history may elicit this information. In this case, we postulate that perhaps the A. alternata was introduced by the reported bee sting. Alternaria species have been isolated from honey bee pollen samples, with the organism being recovered in culture from between 49 and 93% of samples [16,17].

When interpreting the culture results from a specific clinical case, it is important to recognize that Alternaria species are common fungi that can contaminate clinical specimens. In this case, the patient presented with a nodule on the hand that was culture positive for Alternaria species on two separate occasions with the development of a positive 1,3 beta-D-glucan level in the peripheral blood. While 1,3 beta-D-glucan has been used as an adjunct to diagnose Alternaria infections with unusual clinical presentations, studies of fungal species in indoor dust demonstrated that Alternaria are not a significant source of 1,3 beta-D-glucan [18,19]. Thus, while a positive 1,3 beta-D-glucan level can be helpful, a negative level should not exclude the diagnosis. In cases where the diagnosis is difficult, PCR has been used successfully to facilitate identification of the two predominant clinical species, A. alternata and A. infectoria [7].

In addition to 1,3 beta-D-glucan, the other fungal marker that is commonly used in clinical practice is galactomannan. While galactomannan is felt to be fairly specific for Aspergillus species, there is some cross-reactivity with other fungi. While one prior report suggested that serum samples contaminated with A. alternata could cause a false positive galactomannan result, more recent data suggest that Alternaria should not cause a positive galactomannan in the peripheral blood and that such a finding should prompt an evaluation for concomitant invasive aspergillosis [20,21]. Our patient had a negative galactomannan level throughout his course, including when he was initiated on voriconazole treatment for a suspected Aspergillus pulmonary infection.

Our patient was still on voriconazole at the time that the Alternaria infection developed in his hand. In vitro antifungal susceptibility data have typically reported Alternaria as susceptible to voriconazole, although voriconazole has a higher MIC50 of 2 μg/ml compared with a MIC50 of 0.125 μg/ml and 0.5 μg/ml for posaconazole and itraconazole, respectively [12,13]. This suggests a low threshold for the development of resistance to voriconazole, a particular concern in a patient who is exposed to sub-therapeutic levels of this drug. As mentioned above, therapeutic drug monitoring was not done after this patient was initiated on voriconazole for treatment of his suspected Aspergillus pulmonary infection. Such monitoring has been shown to improve efficacy in patients with invasive mycoses. In one study [22], 6 of 13 (46%) patients with invasive mycoses who had voriconazole serum levels <1 μg/ml had poor responses to therapy. In addition, Boyce et al. [1] noted that two of the eight solid organ transplant patients who developed Alternaria infections were on antifungal therapy when their infections arose. One of these patients was receiving itraconazole as prophylaxis following lung transplant and the other receiving voriconazole as therapy for Aspergillus fumigatus tracheal colonization following heart transplant. Given the presence of these ‘breakthrough’ infections and the higher MIC50 seen with voriconazole, we would recommend using a different azole for treatment, such as posaconazole or itraconazole. We chose posaconazole given institutional availability. It has been thought that there may be less inter-patient variance in drug levels with posaconazole, although there still remains a paucity of data [23]. Fluconazole is not a therapeutic option as it has been demonstrated to have no activity against Alternaria [6].

When developing an optimal systemic antifungal regimen, it is essential to recover the etiologic agent in culture and to investigate its in vitro antifungal susceptibility. When possible, reducing the immunosuppression may also help with wound healing and eradication of the infection.

From a surgical perspective, we recommend an approach similar to that used for sarcoma or other malignancy of the hand. A wide subfascial resection with a clean margin of 2–3 mm is effective. Irrigation of the wound bed with antifungal solution such as amphotericin B, as in this case, may be a helpful addition to surgical extirpation. Following surgery, it is important to continue antifungal therapy to help prevent local recurrence of infection. Even with surgery, however, recurrence has been reported to occur in 20% of cases [24]. The duration of antifungal therapy should be based on response to treatment with an understanding that the azoles are fungistatic rather than fungicidal, with longer courses of therapy required in the immunocompromised population.

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References


