Case Report

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Aureobasidium pullulans infection in a patient with chronic lymphocytic leukemia

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ABSTRACT

Saprophytic fungi are being increasingly recognized as etiologic agents of mycoses in immunosuppressed patients. We report a case of subcutaneous infiltration by *Aureobasidium pullulans*, likely due to traumatic inoculation, in a neutropenic patient during chemotherapy for chronic lymphocytic leukemia. The patient was treated with amphotericin B deoxycholate but was subsequently switched to itraconazole, which improved the lesion. This case highlights the importance of considering unusual fungal infections in critically ill patients such as those who are immunosuppressed due to chemotherapy. Diagnostic techniques and effective antifungal therapy have improved the prognosis of these cases.

Keywords: *Aureobasidium pullulans*. Subcutaneous mycosis. Phaeohyphomycosis. Chronic lymphocytic leukemia.

INTRODUCTION

Aureobasidium pullulans is a saprophytic dematiaceous fungus capable of producing a pigment that is similar to melanin, which accumulates in its cellular wall. It is widely distributed in the environment, and it can be isolated from soil, decaying plant debris, wood, rock, and household dust as well as human hair, skin, and nails[1-3].

The fungus is considered a contaminant when isolated from biological samples from immunocompetent patients. Its pathogenic ability in humans is becoming recognized, especially in situations of immunosuppression. However, its pathogenic mechanism remains unknown[2-6].

Here, we report a case of subcutaneous mycosis caused by *A. pullulans* in a patient suffering from chronic lymphocytic leukemia (CLL) and severe neutropenia during chemotherapy. This is the first report of subcutaneous infection by *A. pullulans* during cancer chemotherapy documented in Brazil, despite a thorough literature review.

CASE REPORT

The patient was a 66-year-old man admitted to the Hematology Care Unit of the *Universidade Federal do Triângulo Mineiro*. He was diagnosed with chronic lymphocytic leukemia (CLL) in July 2007 by absolute lymphocytosis and immunophenotyping of the peripheral blood lymphocytes by flow cytometry (CD5+, CD19+, FMC7+, CD79b+, κ-chain expressed on the surface with weak intensity). Compressive lymphadenomegaly was an indication for chemotherapy. Six cycles of fludarabine were administered between August 2007 and February 2008. The patient's pre-existing symptoms included deformities in the toes as a result of treated leprosy, asymptomatic hyperuricemia, systemic arterial hypertension, and splenectomy as a result of splenic trauma caused by a car accident in June 2007; at that time, the patient also suffered multiple skin abrasions.

In October 2008, the patient experienced a recurrence of lymphadenopathy with compression of the right inguinal region and edema; however, arterial or venous blood flow in the ipsilateral lower limb was not impaired. Excisional biopsy of the right inguinal lymph node confirmed lymphocytic lymphoma/CLL. A new course of chemotherapy based on cyclophosphamide, doxorubicin, vincristine, and prednisone (i.e., the CHOP protocol) was initiated every 21 days. The patient's symptoms were under control during the first treatment cycle.

During the third cycle of chemotherapy, the patient was hospitalized for febrile neutropenia; at this time, the patient was still receiving treatment with cefepime and metronidazole and was afebrile for 72h despite persistent and severe neutropenia (<100 neutrophils/mm^3) and treatment with granulocyte colony stimulating factor (600μg/day filgrastim). The patient complained of pain in his right thigh, and upon examination, a nodule approximately 2cm in diameter was identified in the lateral side of this region. The nodule showed no signs of secretion, fistulization, or inflammation. A punch-needle aspiration biopsy of the nodule was performed, and 2 samples of purulent material were extracted.

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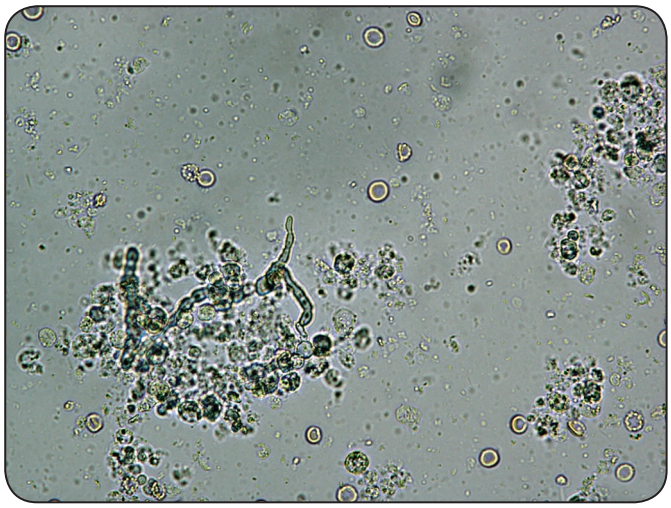

FIGURE 1 - Direct examination of purulent secretion with dematiaceous septate hyphae (*toruloides*-like) compatible with phaeohyphomycosis (KOH stain; 400× magnification).

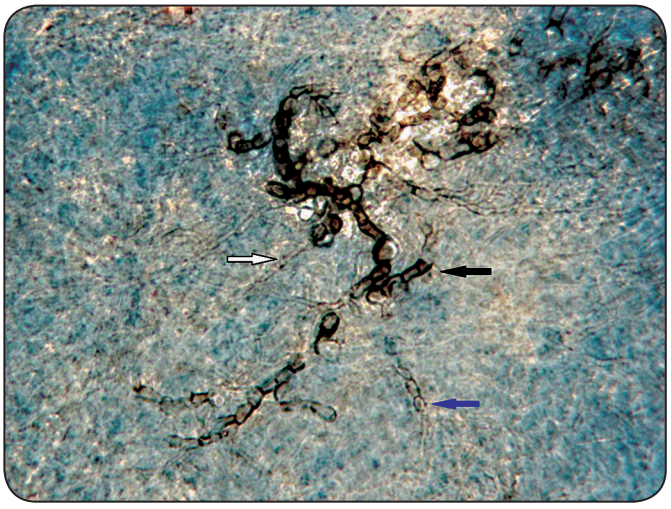

FIGURE 2 - Micromorphology of colonies showing the presence of pigmented arthroconidia with thick walls (black arrow), septate hyaline hyphae (white arrow), and hyaline blastoconidia (blue arrow), compatible with *Aureobasidium pullulans* (lactophenol cotton blue stain; 1,000× magnification).

Tests for mycobacteria and other bacteria came back negative, and direct examination revealed septate hyaline hyphae, thick walls, and irregular pigmented hyphae of varying sizes **(Figure 1)**. The material was seeded on Sabouraud glucose agar (BD DIFCO Sparks, MD, USA) supplemented with chloramphenicol and Mycosel agar (BD BBL Sparks, MD, USA) and incubated at 25°C and 37°C, respectively. The cultures grew clear colonies in approximately 48h. After 7 days of incubation, these colonies exhibited a slightly velvety texture with black coloration on the surface and backside. Macroscopic and microscopic analyses of the colonies prompted microculture in potato glucose agar (BD DIFCO, Sparks, MD, USA), after which hyaline blastoconidia (10-16µm) developed with thick-walled, pigmented arthroconidia (14-22µm) and hyaline septate hyphae (6-12µm) that were in the process of transforming into arthroconidia. These features are consistent with the morphology of *A. pullulans* **(Figure 2)**. There was no microorganism growth in the serial blood cultures. Moreover, screenings for fungal nail beds in the fingers and toes were negative. Antifungal treatment with amphotericin B deoxycholate was started and was continued for 3 days; the treatment was subsequently switched to 400mg/day itraconazole because of the side effects caused by amphotericin B. The nodule shrank, and the patient was discharged while undergoing antifungal therapy (itraconazole).

The patient died during the fourth cycle of chemotherapy due to septic shock and aspiration pneumonia. Blood cultures continued to yield negative results for fungi.

DISCUSSION

Aureobasidium pullulans has been recovered from diverse habitats. Characterized by its morphological variability, mucoid colonies may be white and become filamentous; these colonies later change to dark brown and then black as they mature. Hyphae and dark pigmentation are recognizable after several days[1,5,7]. Three varieties have been described: *A. pullulans* var. *pullulans*, *A. pullulans* var. *melanogenum* and *A. pullulans* var. *aubasidani*[7]. These fungi have biotechnological importance as they produce the extracellular biodegradable polysaccharide pullulan[6,7].

Among the species of fungi belonging to *Aureobasidium, A. pullulans* is the species most commonly recognized as an etiologic agent of human disease[2,8]. Despite its limited pathogenicity, cutaneous and subcutaneous infections (i.e., phaeohyphomycoses and cromoblastomycoses), peritonitis in patients undergoing peritoneal dialysis, pulmonary mycosis, splenic and mandibular abscess, meningitis, eye infections (i.e., episcleritis and keratitis), tonsil infections, and disseminated infections have been described in the literature[1-6,8-11].

Several conditions can predispose patients to infection by fungal saprophytes, including immunosuppression, which can be due to cancer chemotherapy or organ transplantation, the broad and extensive spectrum of antimicrobial therapy, and the disruption of natural barriers, which occurs during major surgery or trauma[2,5,12]. The immunosuppression in the present case was easily attributable to the underlying disease (CLL), asplenia secondary to splenectomy due to recent splenic trauma, and chemotherapy.

Multiple samples isolated from normally sterile sites that exhibit tissue invasion and signs of infection are critical for these diagnoses because growth in blood cultures is uncommon[2,3,9]. The macroscopic aspect and microscopic analysis of colonies, in combination with molecular techniques, are methods for the identification of the fungus responsible for the infection[12]. Concordant with the literature, the automobile-related trauma experienced by this patient could be the point at which the fungus was inoculated into the thigh[2,4]. The progressive growth of the lump in the thigh (skin intact without interruption) during neutropenia, the observation of characteristic hyphae upon direct

examination of the material collected from serial samplings, and the subsequent isolation and identification of *A. pullulans* all support the diagnosis. Molecular analyses were not performed.

Because of its rarity, there is no standard treatment for infections caused by *A. pullulans*. Antifungal drugs used for different sites of infection include amphotericin B, itraconazole, fluconazole, ketoconazole, and flucytosine, and treatment can last between 4 and 8 weeks[1,2,4,6,8-10].

To prevent and treat these infections, critically ill patients should be monitored for possible fungal infections caused by fungi with limited pathogenicity, such as *A. pullulans*; such infections have been increasingly recognized in recent years. Furthermore, improvements in mycological diagnostic methods and the standardization of antifungal therapy are also necessary for the appropriate management in order to minimize the elevated morbidity and mortality caused by this group of infections.

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