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CASE REPORT

Invasive Fungal Infection Due to *Triadelphia pulvinata* in a Patient with Acute Myeloid Leukemia

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***Triadelphia pulvinata* is a rare dematiaceous fungus found in soil. We report the first case of invasive disease in a patient with acute myeloid leukemia who had a bloodstream infection with possibly both lung and brain involvement. Identification was by combined phenotypic features and fungal ribosomal DNA sequence analysis.**

CASE REPORT

A 58-year-old Saudi woman underwent an autologous stem cell transplantation for acute myeloid leukemia (AML) in August 2011. She had few episodes of bacterial septicemia during induction and consolidation chemotherapy, which were treated effectively with broad-spectrum antibacterial agents (1 g meropenem intravenously [i.v.] every 8 h [q8h], 1 g vancomycin i.v. q12h, 200 mg fluconazole *per os*). The peritransplant period was unremarkable other than for the persistent neutropenia, for which she received granulocyte colony-stimulating factor (G-CSF).

Day 146 posttransplant, a peripheral blood smear showed 50% blasts, and relapsed AML was confirmed by flow cytometry.

She was admitted in January 2012 for reinduction chemotherapy with fludarabine and cytarabine (FA), after insertion of a peripherally inserted central catheter (PICC) line. She spiked a fever on hospital day 2 following her FA regimen. On day 9, blood culture grew extended-spectrum beta lactamase (ESBL)-producing *Klebsiella pneumoniae* from the peripheral vein and the PICC line. This was treated with 1 g meropenem i.v. q8h and removal of the PICC line. During this episode of fever, a chest radiograph (CXR) was clear, and a computed axial tomography (CT) of the chest (day 15) revealed slight atelectasis of the left lower lobe and small, scattered bilateral lung nodules (Fig. 1), which were unchanged from an earlier study done 10 months prior.

Her fever resolved after 6 days on meropenem, and subsequent sets of blood cultures were negative, although she remained severely neutropenic with a nadir of 0.04×10^9 cells/liter. She was maintained on 200 mg fluconazole daily from January 2012. The galactomannan (Platelia *Aspergillus* EIABio-Rad Laboratories, USA) and (1,3)-β-D-glucan antigen (Associates of Cape Cod, USA) assays were both negative in the serum.

Fever recurred on hospital day 36, and a CXR revealed new infiltrates in the right lower and the left upper lobes. Fluconazole prophylaxis was then switched to 300 mg voriconazole i.v. q12h. A chest CT on day 37 showed new nodular infiltrates in the left upper and right lower lobes (Fig. 2). The previously noticed small scattered nodules were unchanged.

Two sets of blood cultures taken half an hour apart from both arms on day 41 and another on day 42 (40 h later) were positive for a filamentous fungus. A total of three sets of blood cultures from peripheral sticks became positive in the automated Bactec (BD Diagnostics, USA) system within 4 days at 36.5°C. After incubation on blood and chocolate agar plates, small white yeast-like colonies grew, and a wet prep showed branched septate hyphae. These were subcultured onto Sabouraud dextrose agar plates, which grew more than 30 small colonies that developed a waxy texture with a brownish color and a brown color on the underside of the culture plate as well.

Liposomal amphotericin B at 5 mg/kg of body weight/day was added to voriconazole after the first positive fungal blood culture, as this was considered a breakthrough invasive fungal infection on voriconazole. Therapeutic drug monitoring was not done, as it is not available at our institution. Examination did not reveal any skin lesions other than some ecchymosis.

Serum samples were screened with the galactomannan and (1,3)-β-D-glucan assays twice weekly during the hospital stay. The serum galactomannan assay remained negative (reference cutoff of 0.5); however, the (1,3)-β-D-glucan assay became positive (213 pg/ml; positive threshold of >80 pg/ml). The fungal isolate from the blood culture was sent to the Fungus Testing Laboratory in the Department of Pathology at the University of Texas Health Science Center at San Antonio, where it was identified as *Triadelphia pulvinata*. At this time, a bone marrow sample also showed 1% blasts phenotypically, similar to those seen originally.

On day 44, the patient became stuporous and lapsed into a coma over the next few days. The cause of the encephalopathy remained unclear. Cerebral spinal fluid was not obtained due to the low platelets. A magnetic resonance image (MRI) of the brain showed a small area of restriction on a diffusion-weighted image medial to the left temporal lobe and lateral to the left cerebral

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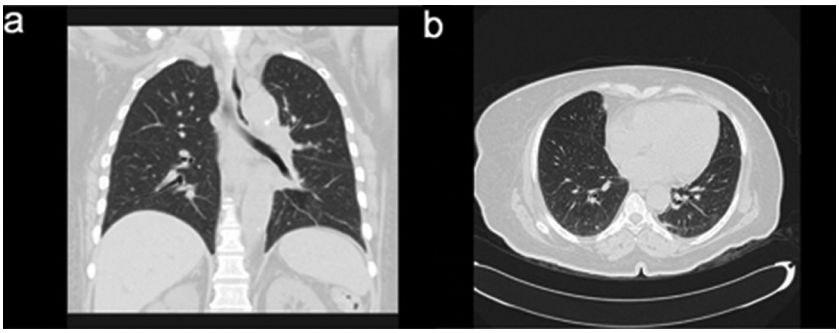

FIG 1 Normal chest computed tomography scan on day 16.

peduncle, which may have represented an early infectious process or a subtle small ischemic insult.

On day 53, her bone marrow was again consistent with a residual/refractory acute myeloid leukemia. At this point, it was the opinion of the hematology team that her prognosis from leukemia was extremely poor and therefore she was placed on palliative care and died on day 68 postadmission. An autopsy was not performed.

Mycology. For morphological studies, the isolate was subcultured onto potato flake agar (PFA) and carnation leaf agar (CLA), prepared in-house, and incubated at 25°C. On PFA, colonies were brownish gray centrally with a white periphery and velvety after 2 weeks of incubation. The type strain of *T. pulvinata*, CBS 590.77, was not obtained for comparison, as features matched those from the original description. Examination of microscopic features from both a PFA slide culture and CLA tease mounts in lactophenol cotton blue revealed three distinct types of conidia consistent with *Triadelphia* species. Brown, one-septate, cylindrical conidia, rounded at both ends and measuring approximately 3 to 3.5 by 9 to 10 μm, were formed in small sporodochium-like areas throughout the culture (Fig. 3). Larger allantoid (curved) to reniform (kidney-shaped) and sometimes asymmetrical dark-brown conidia were also present (Fig. 3b). Also present were long (40- to 60-μm), thin, hyaline, multiseptate conidia (Fig. 3c). These characteristics are consistent with those previously reported in the literature for *Triadelphia pulvinata* (1–3).

The isolate was also characterized by ribosomal DNA sequence analysis. Sequence data were obtained by growing cells on potato dextrose agar (Difco, Detroit, MI) for 24 h at 30°C. Template DNA was prepared and sequenced as described previously to yield the internal transcribed spacer (ITS) and large-subunit (LSU) D1/D2 sequences by amplifying template DNA with the ITS1, (5′-TCCGTAGGTGAACCTGCGG-3′) and NL4 primers (5′-GGTCCGTGTTTCAAGACGG-3′) and then sequencing with the same primers in addition to ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and NL1 primers (5′-GCATATCAATAAGCGGAGGAAAAG-3′) (4–7). The individual sequences were then compared to the ex-type strain of *T. pulvinata* CBS 590.77. Results were considered significant at a query coverage of ≥90% and a percent identity of ≥97%. The ITS sequence was 97% identical to the *T. pulvinata* ex-type strain CBS 590.77. Thus, the identity of this strain was confirmed to be *T. pulvinata* based on the ITS sequence and consistency with the previously reported morphological features described above.

In vitro antifungal susceptibility results as measured according to the Clinical and Laboratory Standards Institute M38-A2 guidelines for filamentous fungi were as follows (8): 0.25 μg/ml amphotericin B, 1 μg/ml voriconazole, 1 μg/ml posaconazole, 2 μg/ml itraconazole, and 0.5 μg/ml caspofungin.

As it is unknown if *T. pulvinata* will be detected by the galactomannan and (1,3)-β-D-glucan assays, we evaluated the potential for these commercially available tests to detect this species. The isolate was inoculated into 5 ml of RPMI growth medium. *Aspergillus fumigatus* inoculated into RPMI and sterile RPMI served as the positive and negative controls, respectively. After ~60 h of incubation at 37°C, supernatants from each tube were collected, and the galactomannan and (1,3)-β-D-glucan assays were performed according to the manufacturers' instructions. The galactomannan assay was negative in the supernatants collected from the *T. pulvinata* and negative-controls tubes (galacto-

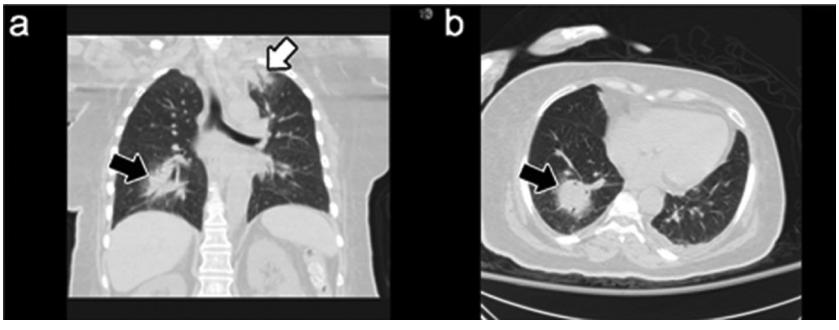

FIG 2 Chest computed tomography scan on day 37 showing a nodular infiltrate in the right lower lobe (black arrow) and a left upper lobe infiltrate (white arrow).

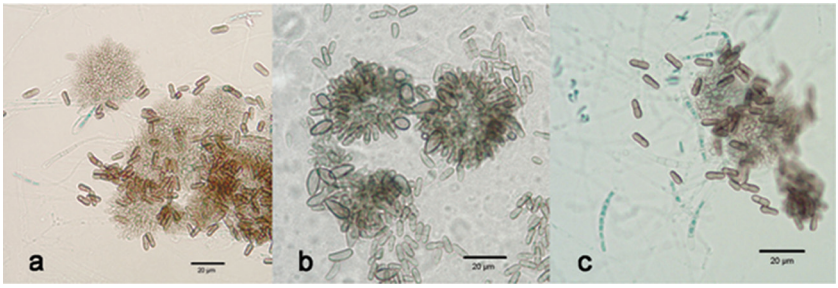

FIG 3 Photomicrograph of *Triadelphia pulvinata* isolate. (a) Brown, 2-celled conidia and sporodochium-like areas showing the conidiogenous cells from which the conidia were produced; (b) sporodochium-like areas, smaller, brown 2-celled conidia and larger, brown, asymmetrical conidia; (c) small, brown, 2-celled conidia and long, hyaline, curved conidia.

mannan index of <0.2), while this assay was positive for *A. fumigatus* (galactomannan index of >5). In contrast, the (1,3)-β-D-glucan assay was positive in the supernatants collected from the *T. pulvinata* and *A. fumigatus* tubes (>500 pg/ml) and negative in the RPMI control (<80 pg/ml). These findings are in agreement with the serum galactomannan and (1,3)-β-D-glucan results in this patient.

This is the first case of invasive disease due to *Triadelphia pulvinata*, which is a rare dematiaceous hyphomycete first described in 1978 by Maggi et al., following its isolation from the rhizosphere of the grass *Loudetia simplex* in the Ivory Coast (3).

Al Hedaithy and Leathers isolated the fungus in Saudi Arabia from soils contaminated with bat guano (2), and Al Hedaithy reported the first and only case of human infection due to *Triadelphia pulvinata* (1). It was isolated from a superficial eczematoid lesion in an expatriate Indian laborer, and whether the patient acquired the disease from India or from Saudi Arabia is unknown.

Our patient with relapsed leukemia had systemic signs of infection coinciding with the isolation of *Triadelphia pulvinata* from multiple sets of blood cultures collected over several days and new nodular infiltrates characteristic of fungal infection on chest CT scan. The (1,3)-β-D-glucan assay became positive during fungemia. Although false positives with this assay can occur with serous exposure to gauzes and hemodialysis membranes, this is unlikely in this patient. These findings highly suggest *Triadelphia pulvinata* to be a true invasive pathogen in this patient, although diagnosis is not definitive without a biopsy. Severe thrombocytopenia precluded any invasive procedures.

One can surmise that the encephalopathy and the brain MRI, suggestive of an infectious process at the time of the fungemia, was probably caused by *T. pulvinata*. Only an autopsy could have confirmed this and also revealed the true extent of the involvement. However, per the EORTC/MSG definitions, this could be classified as a proven invasive fungal infection due to multiple positive blood cultures in a neutropenic patient (9).

This is to be considered a hospital-acquired infection, as the woman was an inpatient for a month prior to the relapse of fever and the isolation of the fungus from blood. Moreover, she was a homemaker, living in the city, with no particular hobbies. The place of residence/occupation of the only reported case of *T. pulvinata* could not be traced. We speculate that the portal of entry of this dematiaceous fungus is likely by inhalation. However, no construction work was ongoing in the vicinity of the ward.

At our institution, fluconazole is still used as antifungal prophylaxis in neutropenic patients. Although posaconazole has been shown to be effective for this indication (10), it is unknown if prophylaxis with this agent would have been effective given the elevated MIC against this particular isolate.

Nucleotide sequence accession numbers. The case isolate has been preserved at the King Faisal Specialist Hospital and Research Center under the accession number 12-043-03801. ITS and D1/D2 sequence data have been deposited into GenBank under accession numbers KC489510 and KC489511.

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