Cutaneous Phaeohyphomycosis Caused by *Paraconiothyrium cyclothyrioides*

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Paraconiothyrium cyclothyrioides is a recently described coelomycetous fungal species. We present a case in a renal transplant patient with chronic skin lesions of the lower extremities caused by *P. cyclothyrioides*. Treatment with posaconazole led to complete resolution of the lesions. *P. cyclothyrioides* should be considered an opportunistic human pathogen in immunocompromised patients.

CASE REPORT

A 49-year-old Latin American man with end-stage renal disease and a cadaveric renal transplant (2 years prior to the current admission) was hospitalized with cutaneous lesions in his lower extremities. The patient had history of type II diabetes, hypertension, and atrial fibrillation. One and half years previous to admission, the patient received thymoglobulin for acute T-cell rejection, with improvement of the episode. Approximately 6 months before the current admission, the patient first noticed a painless, nonpruritic, mildly tender scaly papule on the left tibia just inferior to the knee joint and applied a local adhesive band to prevent friction injury. Over the next few months, other lesions appeared on both tibial surfaces which then began to coalesce into violaceous, necrotic, plaque-like lesions with some areas of ulceration and crusting and mild self-limiting sanguineous discharge without accompanying fevers. The patient lives in Brownsville, TX, works in an office as a manager, and denied any travel outside Texas, trauma, or animal or insect bites. The patient's hobbies include performing mechanical work on car engines. Medications included leflunomide, prednisone, tacrolimus, doxazosin, clonidine, carvedilol, minoxidil, amlodipine, trimethoprim-sulfamethoxazole, insulin, famotidine, gemfibrozil, and pravastatin. His complete blood count was notable for a platelet count of 73 and creatinine of 2.4 mg/dl. On physical examination, he was afebrile and vital signs were stable. A cardiac examination evidenced an irregular rate and a II/VI murmur loudest in the apex. The lungs were clear, and the rest of the examination was unremarkable except for the lower extremities. He had a crusted, ulcerated plaque on the anteromedial aspect of the left knee (measuring 6 by 3 cm) and several similar-appearing lesions of smaller size on the same area (Fig. 1A). The patient was started on vancomycin (1.25 g every 24 h); radiographs of the knee did not reveal any bone abnormality. A skin biopsy specimen yielded a presumptive diagnosis of pyoderma gangrenosum, antibiotics were stopped, and the patient was discharged with wound care instructions and silver sulfadiazine.

Two months prior to the current admission, a fungal culture from the skin tissue was positive for a single mold which grew on

Sabouraud dextrose (Sab Dex) agar but was not identified at the time. Oral voriconazole (200 mg orally every 12 h) administration was initiated. A month after his discharge, he presented to the dermatology clinic with evidence of progression of his skin lesions and was again admitted to the hospital. He denied new recent trauma or travel history and reported compliance with the voriconazole regimen. New punch biopsy specimens revealed pseudoepitheliomatous hyperplasia and suppurative granulomas, and fungal elements were seen invading the dermis (Fig. 1B). Cultures yielded a mold (only one colony type) with the same morphology as the previous culture in Sab Dex agar that was subsequently identified as *Paraconiothyrium cyclothyrioides* (see below). The patient was started on amphotericin B lipid complex (400 mg intravenously [i.v.] every 24 h) and micafungin (100 mg i.v. every 24 h). Due to the elevation in the creatinine level, amphotericin B was stopped and posaconazole (400 mg orally every 12 h with meals) treatment was initiated. Micafungin administration was discontinued. The skin lesions markedly improved 10 days after initiation of posaconazole therapy. Follow-up at 3 weeks after hospital discharge showed complete resolution of the skin lesions with only some remaining scaling of the skin in the area. Posaconazole was continued for an additional 4 weeks.

Extensive morphological and molecular characterization of the isolate was required to obtain a definitive identification. Added to the Fungus Testing Laboratory culture collection as UTHSC 11-2294, the isolate was subcultured onto potato flake agar (PFA), V-8 agar, and carnation leaf agar (CLA), all prepared in-house, at 25°C. A photomicrograph of the macroscopic morphology on PFA after 6 days of incubation at 30°C

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FIG 1 Panel A, crusted, ulcerated skin lesions of the left knee; panel B, periodic acid-Schiff (PAS) stain showing fungal elements (hyphae; black arrows) invading the dermis ($\times 60$ magnification).

shows a woolly, olivaceous-gray colony with a white periphery (Fig. 2A). A brown diffusing pigment developed after 3 weeks of incubation at 25°C. Temperature studies on PFA indicated optimal growth at 27°C and minimal growth (1+) at 35°C. After 4 weeks of incubation on CLA at 25°C, thick-walled, submerged, reddish-brown conidiomata were present on CLA (Fig. 2B). The hyaline, discrete or integrated, phialidic conidiogenous cells (Fig. 2C) producing brown, cylindrical, 1-celled conidia measuring approximately 1.5 by 3 to 4 μm (Fig. 2D) were suggestive of taxa known in the pycnidial coelomycete genera *Microsphaeropsis*/*Paraconiothyrium*/*Coniothyrium*. In order to accurately identify the isolate, molecular characterization by DNA sequencing was performed and the sequence was deposited under accession number R-4779 at University of Texas Health Science Center (UTHSC) Fungus Testing laboratory culture collection. Briefly, DNA was recovered from a 24-h culture grown on potato dextrose agar (Difco, Detroit, MI) and amplified by PCR using internal transcribed spacer 1 (ITS1) and NL4 primers as previously described (3). The amplicon was sequenced in the UTHSC, San Antonio (UTHSCSA), Advanced Nucleic Acids Core facility to yield ITS and D1/D2 sequences (3). A BLASTn search of GenBank was performed using the ITS sequence, and a cutoff value of $\geq 97\%$ identity at a query length of $\geq 90\%$ was used for consideration of conspecificity. The results of this search identified *Microsphaeropsis arundinis* (accession no. JN851034.1; 99% identity over 551 nucleotides), *Bipolaris sorokiniana* (accession no. DQ337383.1; 99% identity over 541 nucleotides), and *Paraconiothyrium cyclothyrioides* (accession no. AY642529.1; 99% identity over 505 nucleotides). The D1/D2 sequence was not informative, because there is no GenBank deposit for *Paraconiothyrium cyclothyrioides*. As ITS and D1/D2 sequencing failed to discriminate between *P. cyclothyrioides* and *M. arundinis*, both of which showed 99% nucleotide identity to the case isolate, our sequence data were forwarded to the Centraalbureau voor Schimmelcultures (CBS) for further analysis by G. J. M. Verkley and J. B. Stielow and subsequent comparison with their nucleotide sequence database of known isolates (4). With additional sequencing of γ -actin and β -tubulin loci (using

primer pair ACT512f and ACT783r and primer pair Btub4rd and Btub4fd, respectively) (4), the levels of sequence identity of R-4779 with the type strain of *P. cyclothyrioides* (CBS 972.95) were 100%, 97%, and 99.1% for ITS, actin, and β -tubulin, respectively, suggesting that the case isolate was *P. cyclothyrioides*.

Coelomycetous fungi are common, anamorphic (asexual) plant and soil organisms that produce conidia within some type of closed (pycnidial) or semiclosed (acervular) structure.

The coelomycete genus *Paraconiothyrium* was proposed to accommodate species resembling those in *Microsphaeropsis* and *Coniothyrium* that do not match species in those genera morphologically and phylogenetically (3). Due to the variability and plasticity in morphological characters, especially in culture, it is difficult to distinguish members of these genera morphologically, but, generally, the *Coniothyrium* and *Microsphaeropsis* genera mostly include species with true pycnidia (conidiomata with an opening), while most species of *Paraconiothyrium* form more complex conidiomata lacking a distinct ostiole. *Coniothyrium* spp. exhibit annellidic conidiogenesis, while those of the other two genera mostly show phialidic conidiogenesis, sometimes with percurrent proliferations. The species thus far included in *Paraconiothyrium* were formally described based on their anamorphic or asexual reproductive stage *in vitro* and currently include *P. cyclothyrioides*, first isolated from a soil sample collected in Papua New Guinea in 1995, *P. estuarinum*, *P. brasiliense*, and *P. fungicola* (4). Molecular phylogenetic data place all three genera within the order *Pleosporales* and resolve them as three distinct clades (4).

To the best of our knowledge, this is the second reported case of infection with *Paraconiothyrium cyclothyrioides*. We present unequivocal evidence that the organism was the pathogen in this case, since it invaded the skin of the patient and lesions resolved with appropriate antifungal therapy. Balajee et al. (1) described a case in which a 3-year-old child presented with crusted nodules on a lower extremity 1 year after receiving a liver transplant. ITS

FIG 2 Macroscopic and microscopic features (lactophenol cotton blue mounting medium) of case isolate of *Paraconiothyrium cyclothyrioides*. Panel A, colony after 6 days of incubation at 30°C on potato flake agar; panel B, thick-walled conidioma, showing conidia that are reddish-brown, in mass, within the cavity; panel C, phialidic conidiogenous cells protruding into the cavity of the conidioma; panel D, small, 1-celled, brownish cylindrical conidia.

sequencing and comparison with a large coelomycete database at the U.S. Department of Agriculture (accessible at <http://nt.ars-grin.gov/sbmlweb/fungi/index.cfm>) identified the isolate as *P. cyclothyrioides*. Localization of the lesions (lower extremities) was very similar to that in our case, but no information was provided in terms of the clinical evolution or treatment of the infection (1). Interestingly, a fungus isolated from a skin lesion of a 56-year-old immunocompromised patient was recently identified as *Paraconiothyrium maculicutis* (2), a close relative of *P. cyclothyrioides*, supporting the conclusion that *Paraconiothyrium* spp. are potential human pathogens.

Our case highlights the importance of obtaining tissue samples for culture when immunocompromised patients present with chronic indolent skin lesions, since the differential diagnosis is broad and includes fungi, mycobacteria, and *Nocardia* spp., among other pathogens. In fact, several biopsy specimens were taken during the course of this infection, which increased the likelihood of isolating the etiologic agent. In our case, although susceptibility analyses were not performed, the clinical course for our

patient suggested that *P. cyclothyrioides* has important differences in susceptibility to azole antifungals; indeed, our patient did not respond to voriconazole but showed a remarkable improvement when switched to posaconazole, with resolution of the lesions. We were unable to evaluate the response to amphotericin B, since administration of that compound had to be stopped due to renal toxicity.

In summary, the rare soil fungus *P. cyclothyrioides* has pathogenic potential in chronic, crusted, and ulcerated skin lesions in transplant patients and should be considered in the differential diagnosis of invasive fungal infections in immunocompromised patients. The most accurate identification among *Coniothyrium*-like fungi and allied genera can be achieved by β -tubulin sequencing using the suggested primer pair. Posaconazole appears to be effective in treating these infections.

Nucleotide sequence and microfungus accession numbers. The ITS and D1/D2 sequences were deposited in GenBank under accession numbers [JQ681303](#) and [JQ681304](#), respectively. The case isolate has also been deposited in the University of Alberta

Microfungus Collection and Herbarium under accession number UAMH 11641.

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