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Clinical Transplantation

Case Report

Aspergillus flavus myositis in a patient after liver transplantation

Li DM, Xiu DR, Li RY, Samson RA, de Hoog GS, Wang DL. *Aspergillus flavus* myositis in a patient after liver transplantation.
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Abstract: We describe the first case of *Aspergillus* myositis caused by *Aspergillus flavus* in a liver transplant patient. The patient was a 43-year-old man who underwent liver transplantation because of end-stage hepatic cirrhosis. He experienced pain in his left calf two months after the operation. Nodules with weakness, swelling, and flaring were found in the calf two wk later. Color ultrasonic examination showed uneven resonance in the left gastrocnemius. Needle aspiration and biopsy of the muscle revealed septate hyphae consistent with *Aspergillus* species and focal necrosis of the muscle cells with inflammatory cell infiltration. A culture subsequently yielded *A. flavus*, confirming histopathologic diagnosis. Sequencing of the internal transcribed spacer region confirmed the morphologic identification. The patient was first given itraconazole 0.2 g twice daily for one wk and was then switched to terbinafine 0.25 g once a day. A three-month regimen of terbinafine therapy cured the infection, though the cultured fungus showed resistance to a number of antifungal agents. *Aspergillus*, a genus of ubiquitous molds, may cause invasive and even fatal disease in immunosuppressed patients.

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Invasive aspergillosis, the most common invasive fungal infection, is the most feared complication in patients after organ transplantation or in cases of severe immunosuppression (1–4), such as occurs in patients undergoing high-dose chemotherapy. *Aspergillus flavus* is the second most common species associated with these infections (5). However, while systemic *A. flavus* infections have been reported in the literature (2, 5, 7), to our knowledge, intramuscular infection by this fungus has not yet been reported. In this article, we describe a successfully treated case of myositis caused by *A. flavus* in a liver transplant patient.

Case report

The patient was a 43-year-old man living near Beijing, China, who underwent liver transplantation because of end-stage hepatic cirrhosis. His immunosuppressive regimen included prednisone (10 mg twice daily) and cyclosporine (200 mg twice daily). Fluconazole was given (50 mg daily) to prevent fungal infection. Two months after surgery, the patient developed muscle pain with increasing fatigue, profound weakness, and fever. Two wk later, nodules formed in his left calf, accompanied by swelling and flaring. His symptoms initially limited his daily activities and finally confined him to bed.

Physical examination showed two 2×2 cm nodules in his left calf (Fig. 1). Color ultrasonic examination reflected uneven resonance in the left gastrocnemius. The muscle tissue was biopsied with needle aspiration, and histopathologic analysis showed focal necrosis of the muscle cells with inflammatory cell infiltration. Septate hyphae and conidia with hematoxylin and eosin were noticed among the muscle cells (Fig. 2). The muscle specimen was inoculated on medium, and culture

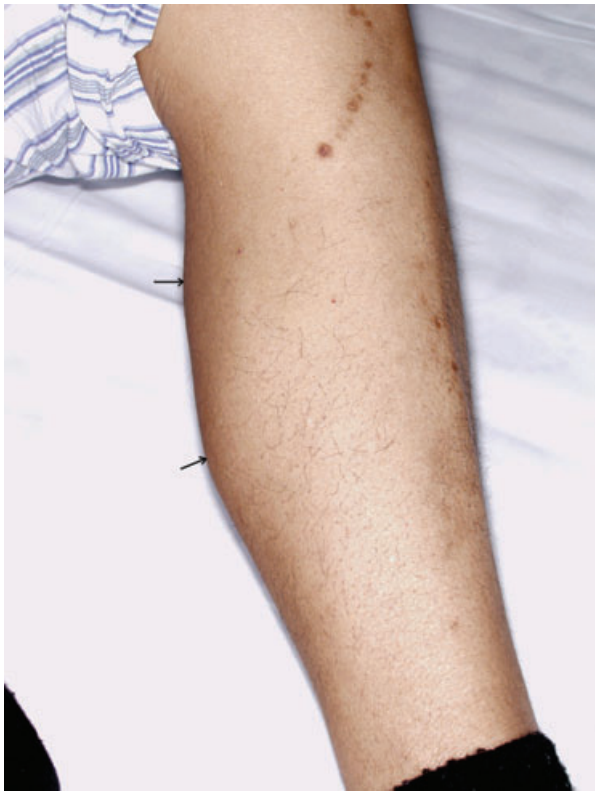

Fig. 1. Two 2 × 2 cm nodules were found in the patient's left calf.

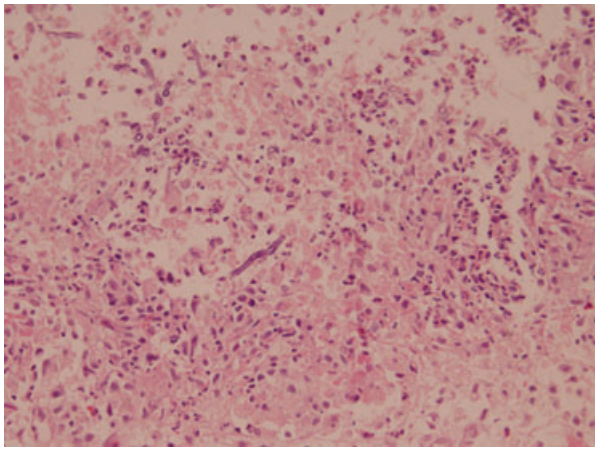

Fig. 2. Histopathologic analysis of muscle tissue showed focal necrosis with inflammatory cell infiltration. Cells were stained with hematoxylin and eosin and periodic acid-Schiff, which revealed septate hyphae and conidia among the muscle cells. Magnification ×400.

grew *A. flavus*, confirming the histopathological diagnosis.

Antifungal susceptibility tests were performed using the broth microdilution method according to National Committee on Clinical Laboratory Standards (NCCLS) guidelines and described previously (8). The patient was first given itraconazole 0.2 g twice daily for one wk; his condition did not improve, so he was switched to terbinafine 0.25 g once daily. Three d later, his symptoms began to lessen. After a three-month regimen of terbinafine, the patient's infection resolved.

Mycology

Tissue from the leg lesion biopsy was plated on Sabouraud dextrose agar with chloramphenicol and incubated at 25°C. Strain T19 (BMU 35035 and CBS120264) was isolated from the muscle specimen and transferred to Czapek agar and malt extract agar (MEA), which were cultured at 25 and 37°C.

Strain T19 showed good growth at both temperatures. Colonies on Czapek agar at 25°C attained a diameter of 3 cm within seven d and consisted of a dense felt of yellow-green conidiophores (Fig. 3). Colonies on MEA grew faster; otherwise, their characteristics were similar to those of colonies grown on Czapek agar. This strain was identified as *A. flavus* and was collected

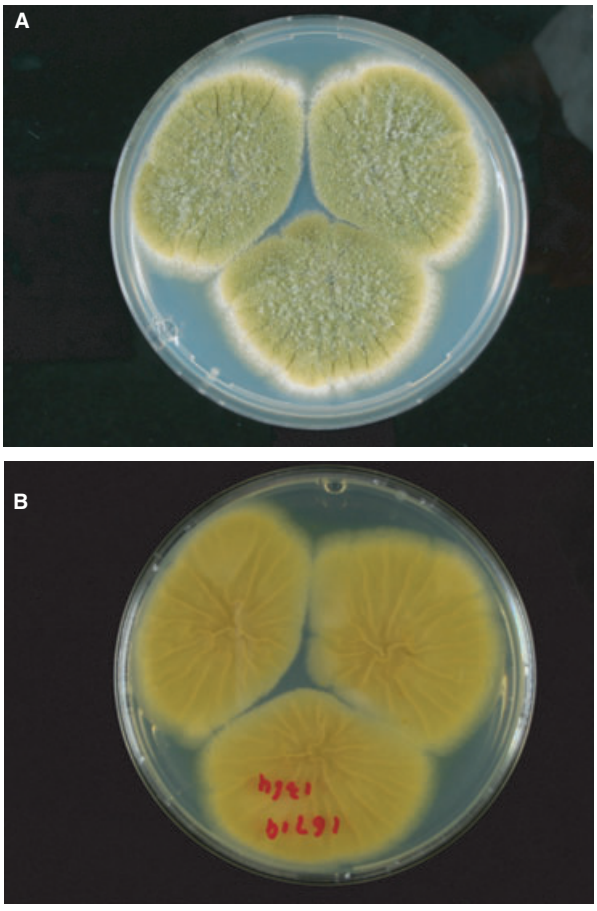

Fig. 3. Colonies on Czapek agar at 25°C attained a diameter of 3 cm within seven d (A), with dense felt of yellow-green surface (B).

in Centraalbureau voor Schimmelcultures and given the accession number CBS120264.

Antifungal susceptibility studies

The case isolate was evaluated by using the NCCLS-based method. Mature cultures were overlaid with sterile, distilled water, and suspensions were made by gently scraping the colonies with the tip of a Pasteur pipette. Heavy hyphal fragments were allowed to settle, and the upper, homogeneous conidial suspensions were removed. Conidia were counted with a hemocytometer, and the inoculum was standardized to 1.0×10^3 CFU/mL (8). The results of MICs were as follows: itraconazole 0.5 μg/mL, amphotericin B ≥ 16 μg/mL, terbinafine 0.03 μg/mL, fluconazole 32 μg/mL, 5-flucytosine >64 μg/mL, miconazole 4 μg/mL, ketoconazole 0.5 μg/mL, nystatin 4 μg/mL.

DNA extraction, amplification and sequencing

Approximately 1 cm^2 of fungal material was transferred to a 2-mL Eppendorf tube containing a 2:1 (w/w) mixture of silica gel and Celite (silica gel H, Merck 7736/Kieselguhr Celite 545, Machery, Merck, Amsterdam, The Netherlands); DNA was extracted according to methods described previously (9).

Polymerase chain reaction was performed on 50-μL volumes of a reaction mixture containing 10 mM Tris HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2 \cdot 6H_2O$, 0.01% gelatin, 200 mM of each deoxynucleotide triphosphate, 25 pmol of each primer, 10–100 ng rDNA, and 0.5 U Taq DNA polymerase (Bioline, GC Biotech, Alphen a/d Rijn, The Netherlands). Primer sets ITS1 and ITS4 were used to amplify and sequence the internal transcribed spacer (ITS) region of rDNA. Amplification of ITS was performed as follows: 95°C for four min, followed by 35 cycles consisting of 94°C for 45 s, 52°C for 30 s, and 72°C for 2 min. Amplicons were cleaned with GFX columns (GE Healthcare, Sweden). Sequence PCR was performed as follows: 95°C for one min, followed by 30 cycles consisting of 95°C for 10 s, 50°C for five s, and 60°C for two min. DNA was purified with Sephadex G-50 Superfine. Purified amplicons were then sequenced on both strands using the same primers described above. BigDye terminator cycle sequencing Ready Reaction kits (Perkin Elmer Applied Biosystems, Applied Biosystems, Nieuwerkerk a/d Ijssel, The Netherlands) were used according to the manufacturer's instructions, and DNA was sequenced using an DYE-ET terminator. Sequences were compared with GenBank and a research database available at CBS. The entire ITS region was 520 base pairs, which showed high similarity to *A. flavus* strains in GenBank.

Nucleotide sequence accession number

The nucleotide sequence of the ITS region was deposited in the GenBank database under accession number EF205326.

Discussion

Aspergillus flavus is ubiquitous in nature and can cause a variety of aspergilloses, including central nervous system infections (10). *Aspergillus flavus* is one of the main agents of human pulmonary infections in immunocompromised patients (6) and is also a common cause of endocarditis. *Aspergillus flavus* can be found in the externae of ears and may be involved in otitis, invasive sinusitis, keratitis, and scleritis (6). Only two cases of *A. flavus* infection have been reported in China, one associated with cutaneous nodules (11) and the other with fever, cough, and stomachache after fatigue. Both of them were successfully treated with terbinafine (11).

Fungal myositis is an uncommon infection of the muscles, which is caused mostly by *Candida* spp., *Cryptococcus* spp., and *Sporotrix shenchii* (12, 13). To date, six cases of muscle aspergillosis have been reported worldwide (7, 14–18); all of them occurred in severely immunosuppressed patients, and all were fatal. To our knowledge, this is the first case of *A. flavus* myositis.

Fungal infection is a major contributor to death after liver transplantation. Alhough most fungal infections in liver transplant patients are caused by *Candida* species, *Aspergillus* infections are second most common. In one study, the incidence of *Aspergillus* infection was as high as 8.21%, while the mortality rate was 3.4% (6). Among retransplant liver recipients, the mortality rate of *Aspergillus* infections was as high as 63%–100% with time elapsed (19).

Fungal myositis can be fatal whether or not the patient is immunocompromised. Antifungal susceptibility tests, however, can inform treatment (20, 21). Most clinical isolates of *A. flavus* are susceptible to amphotericin B and itraconazole (22, 23), resistance to these agents in some isolates can lead to treatment failure if their MICs are not detected in time (24). In the present case, itraconazole therapy (0.2 g/d) for one wk resulted in no

improvement, but his symptoms decreased after he was switched to terbinafine. MIC results showed that the isolate was much more susceptible to terbinafine (0.03 μg/mL) than itraconazole (0.5 μg/mL); it also showed resistance to several antifungal agents (fluconazole 32 μg/mL, 5-flucytosine >64 μg/mL, miconazole 4 μg/mL, and amphotericin B 16 μg/mL), showing a correlation between *in vitro* MIC results and *in vivo* effectiveness. Early diagnosis, monitoring susceptibilities to antifungal agents, and sufficient antifungal therapy are essential to improve the survival rate in patients with fungal infection after organ transplantation.

High similarity in ITS regions with those of other *A. flavus* strains in GenBank indicates that the infection was acquired in the hospital. As liver transplantation is increasingly common, preventing and treating fungal infections will become increasingly urgent.

Acknowledgements

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