Disseminated *Trichosporon mycotoxinivorans*, *Aspergillus fumigatus*, and *Scedosporium apiospermum* Coinfection after Lung and Liver Transplantation in a Cystic Fibrosis Patient

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*Trichosporon mycotoxinivorans* is a novel pathogen recently found in cystic fibrosis patients. We report the first case of a disseminated fatal infection with *T. mycotoxinivorans* associated with invasive *Aspergillus fumigatus* and *Scedosporium apiospermum* infection after lung and liver transplantation in a cystic fibrosis patient.

**CASE REPORT**

A 35-year-old man with cystic fibrosis (CF) was admitted for rapid respiratory deterioration and high-level oxygen requirements with respiratory acidosis. He was a nonsmoker and was still working actively as a farmer until a few days before his admission. His past medical history included sinonasal polyposis and diabetes mellitus. He was in good general condition despite a severe obstructive pulmonary disease that had been stable over the previous 5 years, with a forced expiratory volume in the first second at 20% of that predicted. He also had a 5-year bronchial colonization with *Pseudomonas aeruginosa* and *Aspergillus fumigatus*.

Because of respiratory deterioration despite noninvasive ventilation and antibiotics, an extracorporeal membrane oxygenation, inotropic support, and hemodialysis were started and an emergency double lung transplantation (LT) was performed. The immunosuppression included basiliximab and high-dose methylprednisolone for induction, and methylprednisolone for maintenance. A standard dose of tacrolimus, mycophenolate mofetil, and methylprednisolone were given. Antifungal prophylaxis with caspofungin (70 mg) started on the day of LT was discontinued the next day due to liver dysfunctions. Amyloidosis AA (deposition of amyloid protein A) was suspected on explanted liver.

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Because of respiratory deterioration despite noninvasive ventilation and antibiotics, an extracorporeal membrane oxygenation, inotropic support, and hemodialysis were started and an emergency double lung transplantation (LT) was performed. The immunosuppression included basiliximab and high-dose methylprednisolone for induction, a standard dose of tacrolimus, mycophenolate mofetil, and methylprednisolone for maintenance.

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The patient’s condition remained stable, although invasive ventilation and hemodialysis were maintained. Routine bronchoalveolar lavage specimens at days 1 and 6 post-LT were positive for *P. aeruginosa* (treated with ceftazidime, ciprofloxacin, and meropenem) and *A. fumigatus*. Caspofungin was resumed (50 mg daily) at day 6 post-LT because an *Aspergillus* galactomannan detection test (Platelia *Aspergillus* antigen; Bio-Rad) became positive in serum.

On day 9 post-LT, nonfebrile sleepiness was noted. Within 4 days, the neurologic condition worsened, with tetraparesis and coma. Cerebral magnetic resonance imaging (MRI) showed multiple foci of cortical, subcortical, and periventricular hyperintensities, consistent with ischemic or septic embolism. Blood cultures became positive after 5 days of incubation, showing budding and arthrosporic fungal elements at direct examination. *Trichosporon* species was suspected based on the macroscopic aspect of the colonies subcultured on ChromID Candida agar (bioMérieux) and on microscopic morphology on potato–carrot–bile (Bio-Rad) slide culture showing true hyphae and arthroconidia. Routine methods (AuxaColor2 [Bio-Rad], ID32C [bioMérieux], and matrix-assisted laser desorption ionization–time of flight [MALDI-TOF] [Bruker]) did not allow species identification. Identification of *T. mycotoxinivorans* was finally obtained on day 23 post-LT by double-strand sequencing of the ribosomal DNA internal transcribed spacer (ITS) region using the ITS1 and ITS4 primers. Analysis of the 423-bp sequence was done using a BLAST search against the database, including all GenBank, EMBL, DDBJ, and PDB databases. One hundred percent identity was obtained with the GenBank *T. mycotoxinivorans* sequences under accession no. JQ266092.1, FJ416595.1, DQ325456.1, and DQ325457.1 and the EMBL sequence under A6f01389.1. MICs were measured by the Etest method on RPMI medium for amphotericin B (4 μg/ml), itraconazole (>32 μg/ml), voriconazole (>32 μg/ml), posaconazole (24 μg/ml), caspofungin (>32 μg/ml), and fluconazole (>32 μg/ml).

On day 14 post-LT, the respiratory condition worsened, with progressive subcutaneous emphysema. *Aspergillus* antigenemia remained positive. Intravenous voriconazole (400 mg twice daily) was added to caspofungin. Voriconazole residual concentrations in plasma, determined by a high-performance liquid chromatography (HPLC) method with UV detection, remained within the target range. In the following days, chest wall dehiscence occurred,
and bronchopleural fistula was confirmed at bronchoscopy. Multiple cavitary masses developed on chest X-ray (Fig. 1). Surgical toilet of the thoracic wound was performed. All 12 blood cultures drawn between day 9 and day 28 post-LT remained positive for *T. mycotoxivorans*. Aspergillus antigen was still positive in serum (index, 2.8 to 8.8). In the following days, ascites and abdominal wound dehiscence developed. Clinical deterioration continued, and the patient died on day 29 post-LT.

Autopsy confirmed at culture a disseminated fungal coinfection with *A. fumigatus* (lung abscess, thoracic wound, small bowel, heart, trachea, thyroid, pancreas, and mesenteric lymph nodes) and *T. mycotoxivorans* (thoracic wound, heart, pericardium, and bronchi) and also allowed the isolation of an unexpected *Scedosporium apiospermum* strain (thoracic wound, lung abscess, bronchi, heart, trachea, thyroid, liver, kidney, and small bowel). Brain tissue was fixed in formalin for histopathological examination without any previous culture. All the postmortem specimens were initially plated on ChromID Candida agar, Sabouraud-dextrose-chloramphenicol agar with cycloheximide (SCC), and Sabouraud-dextrose-chloramphenicol agar supplemented with 1 μg/ml voriconazole (SCV) as selective media for *T. mycotoxivorans*. *A. fumigatus* was isolated mainly on ChromID Candida agar, *T. mycotoxivorans* on SCV, and *S. apiospermum* on SCC.

Double-strand sequencing of the ITS region and of the β-tubulin gene was performed on the *A. fumigatus* (ITS1/ITS4 and βTUB1/βTUB2 primers) and *S. apiospermum* (ITS1/ITS4 and βTUBF/βTUBR primers) isolates in order to detect the cryptic species recently described for *Aspergillus* belonging to the *fumigati* section (especially the human pathogens *A. lentulus*, *Neosartorya fischeri*, *Neosartorya pseudofischeri*, *Neosartorya hiratsukae*, and *Neosartorya udagawae*) and within the complex *S. apiospermum*/*Pseudallescheria boydii* (2, 12, 18). The sequences were compared to the GenBank and CBS databases. *A. fumigatus* species was confirmed by both ITS and β-tubulin sequencing and both databases. The results were not discriminant for *S. apiospermum* between *S. apiospermum* and *P. boydii*.

Histopathological examination revealed the presence of fungi in the transplanted lungs and liver, myocardium and posterior papillary muscle of the mitral valve, trachea, right stem bronchus, mesenteric lymph nodes, and thyroid gland. Macroscopic examination of the brain revealed a massive hemorrhagic lesion in the right hemisphere and multiple small ischemic or hemorrhagic lesions disseminated in both hemispheres. Most lesions proved to
be fungal microabscesses (Fig. 2A) and fungal arteritis with secondary hemorrhage (Fig. 2B) on histopathology examination. To further determine the identity of the fungal agents found in the brain, DNA sequencing was attempted, after fixation in formalin, but was not informative.

Yeasts of the genus *Trichosporon* are widely found in the environment. The 1992 taxonomy distinguished six medically relevant species (formerly *Trichosporon beigelli*) (4, 7, 8). *Trichosporon ovoides*, *Trichosporon cutaneum*, *Trichosporon inkin*, and *Trichosporon asteroides* are responsible for superficial skin lesions. *Trichosporon asahii* and *Trichosporon mucoides* have been described as agents of invasive diseases in leukemic patients and more recently in liver, kidney, or heart transplant patients (1, 3, 6, 10, 14, 15, 16, 20). One case of fungal arteritis with arterial anastomotic dehiscence after kidney transplantation has been reported (5). In 2004, a new species, *T. mycotoxivorans*, was isolated from a termite and was named due to its characteristics of detoxifying mycotoxins (13). It appeared to be phylogenetically closely related to *Trichosporon loubieri*, a nonpathogenic species. *T. mycotoxivorans* was initially considered nonpathogenic, until the first case of invasive and fatal pneumonia in a nontransplanted CF patient was reported in 2009, followed by the retrospective identification of three additional cases in CF patients from archived clinical samples, suggesting a propensity of *T. mycotoxivorans* for colonizing airways in CF patients (9).

To our knowledge, we report the first case of *Trichosporon* and particularly *T. mycotoxivorans* invasive disease in a LT patient. In our patient, the infection was probably favored by different factors: CF, professional regular exposure to environmental fungi, diabetes, immunosuppressive therapy, and intrinsic resistance of *Trichosporon* spp. to caspofungin preemptive therapy. The portal of entry could have been the respiratory tract or the catheters, but it remains unclear because the yeast was not isolated from sputum before transplantation, nor from the removed catheters.

The invasive fungal disease in our patient was complex, since it associated three different fungal pathogens with sequential diagnosis. *A. fumigatus* was colonizing the airways before and after lung transplantation but was presumed to be invasive only when the antigenemia became positive on day 6 post-LT. *T. mycotoxivorans* fungemia was asserted on the basis of repeated positive blood cultures from day 14 post-LT until death, and finally, *S. apiospermum* was isolated only from thoracic specimens taken on the day the patient died. The multiserial dissemination was confirmed postmortem for all three strains using cultures on standard media and voriconazole-containing media to avoid *T. mycotoxivorans* being overgrown by *A. fumigatus*.

This case illustrates the challenges in diagnosing and treating multiple invasive fungal infections, especially with emerging or rare fungal pathogens. The initial presentation might be puzzling, as in our case, consisting of isolated neurological symptoms and nonspecific imaging; fungal culture may grow very slowly. Phenotypic methods for identification are limited, and molecular tools are necessary for definite identification but are time-consuming, expensive, and difficult to use in routine practice. In our case, *Trichosporon* species was suspected based on morphology, but 16 more days were required for confirmation and species identification.

The prognosis of invasive fungal infections with emerging species is generally very poor (11, 17, 19). *In vitro* data and clinical reports suggest a low efficacy of amphotericin B deoxycholate or its lipid formulations against *Trichosporon* spp. and intrinsic resistance to echinocandins. Triazoles are considered more efficient, and voriconazole or posaconazole might be preferred to itraconazole or fluconazole in patients with neoplastic disease. *Trichosporon* infection in patients with neoplastic disease. Medicine (Baltimore, MD) 65:268–279.

**REFERENCES**