Case Report

Fatal post-operative *Trichoderma longibrachiatum* mediastinitis and peritonitis in a paediatric patient with complex congenital cardiac disease on peritoneal dialysis

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Received 21 February 2011
Accepted 22 July 2011

Trichoderma longibrachiatum is an emerging pathogen in immunocompromised patients. We report a case of *Trichoderma* post-operative mediastinitis and peritonitis in a child with complex congenital cardiac disease and functional asplenia. The patient was treated unsuccessfully, initially with caspofungin alone followed by a combination of voriconazole (systemic and topical), caspofungin and intraperitoneal amphotericin B.

Introduction

The *Trichoderma* spp. were first described in 1794 as green fungi growing on fallen branches, and commonly found in soil, decaying wood and humid environments. They are usually considered plant saprophytes but are increasingly recognized as a cause of human infections, especially in immunocompromised hosts (Chouaki *et al.*, 2002; De Miguel *et al.*, 2005). We report a case of fatal, disseminated *Trichoderma longibrachiatum* post-operative infection in a paediatric patient with underlying complex congenital heart disease.

Case report

A 3-year-old female with a history of complex cyanotic heart disease, abdominal situs ambiguous and functional asplenia was admitted for a modified Fontan procedure (artificial creation of a total cavopulmonary connection by placement of an intra- to extra-cardiac conduit) with fenestration to the right atrium. A peritoneal effusion requiring the insertion of a peritoneal catheter complicated her immediate post-operative course. One day after surgery, she developed haemodynamic instability that required emergency cardiac surgery for a new fenestration. She was started on extracorporeal membrane oxygenation (ECMO) post-operatively. Her chest cavity remained open for 2 days. On post-operative day 2 she developed acute renal failure requiring peritoneal dialysis. Cultures of blood, tracheal

secretions and peritoneal fluid were obtained and she was started empirically on renal adjusted doses of cefepime (50 mg kg⁻¹ every 12 h), vancomycin (15 mg kg⁻¹ every 6 h) and fluconazole (3 mg kg⁻¹ every 24 h). The broad spectrum antibacterial coverage was de-escalated to cefazolin (20 mg kg⁻¹ every 8 h) after 48 h. She was continued on ECMO for a total of 6 days. Fluconazole (20 mg kg⁻¹ every 8 h), administered as prophylaxis against systemic *Candida* infection, was switched to caspofungin (50 mg m⁻² per day) after 2 weeks to prevent interaction with anti-arrhythmic drugs.

Two weeks after the initial surgery, a purulent discharge was noted from the precordial surgical wound. Culture of a sample from the chest wound grew a filamentous fungus, later identified as *T. longibrachiatum*. Culture of the peritoneal fluid, obtained at the same time, remained negative. The patient underwent surgical revision of the infected sternal wound. During this procedure, a defect in the diaphragm was noted, allowing communication between the peritoneal and mediastinal cavities. Intraoperative cultures of samples from the pericardium, sternum tissue and peritoneal fluid showed growth of *T. longibrachiatum*. The caspofungin dose was increased by 30 % (to 67 mg m⁻² per day). Intravenous voriconazole was added due to concerns that oral voriconazole would be poorly absorbed. The dose of voriconazole was adjusted to maintain a trough level ≥ 5.5 g l⁻¹. Wet to dry dressings placed on the mediastinal wound were also soaked in voriconazole (0.5 g l⁻¹). During the sixth week after the patient's initial surgery,

amphotericin deoxycholate instillations into the peritoneal cavity (5 mg l^{-1}) were initiated at 12 h intervals.

When the exudate from the surgical wound was noted, laboratory studies revealed 15.0×10^9 white blood cells l^{-1} , with 92 % neutrophils 3 % lymphocytes and 2 % monocytes. The patient had $137 \text{ g haemoglobin l}^{-1}$ and 40×10^9 platelets l^{-1} . Her C-reactive protein level was 34.7 mg l^{-1} . The peritoneal fluid showed a total cell count less than 52×10^6 cells ml^{-1} .

Three weeks after her Fontan procedure, the patient developed a substantial decrease in both T and B lymphocyte subsets, and a decrease in IgG serum level (1.9 g l^{-1} ; normal range $2.95\text{--}11.56 \text{ g l}^{-1}$). Serum IgA and IgM levels were within normal limits. She received supplementary IgG (400 mg kg^{-1} intravenous) and *Pneumocystis* pneumonia prophylaxis with trimethoprim-sulfamethoxazole (5 mg kg^{-1} every 48 h).

Computed tomography of the chest 4 weeks after her initial surgery revealed extensive coarse pulmonary infiltrates with bilateral pleural effusions that also grew *T. longibrachiatum*. The patient's Fontan was taken down (reversed), with removal of the intra- to extra-cardiac conduit. The peritoneal dialysis catheter that had been in place for 5 weeks was removed and she was started on continuous venovenous haemodialysis. Due to intermittent febrile episodes, the patient had been restarted on empiric broad-spectrum antibiotics [vancomycin (dose adjusted according to trough levels) plus cefepime (50 mg kg^{-1} every 12 h) or meropenem (20 mg kg^{-1} every 8 h)] on three different occasions. Multiple blood cultures obtained during this time were negative for bacterial or fungal growth. Despite aggressive combination antifungal therapy and multiple surgical wound debridements, and antifungal irrigations of the chest and the peritoneal cavities, the patient deteriorated, prompting withdrawal of care 8 weeks after her initial surgery. Cultures of the mediastinal tissue grew *T. longibrachiatum* until the sixth week of hospitalization and became negative 2 weeks prior to the patient's death. No other organisms were isolated from the clinical sources where *T. longibrachiatum* was recovered. Sternal wound tissue cultures were reported as 'few or 1+' (few= $4\text{--}20$ colonies present). Cultures from sternal wound drainage were quantified as 'many' (many= >20 colonies present). Histopathological studies were not performed.

Methods

Cultures of samples from the sternum, pericardium, extracardiac tissue, mediastinal swabs and peritoneal fluid grew a filamentous fungus within 2–4 days of initial culture. Blood cultures were negative after 14 days. The isolate first appeared as transparent colonies on an IMA (inhibitory mouldagar) plate. These colonies turned white and developed patches of compact or loose tufts in shades of green or yellow within a week. The microscopic morphology was consistent with *Trichoderma* spp., including septate hyphae displaying short, branched conidiophores with flask-shaped phialides situated at wide angles to the conidiophore, and clustered conidia at the tips of the phialides (Fig. 1).

Fig. 1. Microscopic morphology of the *T. longibrachiatum* isolate. The isolate was stained using 20% lactophenol aniline blue dye with $7.5 \text{ mg polyvinyl alcohol ml}^{-1}$. Magnification $\times 1000$.

Sequence analysis of the internal transcribed spacer and D1/D2 region of the 28S rRNA gene (Balajee *et al.*, 2009) further identified the isolate as *T. longibrachiatum*.

Antifungal susceptibility testing for voriconazole, caspofungin, posaconazole and amphotericin B was performed at the Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio, TX, USA. MICs were defined as the lowest concentration of the drug that resulted in 100 % growth inhibition when compared with a corresponding drug-free control. For the *T. longibrachiatum* isolate the drugs had MICs as follows: $\leq 0.5 \text{ } \mu\text{g ml}^{-1}$ for caspofungin, $1 \text{ } \mu\text{g ml}^{-1}$ for voriconazole, and $\geq 2 \text{ } \mu\text{g ml}^{-1}$ for posaconazole and amphotericin B after 48 h of incubation. Consensus breakpoints are not available for the filamentous fungi but, using the Fungus Testing Laboratory-derived interpretive guidelines, the susceptibility results suggested that the isolate was susceptible to caspofungin, borderline susceptible to voriconazole, and resistant to posaconazole and amphotericin B. No synergy testing was performed.

Discussion

There have been few published case reports of invasive *T. longibrachiatum* infections (Alanio *et al.*, 2008; Chouaki *et al.*, 2002; De Miguel *et al.*, 2005; Lagrange-Xélot *et al.*, 2008; Munoz *et al.*, 1997). Patients affected by this fungus are typically immunocompromised or on peritoneal dialysis. Fungal peritonitis caused by *Trichoderma* spp. has been increasingly reported in the literature (Eşel *et al.*, 2003; Goldie *et al.*, 1996; Saran *et al.*, 1996) and is associated with a high mortality (Chouaki *et al.*, 2002; De Miguel *et al.*, 2005). Invasive *Trichoderma* spp. infections have been described in the paediatric population (Alanio *et al.*, 2008; Chouaki *et al.*, 2002; Munoz *et al.*, 1997; Seguin *et al.*, 1995), involving the central nervous system (Seguin *et al.*, 1995), lungs (Alanio *et al.*, 2008; Chouaki *et al.*, 2002) and subcutaneous tissue (Munoz *et al.*, 1997). In one case, the infection mimicked pulmonary aspergillosis, with a chest computed tomography

scan showing multiple peripheral pulmonary nodules with halo and air crescent signs (Alanio *et al.*, 2008). To our knowledge this is the first case of *T. longibrachiatum* in a paediatric patient involving both the peritoneal cavity and the mediastinum. This patient did not have a known underlying immunodeficiency other than functional asplenia, a known risk factor for infection with encapsulated bacteria but not fungi, although she developed lymphopenia and hypogammaglobulinaemia in the post-operative period. It has been observed that patients who undergo open-heart surgery and cardiopulmonary bypass develop lymphopenia and are at increased risk of infection (Shi *et al.*, 2009). The hypogammaglobulinaemia was noted 1 week after the first positive culture for *Trichoderma*, although this could have been an ongoing problem secondary to protein loss due to the peritoneal dialysis.

Trichoderma peritonitis poses a therapeutic challenge. Initially, we chose to use systemic combination therapy with caspofungin and voriconazole as this combination may have an additive to synergistic effect in the treatment of filamentous fungi, as noted previously in a report of the treatment of invasive aspergillosis (Maertens *et al.*, 2006) and in a report that showed a successful outcome in the treatment of *T. longibrachiatum* invasive lung infection (Alanio *et al.*, 2008). Despite the intermediate susceptibility to voriconazole and resistance to amphotericin B, we elected to use those antifungals as we did not find definitive data that clearly correlates antifungal *in vitro* susceptibilities and clinical efficacy for filamentous fungi.

Trichoderma colonization of the peritoneal dialysis catheter and/or the patient's cardiac conduit could have contributed to the therapeutic failure as reflected by the persistence of positive cultures in peritoneal fluid and mediastinal tissue, including tissue surrounding the cardiac conduit. Early removal of the peritoneal catheter or conduit may have allowed better control of the infection but this was not possible due to the clinical instability of the patient.

The primary source of the patient's infection remains unclear. The initial peritoneal fluid was culture negative and had a low leukocyte count at the same time that mediastinal wound cultures grew *Trichoderma*, suggesting that the mediastinum was the initial site of infection. The patient was managed in a cardiac intensive care unit room without a HEPA (high efficiency particulate air) filtration system. It is not known if the patient was colonized with *Trichoderma* before her surgery or acquired the infection within the hospital.

In conclusion, we describe a case of post-operative invasive infection with *T. longibrachiatum* treated unsuccessfully with a combination of caspofungin, voriconazole and amphotericin B. Physicians should be aware of this opportunistic pathogen as a cause of invasive disease in children who are immunocompromised, require peritoneal dialysis or develop complications following cardiac surgery.

Acknowledgements

We wish to thank Thomas Walsh at the National Cancer Institute (Bethesda, MD, USA) for his assistance with the case, Lori K. Steinmetz for assistance with the photograph and the staff of the Mayo Clinic Mycology Laboratory for technical assistance.

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