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CASE REPORT

# Trichoderma: an unusual bystander in invasive pulmonary aspergillosis

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## Abstract

In this case report we present a 64-year old female patient who was admitted to our intensive care unit (ICU) with an abdominal sepsis six days after a laparotomy and a hyperthermic intraperitoneal chemotherapy (HIPEC)-procedure. During her ICU admission she developed a pneumonia and sepsis. Bronchoalveolar lavage (BAL) was positive for *Aspergillus* species and an unknown fungus species. Despite maximum treatment for invasive aspergillosis, the patient died after being on the ICU for 29 days. Evaluation of the unknown species in the BAL showed *Trichoderma*. This case reports focuses on the clinical relevance of finding the *Trichoderma* species in the BAL. In addition, we provide a literature overview concerning Trichoderma species infections.

## Introduction

*Trichoderma* species has always been considered as a contaminant in organ site cultures. However, in the recent literature, *Trichoderma* species has been increasingly reported as etiologic agents in human infections, especially in immunocompromised patients. We describe a case report and discuss recent literature in order to achieve increased awareness of this frequently fatal infection.

## Case

A 64-year old female, with a medical history of hypertension and a right sided hemicolectomy for carcinoma three months prior to admission was admitted to our intensive care unit following a mitomycin-C hyperthermic intraperitoneal chemotherapy (HIPEC) procedure for metastatic carcinoma of the colon. The patient did not receive chemotherapy in the six months before the HIPEC procedure. Six days following the procedure, the patient developed abdominal pain and sepsis. A diagnostic laparotomy was performed which showed a perforation of the small intestine and an extensive faecal peritonitis. The perforation was surgically closed and an abdominal lavage was performed. The patient was hemodynamically unstable and mechanically ventilated at the time of admission to the ICU. Antibiotic treatment was started with amoxicillin, ceftriaxone and metronidazol. In addition, she received selective digestive tract decontamination consisting of amphotericin B, colistin/polymyxin and tobramycin in oral paste and gastric suspension. Blood cultures remained negative except for one anaerobic blood culture, dated just before the re-laparotomy, showing *Bacteroides* species. The patient's condition deteriorated and a second look laparotomy was performed on day two of ICU admission. A necrotizing pancreatitis was found, most probably due to low-flow state during sepsis, and antibiotic treatment was changed to meropenem, dosed 500 mg twice daily (adjusted for renal function). On the third day of ICU admission the cultures of the abdominal fluid showed positive for *Enterococcus faecium* for which vancomycin was added to the meropenem.

Seven days after ICU admission the patient stabilized and infection parameters improved. Antibiotic treatment was stopped after 12 and 11 days of meropenem and vancomycin treatment respectively.

At day 16 of ICU admission, the patient's infection parameters deteriorated. After obtaining cultures from the wound, drain fluids, and sputum, meropenem and vancomycin were re-initiated. The cultures were negative except for the tracheal aspirate which showed less than five colonies of *Aspergillus* fumigatus (*figure 1*). Aerosolized amphotericin B was added to the antibiotic regiment as topical treatment, as the positive tracheal aspirates were considered to reflect colonization. Because the patient could not be weaned from mechanical ventilation and continued to have fever between 38 and 39° Celsius, a computer tomogram of the thorax and abdomen was performed on day 22 of ICU stay. It showed increasing pulmonary consolidations in both inferior lobes and the right superior lobe. Multiple sputum cultures grew *Aspergillus* fumigatus, susceptible for itraconazole (minimal inhibitory concentration [mic] after incubation for 48 hours by E-test and broth microdilution on Sabouraud dextrose agar was 0.75 mg/ml) and amphotericine B (mic 0.38 mg/ml).

Because of potential invasive fungal infection, caspofungin was added on day 22 to the antibiotic regimen, initially dosed at 70 mg

**Figure 1.** *Aspergillus*

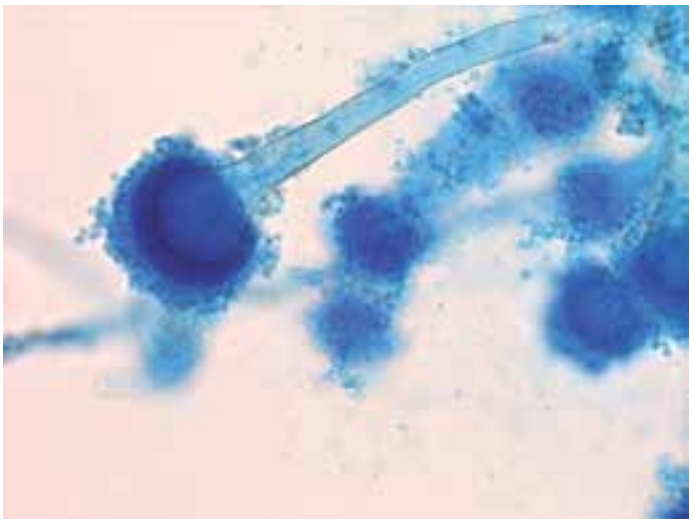

**Figure 2.** *Trichoderma*

daily then reduced to 50 mg daily. Liver function abnormalities precluded the prescription of azoles. At day 27, the chest X-ray showed a cavity in the right middle lobe. The antifungal regime was intensified by adding voriconazole (6 mg/kg twice daily, followed by 4 mg/kg) to the already prescribed caspofungin and a broncho-alveolar lavage (BAL) was performed. The galactomannan test in the BAL was strongly positive (optical density index of 9.3, cut off 0.5 for serum). Galactomannan in serum was not performed. A CT-scan of the thorax performed on day 29 showed progression of consolidations compared with the previous one. These consolidations were highly suspicious for invasive aspergillosis because of extensive cavitation. The patient's condition worsened and she subsequently died on day 29 in the ICU.

Cultures from the tracheal aspirate taken two days before her death revealed the same *Aspergillus* species and another fungus which was difficult to identify. It was ultimately determined by the Dutch tertiary reference centre for fungal infections (Department of Medical Microbiology Radboud University, Nijmegen) as *Trichoderma* species (*figure 2*), which showed relative susceptibility to voriconazole and anidulafungin. The mic for *Trichoderma* as were (in mg/ml): itraconazole: >16, fluconazole: >64, amphotericin B: 2.0, 5-flucytosine: >64, voriconazole: 1.0, anidulafungin: 0.25 and posaconazole: >16.

An autopsy was performed. The pulmonary slides showed (bilaterally) necrotizing pneumonia with clusters of mould-cords consistent with *Aspergillus* with vascular invasive growth. *Trichoderma* could not be detected in the obtained tissue samples.

## Discussion

The diagnosis of invasive fungal disease (IFD) is challenging, since moulds are ubiquitously present in the environment and detection in a clinical sample does not necessarily represent invasive fungal infection. Criteria for IFD have been developed by the EORTC (European Organization for the Research and Treatment of Cancer) for patients with haematological malignancies to standardize clinical and epidemiological research (*table 1*)[1]. Despite this they are often applied to such patient groups[2], as we did on day 22, facing an immunocompromised patient with increasing pulmonary consolidations on CT and moulds found in multiple sputum cultures. The autopsy results confirmed our diagnosis by showing a necrotising pneumonia with clusters of hyphae conform *Aspergillus* with invasive growth in vascular structures.

In our patient, Trichoderma was found in combination with *Aspergillus* in the BAL-material. In the tissue samples obtained by autopsy, *Trichoderma* could not be identified. The relative contribution of *Trichoderma* in the clinical course is undetermined. Indeed, *Trichoderma* could just be an unusual bystander in invasive pulmonary Aspergillosis, emphasizing the severe immune-incompetence of our patient. However, the clinician confronted with a culture containing *Trichoderma* should be aware of this emerging pathogen in immunocompromised patients, as we have described, and should make a therapeutic decision.

Previously, *Trichoderma* species were considered to be contaminants. *Trichoderma* infections in humans appeared to be rare, but they are increasingly reported as emergent pathogens, probably due to their opportunistic behaviour, but also because of the increasing number of immunocompromised patients nowadays[3,4].

The first case of an invasive *Trichoderma* infection was described by Robertson, concerning an accidental intravenous infusion of contaminated fluid[5]. In the last decades of the past century, the majority of described patients suffered peritonitis as a result of peritoneal dialysis. Mortality was high, with less than fifty percent survival rate. The majority of the non-peritonitis cases contain

**Table 2.** IFD criteria by the EORTC

| Host criteria | Clinical criteria (of the respiratory tract) | Microbiologic criteria |
|---|---|---|
| • Recent history of neutropenia (0.5x109 neutrophils/L for >10 days) temporally related to the onset of fungal disease<br>• receipt of an allogeneic stem cell transplant<br>• prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for 13 weeks<br>• treatment with other recognized T cell immunosuppressants, such as cyclosporine, TNF-a blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days<br>• inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency) | • The presence of 1 of the following 3 signs on CT:<br>- dense, well-circumscribed lesions(s) with or without a halo sign<br>- air-crescent sign<br>- cavity<br>or<br>• tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis | • Direct test (cytology, direct microscopy, or culture)<br>- mold in sputum, bronchoalveolar lavage fluid, bronchial brush, or sinus aspirate samples, indicated by 1 of the following:<br>• presence of fungal elements indicating a mold<br>• recovery by culture of a mold<br>• indirect tests (detection of antigen or cell-wall constituents):<br>- aspergillosis<br>• galactomannan antigen detected in plasma, serum, bronchoalveolar lavage fluid, or CSF<br>- invasive fungal disease other than cryptococcosis and zygomycoses<br>• β-d-glucan detected in serum |

*Proven IFD is defined as recovering a fungal species from a sterile compartment of the body (e.g. positive microscopy or culture from blood, cerebrospinal fluid or sterile tissues).*
*Probable IFD requires the presence of a host factor, a clinical criterion and a microbiological criterion.*
*Possible IFD is established when cases meet the criteria for a host factor and a clinical criterion but for which mycological criteria are absent.*

opportunistic infections complicating the course of haematological malignancies and solid organ transplantations.

Little is recorded about the clinical manifestations of *Trichoderma*, perhaps because the clinical image seems very un-specific. Symptoms appear predominantly in immunocompromised patients as nodular pulmonary infiltrates, sometimes mimicking invasive aspergillosis[6], localized (ulceronecrotic) cutaneous lesions or disseminated infection, including the central nervous system. In the case of peritonitis (due to infected peritoneal dialysis catheters) the symptoms range from mild (abdominal discomfort) to more severe (bowel obstruction). Sometimes fever is present. None of these symptoms could suggest a fungal rather than a bacterial origin. This makes the definitive diagnosis of *Trichoderma* difficult. The diagnosis relies on the demonstration of hyphae in tissue sections associated with positive culture results in non-biopsy specimens, obtained from accessible sites, e.g. skin, upper- or lower airways, or urine, sputum cultures, wound swabs etc. Once hyphae have been demonstrated in tissue sections, *Trichoderma* infection can easily be misdiagnosed as aspergillosis or other hyalohyphomycosis, because the hyphae are morphologically quite similar. Guarro et al. emphasized the complexity of the branching pattern of *Trichoderma* hyphae in tissue, compared to the other hyalohyphomycosis[7].

Also the identification of *Trichoderma* isolates at species level may be difficult by only relying on morphology, sometimes leading to erroneous species identification. Nowadays molecular techniques are used for species identification, since Kuhls et al. identified the human pathogenic *Trichoderma* isolates by PCR-fingerprinting[8].

The prognosis for patients with *Trichoderma* infections is poor. For disseminated infections the mortality is as high as 100%. Favourable outcome of the infection is associated with immunocompetence, catheter or drain removal in cases of peritonitis and surgical debridement of the localized lesion. The major problem with *Trichoderma* genus is its poor susceptibility to antifungal drugs. The minimal inhibitory concentrations for fluconazole and 5-fluorocytosine are far above the human toxicity level[9,10,11,12]. Possible susceptibility (i.e. fungal mic for which non-toxic blood levels are achievable) is reported for itraconazole, ketoconazole and miconazole, although for itraconazole and ketoconazole high mic levels have also been reported[7]. Apparent susceptibility to voriconazole has been described[13,14]. Recent data indicate that there may be resistance to amphotericin B[15,16,17]. There is a lack of information about the efficacy of caspofungin, although low mics have been recorded[6,18]. No clinical trials have been performed directing the treatment of *Trichoderma* infections. Susceptibility testing is of utmost importance, and preferably antifungal drugs with low mics should be used. There is no evidence for advising a specific antifungal agent in the empirical setting (i.e. before susceptibility testing). The most recent case report uses a combination of an echinocandine (caspofungin) in combination with voriconazole, both more tolerable than amphotericin B, with the aim that *Trichoderma* will at least be susceptible to one of these antifungals[18]. Since for other susceptible fungal infections duo therapy is not proven to be superior to monotherapy, we advise switching to monotherapy when results for susceptibility testing become available from the laboratory[19,20,21].

## Conclusion

A case of an invasive opportunistic pulmonary infection with *Aspergillus* and possibly *Trichoderma* spp. is presented. Awareness of this frequently fatal invasive *Trichoderma* infection is warranted for critical care physicians. Clinical backgrounds and considerations concerning the diagnosis have been discussed and suggestions for treatment are given in this article.

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