**Case Report**

**Scedosporium apiospermum**: a fungal pathogen causing pneumonia in a patient with cystic fibrosis

Carsten Schwarz, Anja Thronicke, Doris Staab and Kathrin Tintelnot

**Correspondence**
Carsten Schwarz
carsten.schwarz@charite.de

1 Division of Cystic Fibrosis, Pediatric Pneumology and Immunology, Charité – Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

2 Reference laboratory for cryptococcosis, scedosporiosis and imported systemic mycoses, FG16, Mycotic and Parasitic Agents and Mycobacteria, Robert Koch Institute, Nordufer 20, 13353 Berlin, Germany

**Introduction:** *Scedosporium apiospermum* is a slow-growing pathogenic fungus that can cause pneumonia. Diagnosis of this rare filamentous fungus is challenging. To the best of our knowledge, this is the first case describing an acute pulmonary infection caused by this fungal pathogen in a patient with cystic fibrosis (CF).

**Case presentation:** A 35-year-old female with CF, with a 1-month history of irritating cough, increased sputum production and dyspnoea, was admitted to hospital. A chest computed tomography scan revealed new bilateral infiltrations, mediastinal lymphadenopathy and bronchiectasis. *Pseudomonas aeruginosa* and *S. apiospermum* were detected in the bronchoalveolar lavage. The patient's clinical status was not improved by standard antibiotic therapy. Antifungal systemic therapy consisting of systemic caspofungin and oral posaconazole against the highly suspected scedosporiosis was initiated, but the clinical outcome was not significantly improved. Antibodies against the *S. apiospermum* complex were present in the patient’s serum. Only after the addition of 50 mg once a day of inhaled liposomal amphotericin B the patient was successfully treated.

**Conclusion:** The patient was cured by a combined therapy of two systemic antifungal drugs and one local antifungal drug.

**Keywords:** *Scedosporium apiospermum; Cystic fibrosis; pneumonia.*
woman had been diagnosed with CF carrying the compound heterozygote R347P/1078delT deletion of phenylalanine at position 508 of the CFTR gene (delF508) mutation (class IV/class I mutation). Even though it appeared that the patient had a ‘mild’ phenotype, it was unusual that she had been colonized by *P. aeruginosa*. The patient was treated once to twice per year with a prophylactic *Pseudomonas*-effective therapy without significant problems. Informed consent was given by the patient. As this patient was included in a study on pulmonary infection in CF, Institutional Review Board (IRB) approval from the local Charité – Universitätsmedizin Berlin ethics committee was also obtained (EA2/080/11). Haematology testing showed enhancement of the following parameters indicating acute infection: leukocytes 17.9 cells/nl (normal range 4.4–11.3 cells/nl), thrombocytes 449 cells/nl (normal range 170–394 cells/nl), absolute neutrophils 13.7 cells/nl (normal range 1.6–7.1 cells/nl) and absolute monocytes 1.5 cells/nl (normal range 0.2–0.6 cells/nl). Liver and kidney function parameters were normal. Blood gas parameters were normal. A chest computed tomography (CT) scan revealed new bilateral infiltrations, mediastinal lymphadenopathy and bronchiectasis as the result of chronic *P. aeruginosa* colonization (Fig. 1a).

**Investigations**

Cultivation of bronchoalveolar lavage (BAL) on diagnostic culture medium in our microbiological institute yielded the growth of *P. aeruginosa* and *S. apiospermum*. Further microscopy, cultivation on highly selective SceSel+ medium and molecular methods repeatedly identified *S. apiospermum* in BAL and sputum. *S. apiospermum* was confirmed by sequencing of the internal transcribed spacer regions of rRNA genes. Multilocus sequence typing revealed the identical sequence type #532 in consecutive isolates of this patient (Bernhardt et al., 2013). Repeated *in vitro* susceptibility testing of this filamentous fungus against voriconazole, posaconazole and amphotericin B by a microdilution method according to the Clinical and Laboratory Standard Institute guidelines M38-A2 (CLSI, 2008) resulted in MIC values of 1, 2 and 2–4 µg ml⁻¹, respectively. The minimal effective concentration of caspofungin was 4 µg ml⁻¹.

Counter-immunoelectrophoresis revealed six bands of serum antibodies directed against the *S. apiospermum* complex. This result demonstrated an immunological interaction between the host and the pathogen, and reinforced the suspicion of an infection caused by this fungus. Serum antibodies were determined using a cytosolic antigenic extract obtained from strain IHEM 15.155 (Institute of Hygiene and Epidemiology – Mycology Section; Institute of Public Health, Brussels, Belgium). This strain has been identified as *Pseudallescheria boydii*, which is the most frequently isolated species of the *S. apiospermum* complex in CF in France (Zouhair et al., 2013). The antigen has been used previously in several studies (Bertrand et al., 2009; Larcher et al., 1996; Parize et al., 2014b).

The combination of pathological findings in the CT scan and in BAL indicated a strong suspicion of pulmonary fungal infection. As fungal infections are known to develop preferentially in immunocompromised patients, we performed immunological assays, which revealed the following results: normal distribution of T-cells, B-cells and natural killer cells (assessed by expression of cluster of differentiation markers CD3, CD4, CD9, CD16/CD56 surface markers in peripheral blood), and normal proliferation of peripheral blood lymphocytes upon activation with mitogens (phytohaemagglutinin and pokeweed mitogen) and recall antigens (tetanus and *Candida*). Elevated neutrophils at 76% were the only elevated parameter indicating neutrophilia caused by the already existing bronchopulmonary infection. The immunoglobulins IgA and IgM were not reduced. The subclasses of IgG were normal, and IgG was enhanced at 19.5 g l⁻¹ (normal range 7–16 g l⁻¹), presumably due to chronic infection. The above-mentioned immunological methods provided no evidence of a primary immunodeficiency. Diabetes mel-

![Fig. 1.](image-url) (a) Chest CT scan before treatment, showing bilateral infiltrations, mediastinal lymphadenopathy and bronchiectasis. (b) Chest CT scan after 8 weeks of treatment showing regression of the pulmonary changes.
litus, human immunodeficiency virus infection, systemic steroid therapy and alcohol abuse could be excluded as secondary immunodeficiencies for this patient. Further investigations are needed to focus on immunodeficiency in CF (e.g. at airway level or innate immune status; Hartl et al., 2012).

Treatment
After admission to hospital the patient was treated with standard antibiotic agents: systemic ceftazidime 3 g three times per day and tobramycin 560 mg once per day (for 2 weeks) (Fig. 2). The therapy failed and was changed to new treatment including systemic fosfomycin 4 g three times per day and aztreonam 2.5 g three times per day (for 16 days). As the patient’s clinical status was not improved by this standard antibiotic therapy and the patient was severely ill, a diagnostic flexible bronchoscopy was performed to identify the pathogenic profile. Cytologically, 1% macrophages and 99% granulocytes (neutrophils) were found in the BAL.

After the CT, flexible bronchoscopy and laboratory investigations, antifungal systemic therapy against the highly suspected scedosporiosis was started with i.v. caspofungin at 50 mg once per day and oral voriconazole 400 mg twice per day directly after the second antibiotic treatment (with an overlap of 3 days of antibiotic treatment and antifungal regimen) and was continued with i.v. caspofungin at 50 mg and oral posaconazole (due to side effects under therapy with voriconazole) at 400 mg twice per day (Fig. 2). The clinical status stabilized but did not improve. Inhaled liposomal amphotericin B (50 mg once per day) was added to the caspofungin and posaconazole regimen. Standard basis treatment was continued after i.v. antibiotic treatment was stopped, comprising azithromycin at 500 mg three times a week, pancreas enzyme daily as necessary and inhalation treatment of: (i) 5.85% NaCl and Salbutamol twice per day; (ii) Pulmozyme at 2.5 mg once per day; and (iii) tobramycin at 300 mg twice per day (Fig. 2).

Outcome and follow-up
After the addition of inhaled liposomal amphotericin B, the patient’s clinical status improved for the first time. The radiological follow-up (Fig. 1b) showed a complete regression of the pulmonary changes. Lung function improved as shown by an FEV1 value of 91% of the predicted value. The patient was discharged after 6 weeks of treatment from hospital. During the 2-year follow-up, the patient remained stable according to pulmonary function tests, which revealed no obstructive ventilatory disturbance (FEV1 of 88% of the predicted value).

Discussion
Besides bacteria, pathogenic fungi are increasingly acknowledged in association with bronchopulmonary exacerbations in patients with CF (Amin et al., 2010; Pihet et al., 2009). Pulmonary fungal infections are difficult to diagnose, as a biopsy for histological examination is required, which is not always feasible due to the risk of bleeding. Furthermore, the diagnosis can be challenging due to unsuitable, insensitive, non-specific techniques or delayed results (Ostrosky-Zeichner, 2012). In many cases, Scedosporium/Pseudallescheria spp. are not detected, as the cultivation is done mostly on routinely used diagnostic fungi-selective media such as Sabouraud’s dextrose agar and beerwart (Bierwürz) agar. Particularly in the presence of colonizing Aspergillus spp. in the respiratory tract, slow-growing Scedosporium spp. are overgrown by fast-growing pathogens (compared with Aspergillus spp. colonies). Therefore, for the microbiological detection of this species complex, a Scedosporium-selective medium, e.g. SceSel+ agar, is recommended (Horré et al., 2009; Rainer et al., 2008; Sedlacek et al., 2015).

In our case, this highly selective agar was utilized for the detection of S. apiospermum. The detection of precipitating antibodies of a S. apiospermum complex in a counter-immunoelectrophoresis assay supported our suspicion of pulmonary fungal infection caused by S. apiospermum.

As indicated in the literature, differentiation has to be made between lung colonization, infection with fungi, allergic sensitization to fungi and clinically proven allergic bronchopulmonary aspergillosis (ABPA), the most common allergic bronchopulmonary mycosis (Mastella et al., 2000; Slavin et al., 2004). For further differential diagnosis, allergic reactions due to ABPA/allergic bronchopulmonary scedosporiosis were ruled out, indicated by the normal status of IgE and the absence of specific IgEs for Aspergillus fumigatus and absence of serum IgE to rAsp f4 and rAsp f6.
These tests have been shown to be predictive for ABPA in previous publications (Knutsen et al., 2004).

Last but not least, risk factors and infection routes were investigated. Scedosporium/Pseudallescheria spp. are commonly found in soil, water bodies and sewage. As it was known that the patient was an enthusiastic gardener, we tested soil from the patient’s raised herb bed, flat flower bed and green house, water from the rain barrel and water from the pond. Cultivation tests in the reference laboratory resulted in the detection of Scedosporium prolificans in soil from the raised herb bed, whereas S. apiospermum was not found. Nevertheless, we hypothesize that one of the possible aetiologically significant risk factors in the presented patient could have been her frequent gardening (Table 1). Potted plants, next to gardening, also seem to be a risk factor for acquisition of fungi. The immunodeficient status of patients with CF at the airway level may be a risk factor for acquiring a pulmonary fungal infection of the Pseudallescheria/Scedosporium complex. CF has been described as a mucosal immunodeficiency in recent literature (Cohen and Prince, 2012). It is increasingly being acknowledged that up to approximately 14% of CF patients are colonized by Scedosporium/Pseudallescheria spp. (Horré et al., 2009; Pihet et al., 2009). To understand the mechanisms why rare fungi such as those of the Pseudallescheria/Scedosporium complex colonize the lungs of patients with CF and not other patients, the underlying host–pathogen and pathogen–pathogen interactions should be studied in detail (Hartl et al., 2012). Further clinical studies will need to be performed in order to significantly link CF with community-acquired fungal pneumonia caused by rare fungi such as Scedosporium/Pseudallescheria spp. These fungi represent a therapeutic challenge. Because of their distinct resistance to most antifungal agents and because of the seriousness of an infection, a broad antifungal combination therapy would be the suitable clinical therapy. First-, second- and third-generation triazoles, amphotericin B and the echinocandines are the classically available antifungal agents. In our case, antifungal treatment with systemic caspofungin and oral voriconazole as well as systemic caspofungin and oral posaconazole failed to show an adequate response. We would like to note that antifungal treatment potentially has many side effects (e.g. liver and cardiac toxicity) (Ashbee et al., 2014), and up-to-date data about combined antifungal therapy in CF is scarce (Eickmeier et al., 2013).

Clinical success was achieved only with the off-label utilization of inhaled liposomal amphotericin B in addition to combined systemic antymycotic treatment of i.v. caspofungin and oral posaconazole. In vitro susceptibility testing showed a MIC of 2–4 μg mL⁻¹ for amphotericin B, equivalent to resistance against systemic treatment. According to our results and experiences, azoles and echinocandines in combination with the inhaled liposomal amphotericin B would have a therapeutic advantage over a course of at least 4 weeks. In some cases, a longer therapy would be helpful. In our case, 3 months of therapy were necessary for complete regression of the fungal infection. We would suggest not using voriconazole as a monotherapy as this could trigger breakthrough infections.

In summary, clinical relevant pulmonary fungal infections should be considered in patients with CF in the case of therapy-resistant pneumonia, detection of multiple fungi and new pulmonary infiltrates. In such a case, highly selective agars may be used for identification of the relevant pathogen. In addition, the detection of precipitating antibodies against the relevant fungus may confirm the diagnosis, especially if longitudinal detections have been performed. The patient was cured by a combined therapy of two systemic antymycotic drugs and one local antymycotic drug. As there is no universal antymycotic drug that might be useful for treatment – independent of fungal species – it is indispensable to identify the pneumonia-causing species. We speculate that the combination of systemic and local treatment may increase the likelihood of successfully treating severe fungal infections. To the best of our knowledge, there is only other case that has documented pulmonary fungal infection caused by S. apiospermum in a patient with CF (Holle et al., 2014). Successful treatment of the S. apiospermum infection, the survival and the good long-term response of the patient in our case was attributed to early clinical suspicion, microbiological and molecular identification, and susceptibility testing with initiation of an adequate combinational antifungal treatment (see also Table 1).

**Acknowledgements**

We would like to thank Dr Jean-Philippe Bouchara, Departement Biologie des Agents Infectieux et Pharmaco-toxicologie, CHU d’Angers, for performing the counter-immunoelectrophoresis. Informed consent was given by the patient. As this patient was included in a study on pulmonary infection in CF, Institutional Review Board (IRB) approval from the local Charite – Universitats-
medizin Berlin ethics committee was also obtained (EA2/080/11). The authors declare no conflicts of interest.

References


