Fungal infections in patients with cystic fibrosis

Glivne okužbe pri bolnikih s cistično fibrozo

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Abstract

In the last two decades prevalence of fungal infections is increasing for various reasons. One of them is the advance of medical science and the associated longer life expectancy in some patient groups. This includes cystic fibrosis patients who encounter fungal diseases already in their childhood. Fungal pathogens isolated in high frequencies from the respiratory tract include *Aspergillus fumigatus*, *Candida albicans* and *Scedosporium apiospermum*. In the case of cystic fibrosis, these organisms normaly colonise the respiratory and intestinal mucosae and can cause hipersensitivity reactions and invasive diseases. The fungus-patient interactions are complex and depend on several different factors which determine what course will the colonisation/infection take.

Izvleček

V zadnjem času se pojavnost glivnih okužb povečuje iz različnih razlogov. Eden od njih je napredek v medicinski znanosti in z njim povezana daljša pričakovana življenjska doba določenih skupin bolnikov. Sem spadajo tudi bolniki s cistično fibrozo, ki se s problematiko glivnih patogenov srečujejo že v otroštvu. Največjo vlogo imajo glive *Aspergillus fumigatus*, *Candida albicans* in *Scedosporium apiospermum*. Pri cistični fibrozi so ti organizmi kolonizatorji dihal in prebavil, povzročitelji preobčutljivostnih reakcij in v redkih primerih povzročitelji invazivnih okužb. Do kakšnega poteka bo prišlo, je odvisno od značilnosti posameznega bolnika, glive in okolja ter izredno kompleksnih interakcij med njimi.

Cystic fibrosis and fungi

Cystic fibrosis (CF) is one of the most common autosomal recessive genetic diseases. One in every twenty-seven people carries a recessive allele. The disease occurs as the result of mutations in the CFTR gene (Cystic Fibrosis Transmembrane Conductance Regulator), which affects the irregular synthesis of the protein involved in the transport of chloride ions across the membrane of the epithelial cells. Consequently, many organ systems are affected, especially the respiratory tract (1). Disturbances in the transport of ions on the respiratory epithelium and the resulting change in viscosity of the secretions hinder the functioning of the mucociliary escalator which creates favourable conditions for the development of various infections. They are most frequently caused by *Staphylococcus aureus, Haemophilus influenzae, Burkholderia cepacia* and *Pseudomonas aeruginosa*. The latter is considered one of the main causes for the progressive reduction in pulmonary functions in many CF patients (1,2). In

recent times, more interest has been paid to infections caused by opportunistic pathogenic fungi.

Fungi are ubiquitous eukaryotic organisms, which may colonise the respiratory and digestive tracts and causehypersensitivity reactions and infections in CF patients (3). The most common types of fungi isolated in CF patients include *Aspergillus fumigatus*, *Candida albicans* and *Scedosporium apiospermum* (3). Initially, disorders or weakening of the immune system are not typical for CF patients, therefore the localisation of fungi is limited to the respiratory epithelium. Irregular mucus secretion from the lower respiratory tract causes long-term exposure to fungal antigens, which leads to the development of hypersensitivity reactions. Consequently, chronic colonisation and hypersensitive reactions are the main types of relationship between fungi and a CF patient. In immunosuppresed state, which normally occurs after lung transplantation, the relationship between opportunistic pathogens and the patient can become more severe. In such cases, though very rare in CF patients, fungi can invade the lung parenchyma and cause an invasive pulmonary mycosis, which, in the absence of an immune response, can progress into multiorgan disseminated infection associated with high mortality.

Clinical mycology in cystic fibrosis

Every day, CF patients are exposed to an environment inhabited by fungi, including moulds and yeasts. A wide variety of fungi can be isolated from respiratory samples but the two most documented and studied agents are *A. fumigatus* and *C. albicans.* Other common and interesting isolates include *S. apiospermum*, *Exophiala dermatitidis* and *Rasamsonia argillacea*, whose role in CF pathobiology has yet to be defined.

Fungi of the genus *Candida*

Species of the genus *Candida* are ascomycetous yeasts, which can normally be found in the oral and intestinal mucosa, moist areas of the skin, the upper respiratory tract and genital mucosa. They are present in people with a normal immune response, as well as in patients with immunodeficiencies. Table 1 summarises interactions between candida and a CF patient.

Factors associated with the isolation of yeasts or candida include pancreatic insufficiency, osteopenia, diabetes mellitus and the concurrent colonisation with the bacteria *P. aeruginosa* (4,5). The high proportion of CF patients colonised with candida (49.4 %) is mainly due to the progression of the underlying disease (6). The most frequently isolated species is *C. albicans*, followed by *Candida glabrata, Candida parapsilosis, Candida krusei* and *Candida tropicalis.* They are often recovered from the sputum, but the clinical significance of these isolates is questionable because of the natural occurrence of yeasts in the oral cavity and pharynx; there is no proof supporting the efficiency of antifungal treatment in these cases (7,8).

In certain situations, the commensal relationship in CF patients turns into parasitism. The most commonly encountered are superficial infections that affect the oral and/or genital mucosa. There have been documented cases of oropharyngeal candidosis, the development of which is enhanced by the impaired functioning of the salivary glands, CF-related diabetes, steroid treatment and broad-spectrum antibiotic treatment (6). Since there may also be other causes for a similar symptomatology in

CF patients (e.g. vitamin B complex deficiency), microbiological confirmation is always advised (6). Female CF patients often suffer from fungal vulvovaginitis; its occurrence is probably underestimated as very little attention is paid to this type of fungal infections in CF (9). Most superficial candidoses are successfully treated with fluconazole, while serious cases and chronic recurrences of the infection are treated with itraconazole (10,11).

In addition to the aforementioned superficial infections, *Candida spp.* can also cause candidaemia and invasive candidosis, which can develop into septic shock if not treated properly. The origin of the infection is usually endogenous – candida are part of the normal gastrointestinal microbiota. The origin of the infection can also be the various vascular catheters on which yeasts form biofilms. Lung transplant patients who receive immunosuppressives after surgery are at particularly high risk for invasive candidosis (5,6). The haematogenous dissemination of candida mainly affects the kidneys, spleen, skin, eyes, heart and meninges (12). Candidaemia that develops into sepsis is associated with a high mortality rate ranging between 30 and 60 % (13). In order to monitor the progression of candidaemia and determine the damage to the organs, a fundoscopy and a transoesophageal echocardiography are advised for neutropenic patients (14). The first choice for treatment are antifungals from the group of echinocandins, usually caspofungin (10,11). The choice of antifungal medication depends on the type of pathogen and the site of infection, among other factors.

Fungi of the genus *Aspergillus*

Species of the genus *Aspergillus* are ubiquitous filamentous ascomycete fungi. They are found in the air, water, dust and decomposing plants. Respiratory infection or colonisation occurs through the inhalation of aerosolised conidia. The latter are small enough (2–5 µm) to enter the lower respiratory tract, but in a healthy person they are expelled regularly by the clearance and defensive functions of the respiratory epithelium. In CF patients these two functions are compromised, therefore various types of

Table 1: Summary of the roles of different species of the genus *Candida* in CF patients.

Infection, colonisation with *Candida* spp.	Colonisation	Surface infection	Systemic infections
Risk factors in CF	Diabetes, osteopenia, colonisation with *Pseudomonas aeruginosa*	Diabetes, treatment with corticosteroids, treatment with broad-spectrum antibiotics	Immunosuppression after transplant surgery
Clinical significance	Short-term: / Long-term: progression of the underlying disease	Candidosis of the oral cavity and pharynx, fungal vulvovaginitis	Candidaemia, disseminated candidosis, invasive candidosis
Medication of choice	/	Fluconazole, itraconazole (10,11)	Echinocandins, fluconazole, amphotericin B or based on the type of fungus and the results of susceptibility testing (10,11)

fungi, most commonly *A.fumigatus,* can be isolated from the respiratory tract of a large number of patients (5). Judging by the cultivation results, the prevalence of *A. fumigatus* is between 6 and 57 % (15). The isolation of this fungus from respiratory samples can indicate colonisation, allergic reactions or an infection (13). Table 2 summarises interactions between aspergilli and a CF patient .

The inhalation of aspergillus conidia leads to different clinical manifestations, which are difficult to distinguish from each other because they overlap to some extent (15,16):

a. intermediate colonisation, when the patient is only temporarily colonised,
b. allergic sensitisation, when a patient becomes sensitive to aspergillus antigens,
c. chronic colonisation, when a patient is colonised over a long period of time with one or more strains,
d. allergic bronchopulmonary aspergillosis (ABPA), which represents the intermediate state between colonisation and infection,
e. aspergilloma,
f. invasive pulmonary aspergillosis, and
g. aspergillus bronchitis.

The leading factors related to the isolation of moulds in CF patients are: the age above 18 years, diminished pulmonary function and inhalation treatment with antibiotics.

Long-term exposure to mould allergens, which is the result of inadequate clearance of the respiratory epithelium, leads to the occurrence of various hypersensitivity reactions. These are most frequently caused by moulds *Alternaria alternata, Cladosporium herbarum, Penicillium* spp. and *A. fumigatus*, the latter playing the most important role in CF patients.

Allergens are either components of the cell wall or secondary metabolites that are secreted by the fungus into its immediate surroundings (17). Classified as *A. fumigatus* allergens are various polysaccharides, elastases, catalase, superoxide dismutase, glycopeptides and nucleo- and proteolytic enzymes. Hypersensitivity reactions are mostly chronic in nature and are not related to a patient's deteriorated immunity; they can lead to the aggravation of the underlying disease in CF patients (17,18). With the onset of immunosuppression, some of the aforementioned allergens take on the role of virulence factors, which enable the development of an invasive infection.

Ranked first among the diseases caused by *A. fumigatus* is allergic bronchopulmonary aspergillosis (ABPA), which affects between 7 and 9 % of CF patients (19). It mostly affects adults (15). In ABPA the conidia typically germinate and the mycelium colonises the bronchial tree without invading the lung parenchyma. An inflammatory response to aspergillus antigens develops, regulated by CD4 Th2 cells, which secrete various inflammatory cytokines, especially IL-4 and IL-10 (17,20). This is followed by the invasion of vasoactive substances and the emergence of specific antibodies, especially of the IgE class. Such an inflammatory reaction is clinically manifested as a productive cough, the emergence of pulmonary infiltrates and central bronchiectases, and typically also peripheral and pulmonary eosinophilia and an increase in the concentration of IgE in the serum (17,20). Possible complications include pulmonary fibrosis and chronic cavitary aspergillosis, which can develop into aspergilloma (21). ABPA diagnosis is based mainly on the clinical features and immunological findings . ABPA mostly

affects patients with bronchial asthma and CF patients; in the latter a correct diagnosis is more difficult to make because the symptoms of the underlying disease the manifestations of ABPA overlap.

The minimum diagnostic criteria for ABPA in CF patients are (19):

a. subacute or acute clinical aggravation of the disease in absence of any other possible cause;
b. elevated total serum IgE levels of > 500 IU/mL, except in patients receiving corticosteroid treatment who need to repeat testing after completion of treatment. If the concentration of IgE is between 200 and 500 IU/mL, tests are repeated after one to three months;
c. immediate skin sensitivity to *A. fumigatus* antigens if the patient is not receiving antihistamine treatment or *in vitro* presence of serum IgE specific for *A. fumigatus;*
d. detection of precipitating antibodies or *in vitro* presence of serum IgG specific for *A. fumigatus,* or
e. presence of new or recent radiological irregularities in the thorax that persist despite antibiotic treatment or physiotherapy.

The fungus *A. fumigatus* can be isolated from 60 % of CF patients, but the cultivation has no diagnostic significance.

ABPA is traditionally treated with corticosteroids, which are used in acute aggravation and as maintenance therapy to prevent progressive advancement of the damage to the lung tissue (19,22). Currently, apart from corticosteroid therapy, recombinant monoclonal antibodies (e.g. omalizumab) are being introduced to prevent IgE from binding to effector immune cells (22-24). Because this treatment option for ABPA has only been used for a few years, all the pros and cons of this treatment remain unknown. Apart from the aforementioned groups of medication used to treat ABPA, various antifungal drugs are also used, lessening the load of aspergilli and thus reducing episodes of exacerbation and improving the pulmonary function (22,25).

CF patients also exhibit the so-called allergic sensitisation (AS), which is still poorly defined. This term is used to describe hypersensitity reactions which do not meet all of the criteria necessary for making a diagnosis of ABPA (17). AS supposedly affects between 20 and 65 % of CF patients (16). The problems of establishing the prevalence of AS lie in its overlapping clinical features with ABPA and the use of different diagnostic microbiological methods. Another problem is poor comparability of commercially available tests and in-house tests (16). Recently, differences have been identified that might enable a relatively simple differentiation between ABPA and AS based on the determination of CDC203c basophils concentration, galactomannan antigen in lower respiratory tract samples and pecific IgG in serum. In contrast with ABPA patients, patients with AS have a lower plasma concentration of CDC203c basophils, a negative galactomannan test (< 0.5) and a positive *A.fumigatus-specific* serum IgG (< 75µg/mL) (13,16,27).

In addition to colonisation and hypersensitivity reactions, fungi also cause infections that can affect several organ systems. They are very aggressive and associated with high mortality, but are fortunately relatively rare. Unlike hypersensitivity reactions, invasive mycoses normally affect immunocompromised patients. The most significant risk factor for the development of invasive mycoses

in CF patients is lung transplantation and the accompanying immunosuppressive treatment.

The most common infection is invasive pulmonary aspergillosis (IPA) and only rarely disseminated invasive aspergillosis (8). The most significant risk factors for the development of IPA in CF patients are colonisation of the respiratory tract with *A. fumigatus* before transplant surgery and a positive intraoperative culture (28,29). Post-transplant CF patients are exposed to an increased risk for IPA as a large proportion of patients are colonised with *A. fumigatus* (up to 57 %) even before transplantation (15,29). The occurrence of the infection is enabled by different virulence factors, especially hydrolytic enzymes (elastases, proteases, phospholipases, catalase) and gliotoxin, which further weaken the functioning of the mucociliary escalator, damage the respiratory epithelium and participate in the formation of biofilms (30,31).

IPA usually occurs approximately a month and a half after lung transplantation and affects about one fifth of the patients (29). The infection starts in the lower respiratory tract with invasion of hyphae into the lung parenchyma. Typical of the fungus *A. fumigatus* is angioinvasion, resulting in thrombosis, infarction and necrosis of lung tissue (12). CF patients also exhibit a non-angioinvasive type of IPA, which can occur in the absence of neutropenia (6). IPA is associated with a relatively high mortality (13), which in the case of CF normally does not exceed 16 % (29). Diagnosing IPA is difficult; it demands the consideration of clinical features, and the use of diagnostic imaging – especially CT scans of the thorax – and microbiological methods, such as the cultivation of bronchoscopic samples, detection of galactomannan and β-D-glucan antigenaemia and detection of aspergillus DNA in the affected tissues, bronchoalveolar lavage and blood. The gold standard for diagnosis remains the detection of aspergilli in the primarily sterile clinical sample with cultivation and histopathological evidence of the invasion in the affected tissue (32).

Voriconazole is the drug of choice in CF patients with IPA. When voricona-

Table 2: Summary of the roles of different species of the *Aspergillus* genus in CF patients

Infection, colonisation with *Aspergillus* spp.	**Colonisation**	**Hypersensitivity reactions**	**Infections**
Risk factors in case of CF	Age above 18, inhalation treatment with antibiotics, FEV_1 reduction	Long-term exposure to fungal allergens	Lung transplant immunosuppression, uncontrolled/untreated ABPA, colonisation of respiratory organs with aspergilli before a lung transplant
Clinical significance	Long-term colonisation as a risk for the development of hypersensitivity reactions and infections	ABPA, AS	Invasive (pulmonary) aspergillosis, chronic cavitary aspergillosis, aspergilloma, aspergillus bronchitis
Medication of choice	/	Corticosteroids, recombinant monoclonal antibodies, triazoles (voriconazole or itraconazole) (10,11,22)	Voriconazole, amphotericin B (10,11)

Key: ABPA – allergic bronchopulmonary aspergillosis, AS – allergic sensitisation, FEV_1 – forced expiratory volume in one second

zole is not the optimal choice, amphotericin B is recommended (10,11).

In CF patients, unlike in any other patient group, the fungus *A. fumigatus* creates unique clinical features, referred to as aspergillus bronchitis. It was first described in 2006 and defined six years later (33,34). Aspergillus bronchitis is described as chronic inflammation of the lower respiratory tract (bronchitis) with *A. fumigatus* confirmed in the culture of bronchoscopic samples or detected with molecular methods, and the presence of increased specific IgG in the serum (31,33,34). The most significant factor that helps to differentiate between colonisation and aspergillus bronchitis is clinical improvement in a patient receiving antifungal treatment; the medication proves ineffective in *A.fumigatus* colonisation. The value of antifungal treatment in these cases is not yet well defined, but antifungals from the group of triazoles, especially itraconazole and voriconazole, should be considered (33,34).

Exophiala dermatitidis

Among the fungi often associated with the colonisation of the respiratory tract in CF patientswe also find the fungus *Exophiala dermatitidis*, which the general public usually refers to as »black yeast«. It is a fungus with a melaninated cell wall, which appears in yeast form as a young culture and becomes increasingly filamentous as it matures. In parts of the world with a moderate climate it is rarely isolated from nature; it is mostly associated with the human environment, such as spas, saunas and dishwashers. In short, it can be found in a warm and moist environment (35,36). We assume that the original natural habitat of *E. dermatitidis* is the tropical rainforest (35).

In medicine, *E. dermatitidis* is known as the cause of infections of the skin and subcutaneous tissue, especially in tropical climates. There are also rare documented cases of infections affecting the central nervous system (37). The role of *E. dermatitidis* in CF is still unknown. Table 3 summarises interactions between *E. dermatitidis* and a CF patient.

In Europe, *E. dermatitidis* is isolated from 5 to 19 % of respiratory samples; descriptions of supposed invasive infections are rare (38-41). None of the documented cases meet the criteria for a confirmed invasive mycosis – the described diagnoses are usually based on the isolation of *E. dermatitidis* from the sputum. Improvement was noted with antifungal treatment. The patients can be treated exclusively with amphotericin B or with a combination of amphotericin B and flucytosine. Treatment with itraconazole has proved to be the most efficient (36-

Table 3: Overview of the emerging fungal pathogens and their clinical significance in CF.

	Exophiala dermatitidis	*Scedosporium apiospermum*	*Rasamsonia argillacea*
Risk factors in case of CF	Age above 12, pancreatic insufficiency, colonisation with *A. fumigatus*	Unknown.	Unknown.
Clinical significance	Colonisation, invasive infections during immunosuppression	Colonisation, invasive infections during immunosuppression	Colonisation, invasive infections during immunosuppression
Treatment	Amphotericin B ± flucytosine, itraconazole (36-39)	Voricozanole, posacozanole (50-52)	Echinocandins? (54,56)

39). There are, however, reports of a long-term colonisation of the respiratory tract where even several months of itraconazole treatment did not eliminate from the sputum (42).

Most research indicates that *E. dermatitidis* has the role of a coloniser in CF and that the isolation from the respiratory tract normally does not indicate an infection. At the moment, the identified risk factors for the colonisation with *E. dermatitidis* are the age above 12, pancreatic insufficiency and the colonisation with *A. fumigatus* (40,43). It is interesting that the occurrence of *E. dermatitidis* drops after the age of 35 (41). The colonisation is supposedly also dependent on the patient's genotype: it was most common in patients with a mutation of the CFTR gene at position 508 on chromosome 7 (genotype ΔF508/ΔF508) (40). It is mostly a chronic colonisation, which can be established only after an extended incubation period on classic and/or specific mycological media with the addition of erythritol and chloramphenicol (40). For the isolation of *E. dermatitidis,* clinical samples have to be incubated for 5 to 7 days on average (43).

Fungi from the genus *Scedosporium*

Apart from *A. fumigatus, Scedosporium apiospermum* is another important filamentous fungus that causes invasive infections in CF patients; it causes extremely rapid, aggressive infections quite resistant to antifungal treatment. Table 3 summarises interactions between *S. apiospermum* and a CF patient.

Scedosporium spp. are filamentous ascomycetes found mainly in water and soil all around the world. Their changing taxonomy makes classification difficult: today, the species once belonging to genus *Scedosporium* are reclassified into the genera *Parascedosporium, Lomentospora, Petriella, Petriellopsis*, *Pseudallescheria* and, of course, *Scedosporium* (44). Consequently, the term *Pseudallescheria boydii/Scedosporium apiospermum* complex is usedto refer to most of the frequently isolated, medically relevant species. For easier understanding, we will discuss *S. apiospermum.* Globally speaking, *S. apiospermum* is the second most frequent mould isolated from respiratory samples in CF patients and is associated with chronic colonisation (45,46). The prevalence is between 5.7 and 10 % (47). Invasive scedosporiosis is very rare in CF patients and there have been only eight documented cases since 1996; unfortunately, all of them ended in death (47,48). Most of the patients had a previously detected colonisation with *S. apiospermum.* Therefore the isolation of this mould from the respiratory tract is a contraindication for lung transplantation (47). Apart from invasive infections, there have also been documented cases of allergic bronchopulmonary scedosporiosis (49).

Scedosporiosis is difficult to treat because the fungus is resistant to several antifungal drugs. *S. apiospermum* fungi are intrinsically resistant to amphotericin B and flucytosine, and usually react only to triazole treatment; voriconazole and posaconazole may be used (50). *In vitro* activity against *S. apiospermum* is also exhibited by the more recent isavuconazole (50). For now, voriconazole remains the drug of choice (52).

Rasamsonia argillacea

Rasamsonia argillacea is a filamentous ascomycete fungus. It belongs to the group of the so-called emerging pathogens as its significance in medicine has been researched only in the past few years. *R. argillacea* is a thermotolerant mould, which morphologically resem-

bles the genera of *Penicillium* and *Paecilomyces* (53,54). It is, therefore, often misidentified and its prevalence is thus probably underestimated. 2.6 % of CF patients are supposedly colonised (55). Table 3 summarises interactions between *R. argillacea* and a CF patient.

R. argillacea is most frequently isolated from respiratory samples in CF patients and in patients with chronic granulomatous disease (54). Similarly to *E. dermatitidis, R. argillacea* is isolated using dithiothreitol liquefaction of viscous clinical samples from the lower respiratory tract (43). *R. argillacea* forms a complex of related, morphologically inseparable species, among which the most common are *R. argillacea sensu stricto*, *R. piperina* and *R. aegroticola* (55). The clinical significance of the isolation of species from the *R. argillacea* complex in CF patients is still unknown, but it probably just indicates colonisation of the respiratory epithelium (54,56).

The correct identification of moulds is not only important to obtain epidemiologica information, but is also necessary to choose the correct antifungals in the event of an infection. It is typical for the *R. argillacea* fungus to exhibit high minimal inhibitory concentrations for amphotericin B and antifungals from the azole group, especially voriconazole, while the minimal inhibitory concentrations for the echinocandin group are low (54,56).

Conclusion

People are constantly in contact with fungi as these are normally present in our environment. In CF patients the inhaled fungi are not expelled from the lower respiratory tract because of the impaired clearance function, which enables their growth and leads to different clinical conditions. These usually include hypersensitivity reactions because a normally functioning immune system prevents fungal invasion. The latter can occur after lung transplantation due to immunosuppressive treatment, the most common mycoses being invasive pulmonary aspergillosis and scedosporiosis. The diagnosis and treatment of invasive mycoses are very difficult and demand close cooperation between clinicians and microbiologists. Further research will improve the understanding of infections caused by fungi, which are relatively well-known in medicine, such as *Candida*, *Aspergillus* and *Scedosporium*. At the same time, we will obtain more information on the role of *E. dermatitidis* and *R. argillacea,* the two relatively unknown species compared to candida and aspergilli.

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