

**First Meeting of the ISHAM Working Group
"Fungal respiratory infections in Cystic Fibrosis",
June 7th – 8th 2009 in Angers, France**

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Cystic fibrosis (CF) is the most common genetic inherited disease in the European Caucasian population, and by the number of patients the third orphan disease (at least in France). Based on the UK Cystic Fibrosis Survey, the incidence of this autosomal recessive disorder was evaluated in 1997 being one in 2415 live births (Dodge *et al.*, 1997). The disease is due to mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*), located in chromosome 7, which encodes a chloride channel involved in electrolytic exchanges through the plasma membranes. Regarding the lungs which are the main target organ in this disease, these mutations result in a defective mucociliary clearance and a thickening of the bronchial mucus which are responsible for the entrapment of numerous microorganisms, mainly bacteria (such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Stenotrophomonas maltophilia*). Later in the evolution of the disease, the respiratory tract may also be colonized by various fungal species, including some *Candida* species, especially *C. albicans*, as well as various filamentous fungi and *Pneumocystis jirovecii*. However, our knowledge about the epidemiology, the physiopathology and the clinical relevance of the airway colonisation by fungi in patients with CF is scarce. To collect available data about these topics, the first meeting of the ISHAM Working Group on "Fungal respiratory infections in Cystic Fibrosis" was held at the Faculty of Pharmaceutical Sciences of the University of Angers, France on June 7th – 8th, 2009. Altogether 66 clinicians, mycologists or scientists from 14 different countries participated to the meeting, with numerous PhD students guarantee of dynamism and new synergies. Besides the introduction by the organizer Jean-Philippe Bouchara, 38 oral presentations were given by the attendants about "clinical surveillance and treatment" (13 presentations), "unusual fungal pathogens in cystic fibrosis" (7 presentations), "physiopathology" (5 presentations), "epidemiological and environmental studies" (5 presentations), and "diagnosis" (8 presentations).

Clinical surveillance and treatment. Results from several single or multi-centre studies presented the isolation rates of different fungi from respiratory samples of CF patients from

France, Spain, Italy, Denmark, UK, Australia and Brazil. The main fungal genera or species of interest varied between the studies, but results were not fully comparable because of variability in the methods used. Study duration ranged between 6 months and up to 12 years, but most studies were performed over a period of two years, some of them are still ongoing. Between 54 and 302 adult and paediatric patients were included in the studies with examination of 208 to 8552 respiratory samples, mostly sputum. In all studies, *A. fumigatus* was the most common filamentous fungus with isolation rates ranging from 11.1% (Spain) to 81% (Denmark). Isolation rate of other *Aspergillus* species was highest for *A. flavus* (25%) in the Italian study, but it was significantly lower in all other studies (5% in Denmark, 2.4% in the UK, or lower). Comparing isolation rates from adult and paediatric CF patients in Spain, *A. fumigatus* was more frequent in children than in adults whereas *A. flavus* was more frequent in adult than in paediatric patients. Other *Aspergillus* species isolated were *A. niger*, *A. terreus*, *A. nidulans* and *A. versicolor*, all with isolation rates lower than 5%. Invasive fungal infections during these studies were only summarised from Italy, where three patients were infected by *A. fumigatus* and one patient by *A. flavus*.

In some studies, *in vitro* antifungal susceptibility testing was performed. *Aspergillus fumigatus* strains with decreased azole sensitivity was recognised in France (10-15%) and in Denmark (~ 2.5%). However, compared to the isolates from non-CF patients, no increase in azole resistance was found in the UK.

Identification of the isolates from the section *Fumigati* was mainly based on morphological characteristics and on taxonomy in use until 2005. Nevertheless, in 2005, Balajee described a new species, *Aspergillus lentulus*, morphologically very close to *A. fumigatus*, but resistant to antifungal drugs. The same year, two new species were described: *A. fumigatiaffinis* and *A. novofumigatus* (Hong *et al.*, 2005). In addition, in 2008 *A. turcosus* was described as a new species in the section *Fumigati* together with 3 new *Neosartorya* species (Hong *et al.*, 2008). The differentiation of these new species based on morphological characteristics is difficult and sometimes not possible. However, for concrete recognition of the relevance of these fungi in CF patients, correct identification at the species level is necessary. Furthermore, it may be important for the initiation of an appropriate antifungal treatment, some of these species being known to show resistance to amphotericin B or to some azole drugs.

Unusual fungal pathogens in cystic fibrosis. *Scedosporium apiospermum sensu lato* also colonizes the respiratory tract of CF patients, and it has been described as the second most frequent filamentous fungus in sputum samples from CF patients in France (Pihet *et al.* 2009). However, the clinical significance of its isolation is still unclear. Although it was usually responsible for a chronic colonization of the airways, most patients seem to have an asymptomatic carriage, while some suffer allergic bronchopulmonary disease and others develop serious invasive infections. A significant association between the isolation of *S. apiospermum* and allergic bronchopulmonary disease was mentioned in a French center. According to the recent taxonomic changes within the *Scedosporium* complex based on molecular techniques (Gilgado *et al.*, 2005, 2007, 2008), a re-evaluation of the incidence of the different species in CF patients is necessary. By the use of routine diagnostic methods, detection often fails, since these slow growing fungi are often overgrown by bacteria such *P. aeruginosa* and fungi such as *Candida* and *Aspergillus* species. Therefore, standardised detection methods should be developed and their use recommended in protocols and guidelines. The isolation rate of species of the *Scedosporium apiospermum* complex were 3.4% of 201 patients in France, 9% of 220 patients from Italy, 6% of 302 patients in Spain, 2.3% of 129 Danish patients, 11.9% of 42 patients in Germany, 11.6% of 69 patients in Australia and 5.8% from 218 patients in the UK. *Scedosporium aurantiacum* seemed to be highly prevalent in Australia (5.8%), like *S. prolificans* (5.8%) which was also reported in Germany, but less commonly (2.4%).

Several other fungi have been more or less frequently isolated from CF sputum, such as *Exophiala dermatitidis*, *Geosmithia argillacea* and *Pneumocystis jirovecii*. Isolation rates differed between geographical regions, but these differences may only be related to variations in the sensitivity of the detection methods used. Likewise, misidentification as *Penicillium* or *Paecilomyces* species was reported for *G. argillacea* which was responsible for a chronic colonization of the airways in several French CF patients. *In vitro* antifungal susceptibility testing revealed resistance to voriconazole (VRZ) and, for most of the isolates, to itraconazole, and variable susceptibility to amphotericin B and posaconazole. Conversely, all the isolates were susceptible to caspofungin. In addition, transient colonisation of the airways by unusual *Aspergillus* species in two French CF patients were reported. Morphology was similar to *A. fumigatus*, but none of them grew at 50°C. DNA sequencing allowed identification of the isolates as *Aspergillus lentulus* and *Neosartorya pseudofischeri*. High MIC values were seen for these isolates for the main antifungal drugs, particularly for VRZ.

The relevance of fungal colonisation in lung transplant (LTx) CF patients was discussed during the meeting, as well as special treatment options in CF patients considering the multifactorial care management, the immunosuppressive requirement of lung transplant, the frequent presence of gastro-oesophageal reflux disease, hepatic alterations and pharmacokinetics (PK) specificities of the CF underlying background.

Physiopathology. As a consequence of colonization by *Aspergillus* species, some of the patients develop an allergic bronchopulmonary aspergillosis (ABPA). ABPA is a common complication in patients with CF, with a reported prevalence of 4.4% in the US (U.S. Cystic Fibrosis Foundation Patient, 2006 Annual Data Report to the Center Directors 2007: Bethesda, MD). The diagnosis remains difficult and requires a combination of clinical, radiological, biological and mycological criteria. ABPA develops as a result of an immune mediated response to the presence of *Aspergillus* sp. in airway secretions, with T_H2 lymphocytes playing a central role (Knutsen and Slavin, 1998). Typically patients have elevated IgE levels directed against *Aspergillus* antigens as well as precipitating antibodies. This immune response triggers a destructive process in the airway with consequent significant morbidity. In a French study, ABPA was diagnosed in 18 of 201 patients examined (8.9%). In another French study, from 85 CF paediatrics, eight developed ABPA (9.1%), which was significantly associated with RhDNase therapy, sensitisation to *Alternaria* and *Candida*, and low body mass index (BMI). Multivariate analysis identified an independent association between low BMI and ABPA, and for the first time, between long-term azithromycin therapy and *Aspergillus* colonisation. This last correlation could result from the inhibitory effect of azithromycin on both the recruitment and the activation of neutrophils, which represent the first-line defences against *Aspergillus*. Furthermore, the colonization by *S. apiospermum* and development of ABPA-like signs and symptoms were shown to be closely associated in a French study. In addition, ABPA patients were more frequently colonised by *P. aeruginosa* than non-ABPA CF patients. Treatment of ABPA by IgE blockade using Omalizumab was summarised, suggesting that it has a potential role as adjuvant therapy to control ABPA in corticosteroid-dependent CF patients. To get more concrete data, a randomized, placebo controlled and double-blinded therapeutic study was initiated in several sites in Europe and the U.S.A.

Biofilm production is known for several bacteria and some *Candida* species. The extracellular matrix (ECM) of the biofilm can protect the microorganism against host defences and anti-infective agents. It was demonstrated that *A. fumigatus* can persist in the CF respiratory tract for years and is able to form a biofilm structure *in vitro* on bronchial epithelial cells. Data on the extracellular matrix of *A. fumigatus* and its interactions with *P. aeruginosa* were additionally presented. A study is planned, in which the molecules involved in the interaction between *P. aeruginosa* on *A. fumigatus* *in vitro* in culture medium and on epithelial cell lines will be investigated, especially in conditions as given in CF patients by the use of *cftr*^{-/-} mice. Other results presented reflected the influence on melanin synthesis, as well as differences in fungal host related interactions and immune response and the inhibitory effects of fluvastatin on systemic and local inflammation.

Epidemiological and environmental studies. New methods were presented which could be helpful for epidemiological or environmental studies, especially solid-phase cytometry,

genotyping by analysis of Variable Number of Short Tandem Repeats (VNTR), Multilocus Sequence Typing (MLST) and the SELDI-TOF mass spectrometry.

For detection of pseudallescheriosis and scedosporiosis, IgM and IgG1 k-light chain monoclonal antibodies (MAbs) specific to *P. boydii* and certain closely related fungi were developed and first data presented. The occurrence of *Scedosporium* species in the environment of Australia, Germany, Thailand, Israel and Italy was estimated in several studies. Besides the predominant species *S. apiospermum* and *P. boydii*, *S. aurantiacum*, *S. dehoogii*, *P. minutispora*, and *S. prolificans* have been found in some environmental samples. In Australia, surveys revealed a high prevalence of *S. aurantiacum* in urban areas.

Biological diagnosis. For the optimisation of the microbiological diagnosis in CF patients several new methods were presented, such as specific media and molecular techniques, but also serological tests. Particularly, specific IgE to recombinant *A. fumigatus* antigens Asp f1, f2, f3, f4, and f6 proved to be useful tools for the early detection of sensitisation and the diagnosis of ABPA, especially during an early phase, when clinical symptoms are lacking. The recombinant antigen rAspf4, was shown to have a sensitivity of 80% for ABPA detection.

Data presented during this meeting confirmed that routine methods used for fungal colonisation and infection surveillance of the respiratory tract of CF patients differ from country to country and also from lab to lab in the same country. Therefore, it is difficult to compare the data. This problem became even more complicated, since several genera and species were re-classified during the last years resulting in splitting of formerly one species into up to five new described species, particularly on a molecular basis. This is the case for the *Aspergillus* from the Section *Fumigati*, the *Scedosporium apiospermum* complex and also for the yeast species *Candida parapsilosis* and *C. glabrata*. For optimal monitoring of CF patients correct species identification is necessary, because species of a complex may differ in their ecology or virulence, but also in their susceptibility to antifungal drugs. Further studies are needed to define reliable methods allowing an easy and accurate differentiation of these new species, and to define guidelines for mycological examination of respiratory secretions of CF patients.

Conclusion. As a result of this meeting, you will find the updated list of members of the working group, together with the program of the meeting, the abstracts of the talks and the list of attendants, as well as the pdf-files of nearly all presentations.

Nearly all participants agreed to specify in future publications related to fungal respiratory infections in cystic fibrosis the following sentence as a footnote of the first page: “* Author(s) is/are member(s) of the ISHAM working group on Fungal respiratory infections in Cystic Fibrosis”.

Several collaborative studies were discussed, aiming to evaluate the incidence of particular fungal species (like *Pneumocystis jirovecii*), to get a better knowledge on the epidemiology of *Aspergillus fumigatus* or *Scedosporium* species, or to evaluate the usefulness of some culture media or serological methods. Members of the working group will receive a newsletter listing these proposals.

Likewise, more co-operation is planned with this ISHAM working group and the European Society on Cystic Fibrosis (ESCF) with links from one website to the other, and a special session on fungal infections in CF patients during the next ESCF congress (2010, Valencia, Spain) will be discussed with the organizers of this conference. For example, a quality control regarding the diagnosis of fungi from sputum samples was discussed, similar to the already existing quality control for detection and identification of bacteria which is organized by the ESCF.

A second meeting of the working group was approved by the attendants. As the next ISHAM congress will be held in 2012, the 2nd meeting of the working group should take place in 2011. Then meetings should be held in a three-year cycle.

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