

First Meeting of the ISHAM Working Group on "Fungal respiratory infections in Cystic Fibrosis"

Angers (France), 2009, June 7th - 8th
Faculty of Pharmaceutical Sciences



Organizing Committee:

Jean-Philippe Bouchara, Dominique Chabasse, Gérald Larcher, Raymond Robert,
Laurence Delhaès and Françoise Symoens

Our thanks for their financial support to :

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Program

Sunday June 7th

Greeting participants from 13 h 30 to 14 h 00

14 h 00 - 15 h 30: Clinical surveillance and treatment

- 14 h 00 Introduction talk. **Jean-Philippe Bouchara** on behalf the Host-Pathogen Interaction Study Group.
- 14 h 15: *Aspergillus fumigatus* and cystic fibrosis: a 12-year observationnal cohort. **Judith Fillaux**, François Brémont, Sophie Cassaing, Marlène Murriss, Marie-Denise Linas, Marie-Hélène Bessières, Jean-Luc Rittié, Laurent Tétu, Christine Segonds, Eric Bieth, Michel Abbal, Antoine Berry, Bernard Pipy and Jean-François Magnaval.
- 14 h 30: Characteristics and consequences of fungal respiratory colonization of 201 adults patients with cystic fibrosis (CF). Elise Sauter, Dominique Hubert, Marie-Thérèse Baixench and **André Paugam**.
- 14 h 45: Evaluation and managment of fungal risk in cystic fibrosis : first results of a national French study. **Laurence Delhaes**, Emilie Fréalle, Yolande Lemeille, Bérangère Coltey, Gilles Gargala, Stéphane Dominique, Isabelle Accoceberry, Philippe Domblides, Isabelle Durand-Joly, Guy-André Loeuille, Odile Vagner, Frédérick Dalle, Anne-Lyse Fanton, Amal Boldron, Claudine Pinel, Cathy Llerena, Marc Pihet, Jean-Louis Giniès, Jean-Philippe Bouchara, Christine Person, Nathalie Wizla, Loïc Favennec, Christophe Marguet, Stéphanie Bui and Sylvie Leroy.
- 15 h 00: Risk factors associated with bronchial colonisation or ABPA with *Aspergillus* spp. from the experience of the cystic fibrosis reference centres in Marseille, France. **Stéphane Ranque**, Virginie Jubin, Annie Michel-Nguyen, Nathalie Stremler-Lebel, Jean-Christophe Dubus, Martine Reynaud-Gaubert and Renaud Piarroux.
- 15 h 15: Multicenter study on isolation procedure, species identification and clinical significance of *Aspergillus* spp., *Scedosporium* spp., and other filamentous fungi in italian patients with cystic fibrosis : preliminary results. **Graziana Manno*** and the Italian Working Group for Cystic Fibrosis Microbiology of the Italian Society for the Study of Cystic Fibrosis (SIFC).

15 h 30 - 16 h 00: Coffee break.

16 h 00 - 18 h 00: Clinical surveillance and treatment

- 16 h 00: Prevalence and clinical significance of fungi in respiratory secretions of patients with cystic fibrosis followed at a universitary hospital in São Paulo, Brazil. **Ilma Aparecida Paschoal**, José Dirceu Ribeiro and Carlos Emílio Levy.
- 16 h 15: The identity and antifungal susceptibilities of filamentous fungi isolated from CF patients (2006-2009) at the UK Mycology Reference Laboratory. **Andrew M. Borman**, Michael D. Palmer, Christopher J. Linton and Elizabeth M. Johnson.

- 16 h 30: - *Aspergillus* spp. and other moulds in cystic fibrosis patients in Denmark. **Klaus Leth Mortensen**, Helle Krogh Johansen, Marianne Skov, Tatjana Pressler and Maiken Cavling Arendrup.
- 16 h 45: Fungal isolates in patients with Cystic Fibrosis: 14 years of experience in a single tertiary care hospital. **Javier Peman**.
- 17 h 00: Fungal infections in CF lung transplants from the Lung Transplant and Cystic Fibrosis Unit at the University Hospital La Fe in Valencia, Spain. **Amparo Sole**.
- 17 h 15: - Clinical value of *Aspergillus fumigatus* detection in sputum obtained from 84 patients with cystic fibrosis. **Jean-Pierre Gangneux**, Sylviane Chevrier, Fanny Giroux, Benoît Desrues, Chantal Belleguic, Claude Guiguen and Michel Roussey.
- 17 h 30: Pharmacological aspects of antifungal drugs management in cystic fibrosis transplantation. **Eliane M. Billaud**, Romain Guillemain, Maud Berge, Catherine Amrein, Sandrine Lefeuvre, Véronique Boussaud and Patrick Chevalier.
- 17 h 45: Does IgE blockade have a role in the treatment of ABPA? **Carlos E. Milla**.
- 18 h 00: Departure for the Musée des Beaux Arts (visit of the museum and diner)**

Monday, June 8th

8 h 00 - 9 h 45: Unusual fungal pathogens in cystic fibrosis

- 8 h 00: Transient colonization of the airways by unusual *Aspergillus* species in two cystic fibrosis patients. **Françoise Symoens**, Marc Pihet, Jacqueline Carrère, Hugues Beguin, Nicolas Degand, Laurent Mely and Jean-Philippe Bouchara.
- 8 h 15: *Scedosporium apiospermum* seroprevalence study in a large cohort of patients with cystic fibrosis in France. **Perrine Parize**, Sandrine Nail, Raymond Robert, Anne-Lise Bienvenu, Olivier Lortholary, Gabriel Bellon and Isabelle Durieu.
- 8 h 30: Detection of *Pseudallescheria* / *Scedosporium* species and *Exophiala dermatitidis* in the upper respiratory tract of patients with cystic fibrosis. **Regine Horre**, Rüdiger Siekmeier, Soo-Mi Reiffert, Elisabeth Müller, Sabine Nidermajer, Thomas Gröger, Norbert Schnitzler, Josef Zündorf, Michaela Lackner and Günter Marklein.
- 8 h 45: *Scedosporium aurantiacum* – the Australian perspective. **Wieland Meyer**, Azian Harun, Christopher Blyth, Felix Gilgado, Peter Middleton and Sharon Chen.
- 9 h 00: *Exophiala dermatitidis* in cystic fibrosis : prevalence, risk factors and clinical relevance. Anissa Leonard, Jacques Gigi, Françoise Symoens, Daniel Huang, Grégory Reychler, Teresinha Leal and **Patrick Lebecque**.
- 9 h 15: *Geosmithia argillacea*: an emerging agent of airway colonization in patients with cystic fibrosis? **Sandrine Giraud**, Marc Pihet, Bienvenue Razafimandiby, Jacqueline Carrère, Nicolas Degand, Laurent Mely, Loïc Favennec, Jean-Philippe Bouchara and Alphonse Calenda.
- 9 h 30: *Pneumocystis jirovecii* colonisation among cystic fibrosis patients. **Enrique J. Calderon**.

9 h 45 – 10 h 15: Coffee break

10 h 15 – 11 h 30: Physiopathology

- 10 h 15: *Aspergillus fumigatus*: its extracellular matrix and its interactions with *Pseudomonas aeruginosa*. **Anne Beauvais** and Viviane Balloy.
- 10 h 30: *Aspergillus* biofilm formation on polystyrene and cystic fibrosis bronchial epithelia. **Marc Seidler**, Stefanie Salvenmoser and Frank-Michael Müller.
- 10 h 45:- Proteomic approach of abnormal pigmented strains of *Aspergillus fumigatus* by SELDI-TOF spectrometry. **Claudine Pinel**, M. Arlotto, J.P. Issartel, F. Berger, Hervé Pelloux, Renée Grillot and Françoise Symoens.
- 11 h 00:- Antifungal drug susceptibility of *Aspergillus* spp. isolated from cystic fibrosis patients and immune responses against them. **Maria Simitopoulou**, Elpis Hatziaorou, Elpiniki Georgiadou, John N. Tsanakas and Emmanuel Roilides.
- 11 h 15: Decreased IL-8 secretion and expression by fluvastatin in primary human macrophages and in the whole blood from adult patients with cystic fibrosis. **Jean-Pierre Gangneux**, Stéphane Jouneau, Chantal Belleguic, Mélanie Bonizec, Jeanne Galaine, Graziella Brinchault, Benoît Desrues, and Corinne A. E. Martin-Chouly.

11 h 30 – 12 h 45: Epidemiological and environmental studies

- 11 h 30: VNTR typing and MLST for the epidemiological study of *Aspergillus fumigatus*. Lies M.E. Vanhee, Françoise Symoens, Mette D. Jacobsen, Hans J. Nelis and **Tom Coenye**.
- 11 h 45: Genotypic diversity and colonization patterns of *Aspergillus fumigatus* in patients with cystic fibrosis. **Corné H.W. Klaassen**, Hanneke A. de Valk, Jan-Bart Yntema, Alexandra Hebestreit, Marc Seidler, Gerhard Haase, Frank-Michael Müller and Jacques F.G.M. Meis.
- 12 h 00: Tracking the emerging human pathogen *Pseudallescheria boydii* by using highly specific monoclonal antibodies. **Christopher R. Thornton**.
- 12 h 15: Rapid quantification of human-pathogenic fungi in various samples using solid-phase cytometry. **Lies M. E. Vanhee**, Katrien Lagrou, Wouter Meersseman, Hans J. Nelis and Tom Coenye.
- 12 h 30: Keeping an eye on environmental sources for *Scedosporium* species. **Kathrin Tintelnot**, Elisabeth Antweiler, Wolfgang Altmann, Werner Pohl and M. Seibold.

12 h 45 – 14 h 00: Diner

14 h 00 – 15 h 15: Diagnosis

- 14 h 00: ABPA diagnosis in one brazilian center of cystic fibrosis patients: the clinical utility of IgE specific to recombinant *Aspergillus fumigatus* allergens. **Marina B. Almeida**, Maria Helena C. F. Bussamra and Joaquim C. Rodrigues.
- 14 h 15: ABPA in cystic fibrosis patients: value of biological markers. **Claudine Pinel**, Hélène Fricker-Hidalgo, Bérange Coltey, Catherine Llerena, Jean-Charles Renversez, Renée Grillot, Isabelle Pin and Hervé Pelloux.
- 15 h 00: Improving methods for identification of fungi in CF sputum. **Stuart Elborn**.
- 15 h 15: Microbial diversity in CF lung disease. **Geraint B. Rogers**, Mary P. Carroll and Kenneth D. Bruce.
- 15 h 30: A metagenomic approach for determining the microbiota associated with cystic fibrosis. **Vicente Friaza**, Carmen de la Horra, Luis Maiz, Javier Dapena, Rafael Cantón, Enrique Calderón and Rosa del Campo.

15 h 45: Pathogenesis of *Aspergillus* in cystic fibrosis - a role for molecular diagnostics?
Caroline Baxter, Kevin Webb, Andrew Jones and David Denning.

16 h 00: Identification of Herpotrichiellaceae using a barcode-like sequence of the ITS2.
Gerhard Haase, G. Heinrichs and G. Sybren de Hoog.

16 h 15: Methyl coprogen B, a new potential marker of colonization of the airways of CF patients by *Scedosporium apiospermum*. **Samuel Bertrand**, Gérald Larcher, Pascal Richomme, Olivier Duval and Jean-Philippe Bouchara.

16 h 30 – 17 h 00: Coffee break

17 h 00: Plenary discussion

18 h 00: Departure for the castle of Plessis-Bourrée (visit of the castle and diner).

ABSTRACTS

ASPERGILLUS FUMIGATUS AND CYSTIC FIBROSIS: A 12-YEAR OBSERVATIONAL COHORT

Judith Fillaux^{1,2}, François Brémont³, Sophie Cassaing^{1,2}, Marlène Murriss⁴, Marie-Denise Linas^{1,2}, Marie-Hélène Bessières^{1,2}, Jean-Luc Rittié³, Laurent Tétu⁴, Christine Segonds⁵, Eric Bieth⁶, Michel Abbal⁷, Antoine Berry^{1,2}, Bernard Pipy² and Jean-François Magnaval¹

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Objective: To determine the impact of the presence of *Aspergillus fumigatus* (*Af*) in sputum of cystic fibrosis patients on their force expiratory volume in the first second and to study the link between the presence of *Af* and the presence of *Pseudomonas aeruginosa* (*Pa*).

Design: Data were collected from computerized medical records, “MUCODOMEOS”, from January, 1995 to July 3, 2007. Demographic (age and sex) and medical (sputum production, forced expiratory volume in the first second and body mass index) data, microbiological findings (*Pa* and *Af*) and immune parameters (total serum IgE, specific anti IgE-*Af* and IgG-*Af* antibodies and precipitating antibodies to *Af*) were recorded prospectively. Patients groups were constituted according to the immune parameters and microbiological findings to define the different presentation of the *Aspergillus* disease : “ABPA susceptibility group” (positive anti IgE-*Af* AND precipitin-*Af* ≥ 3 lines AND IgEt ≥ 500 IU/mL), “Sensitized group” (non-“ABPA susceptibility group” AND positive anti IgE-*Af*), “Colonized group” (negative anti IgE-*Af* AND presence of *Af* in sputum or, absence of *Af* in sputum and total serum IgEt < 500 IU/mL and precipitin-*Af* ≥ 3 lines) and “Others” (excluded from the precedent groups). A bivariate analysis was performed. The distributions were displayed as median along with interquartile ranges (med [IQR]). Multivariate analysis was then carried out with FEV₁ as an outcome variable, and a multilevel mixed-effect regression model was set up.

Results: Two hundred and seventy one patients were evaluated 6314 times. The sex ratio was 1.2. The median age at the CF diagnosis date was 8.2 months [1.6 months-3.2 years]. 40 patients (14.7 %) belonged to the “ABPA susceptibility” group, 63 patients belonged to the “Sensitized” group and 39 to the “Colonized” group. 4826 sputum examinations were performed. Culture was positive for *Pa* in 38.8% and for *Af* in 29.7%. Ageing (OR=2.5 [2.2-2.7]), abnormal BMI -“underweight” (OR=9.3 [7.3-11.3]) or “overweight” (OR=10.31 [4.0-16.6])-, *Pa* colonization (OR=3.8 [4.1-16.6]), “Sensitized” group (OR=14.8 [5.4-24.2]) and “ABPA susceptibility” group (OR=18.5 [8.4-28.6]) were significantly and independently associated with FEV₁ worsening. Furthermore, “ABPA susceptibility” group presented a

higher risk of FEV₁ worsening than “Sensitized” group, idem for “Sensitized” compared to “Colonized” group and for “Colonized” compare to “Others” group (OR=6.14 [3.0-9.3]).

Patients with at least one positive *Pa* sputum presented more frequently with at least one positive *Af* sputum compared to those with negative *Pa* sputum (79.2% versus 37.5%, p<0.001). The “ABPA susceptibility” group presented more frequently with *Pa* colonization than the “Others” group (50.0% versus 15.0%, p=0.02). The colonization with *Pa* was not significantly associated either with the “Sensitized” group or with the “Colonized” group.

CHARACTERISTICS AND CONSEQUENCES OF FUNGAL RESPIRATORY COLONIZATION OF 201 ADULT PATIENTS WITH CYSTIC FIBROSIS (CF)

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Improvement in care of patients with CF has resulted in longer survival and a large proportion of patients now reach adulthood. These patients require an age-appropriate care. In this context we have studied the nature and the consequences of fungal respiratory colonisation in a large cohort of adult CF patients.

Frequency and characteristics of fungi isolated from respiratory samples were investigated by a retrospective study. Mycological data have been analysed for a two year period (2005-2006). The study population consisted of 201 patients (53 % male) aged 17 to 65 years (median 26). A total of 657 respiratory samples were collected. Positive fungal samples were observed in 94.5% (190/201) of the patients. During the 2 years of follow-up, 8.9% (18/201) were diagnosed with allergic bronchopulmonary aspergillosis (ABPA).

Filamentous fungi were isolated from 65.6% (132/201) of the patients: one type of filamentous fungus was isolated in 48.2% (97/201) of the patients and more than one species in 17.4% (35/201) of the patients. *Aspergillus fumigatus* was the predominant species in 56.7% (114/201) of the patients. Other *Aspergillus* species (*A. flavus*, *A. niger*, *A. nidulans*, *A. terreus*, and *A. versicolor*) were isolated in 9.4% (19/201) of the patients. *Scedosporium apiospermum* was isolated from 3.4% (7/201) of the patients. A high proportion of samples per patient was positive for *A. fumigatus* and *S. apiospermum*, (71 % and 58 % respectively) indicating a chronic colonization. On the contrary, other filamentous fungi were found only in 23% of the samples which may correspond to a transient colonization. *S. apiospermum* was significantly associated with ABPA (odds ratio = 13, [2- 80]) in a multivariate analysis.

Yeasts were isolated from 72.4% (152/210) of the patients. *Candida albicans* was the predominant species in 72.1% (145/201). The proportion of positive samples by patient was 66 % for *C. albicans* versus 27 % for non-*albicans* spp. As *Candida* spp is a commensal frequently isolated from the oropharynx we interpreted its presence in sputum as a digestive contamination of respiratory samples and not as a respiratory colonization.

A. fumigatus was the most frequently and continuously filamentous fungus isolated from respiratory samples of adult patients with CF. Apart from ABPA, the clinical consequences of the identification of filamentous fungi from respiratory secretions of adult CF patients remain unclear and need to be clarified by further prospective studies.

EVALUATION AND MANAGEMENT OF FUNGAL RISK IN CYSTIC FIBROSIS: FIRST RESULTS OF A NATIONAL FRENCH STUDY

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Background - Objective: Whilst influence induced by bacterial colonization in cystic fibrosis (CF) is established, risk induced by fungal colonization is less defined. Prevalence of *Aspergillus fumigatus*, as well as other species than *Aspergillus*, and factors associated with fungal presence are poorly documented. Our transversal prospective study aimed to determine which fungal species are present in sputum collected from CF patients, and which factors are associated with fungal presence.

Methods: The fungal risk was determined for each patient in a multidisciplinary way: (i) using clinical parameters (Shwachman Score, respiratory function,...), (ii) notifying therapeutics used (including antibiotics, corticosteroids, bronchodilators, and antifungal treatments), (iii) determining fungal presence in sputum using semi-selective growing media according to a unique and standardised protocol of Mycology, and (iv) analysing blood to document patient ABPA status.

Results: 300 CF patients have been included between 2007 and 2008 in our study and will be followed-up during the next 2 years. Among them, the majority were young adults with a median age of 28.1 years (from 17 to 69 years). The age average in our Paediatric population was 12.8 years old (from 6 to 21 years). *Aspergillus fumigatus* was found in about 30% of patients, with a decreased in vitro sensitivity to azoles in 10 to 15% cases. Correlation between fungal, clinical, environmental, therapeutic or microbiological data is evaluated.

Conclusion: Since fungal presence in CF appears frequent, influence of fungal presence on CF course will be characterised. In addition, the 2 years follow-up of our population will allow us to expect a better understanding of filamentous fungus role in CF course, and to establish if patients could benefit from antifungal treatments or preventive measures.

Key-words: Cystic fibrosis, fungal colonization, *Aspergillus*, Moulds, Serology

Acknowledgements: We thank members of the Clinical Investigation Center of Lille: Pr C Libersa, Dr D Delplanque, Sabrina Schilling, and Sarah Surmont, for their valuable help in clinical data collection. We are grateful to Dr F Richard and Julia Salleron (Public Health Department Research of Lille) for statistical analysis.

This work is supported by: PHRC 1902 - Vaincre la mucoviscidose - Pfizer®.

RISK FACTORS ASSOCIATED WITH BRONCHIAL COLONISATION OR ABPA WITH *ASPERGILLUS SPP.* FROM THE EXPERIENCE OF THE CYSTIC FIBROSIS REFERENCE CENTRES IN MARSEILLE, FRANCE

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The incidence of *Aspergillus* colonization (AC) and allergic bronchopulmonary aspergillosis (ABPA) has recently increased in patients with cystic fibrosis (CF). The reasons for this increase are unclear although a number of factors have been suggested to be involved. This study was set up to investigate the association between potential predisposing factors, including new therapies recommended in CF, and the occurrence of AC or ABPA in children with CF.

The medical records of 85 children monitored regularly in the Pediatric Reference Centre for Cystic Fibrosis Care (RCCFC) of the University Hospital of Marseille (France), were analyzed from the first time they attended the RCCFC until either the occurrence of an end event, or their last visit to the RCCFC. Risk factors for AC or ABPA were analyzed by univariate and multivariate logistic regression.

Eight children developed ABPA and 18 had AC. ABPA was significantly associated with RhDNase therapy, sensitization to *Alternaria* and *Candida*, and a low body mass index (BMI). Multivariate analysis identified an independent association between low BMI and ABPA (OR=10.6, 95%CI [2.25-1.8], p=0.004), and for the first time, between long-term azithromycin therapy and AC (OR=6.4, 95%CI [2.1-19.5], p=0.001). This latter 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*.

This comprehensive exploratory study has identified several risk factors associated with AC and ABPA in children with CF that now need to be confirmed in further prospective studies.

MULTICENTER STUDY ON ISOLATION PROCEDURE, SPECIES IDENTIFICATION AND CLINICAL SIGNIFICANCE OF *ASPERGILLUS* SPP., *SCEDOSPORIUM* SPP., AND OTHER FILAMENTOUS FUNGI IN ITALIAN PATIENTS WITH CYSTIC FIBROSIS: PRELIMINARY RESULTS.

Graziana Manno* and the **Italian Working Group for Cystic Fibrosis Microbiology** of the **Italian Society for the Study of Cystic Fibrosis (SIFC)**

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Different rate of Filamentous Fungi (FF) occurrence are described in Cystic Fibrosis (CF) patients (pts) in Europe (France, Germany, Spain, etc.) but there are very little information about prevalence and the clinical significance of FF in CF pts from Italy.

Geographic variation in FF occurrence are described, however the absence of standardised mycological diagnostic protocols specifically addressed for CF together with simple methods to identify the different species recovered, makes difficult to compare the prevalence of FF in pts attending different CF Care Centres as well the importance of these pathogens on clinical course of the colonised pts.

The Italian Working Group for CF Microbiology of the Italian Society for the Study of CF (SIFC) have started a national program with the aim to standardised the microbiological procedures for FF recovery and identification from CF specimens, in all Clinical Microbiology Laboratories working for the 39 CF Care Centres in Italy. The program includes the print and distribution of microbiological recommendation and protocols, training courses addressed for Mycology procedures in CF, regularly distribution of FF QC samples to laboratories and, finally, establish of some Reference Centres for FF identification in Italy.

Moreover a collaborative study between microbiologist and clinicians involved in the care of Pulmonary Infections in CF is ongoing.

Preliminary data about prevalence are available for 220 pts attending the CF Care Centre of G. Gaslini Institute in Genova: 35/220 (15,9 %) of pts where colonized by filamentous fungi. The species distribution was: *A. fumigatus* (64%), *A. flavus* (25%), *S. apiospermum* (9%) others (*Rhizopus oryzae*, *Sporotricum pruinosum*, *Cladosporium spp*) (2%). In 5 pts *A. fumigatus* (3 pts), *A. flavus* (1pts) and *S.pruinosum* (1 pt) recovering was correlated with invasive infection. These preliminary data confirm that fungal colonization is common in CF pts in Italy. This colonization may leads to clinical deterioration when allergy develops. The significant rate of *Aspergillus non-fumigatus* and *S. apiospermum* recovered, suggest that these species, resistant to antifungal drug, are increasing in CF. More study are needed in order to compare mycological results with the clinical outcome of colonized patient. Fungal infections was rare, but may be underdiagnosed, for the lack of standardized protocols available for clinical laboratories for mycological examination of airways sample in CF.

PREVALENCE AND CLINICAL SIGNIFICANCE OF FUNGI IN RESPIRATORY SECRETIONS OF PATIENTS WITH CYSTIC FIBROSIS FOLLOWED AT A UNIVERSITY HOSPITAL IN SÃO PAULO, BRAZIL.

Ilma Aparecida Paschoal, José Dirceu Ribeiro and Carlos Emílio Levy.

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A search of our hospital database (the Teaching Hospital of the Medical School of the State University of Campinas- UNICAMP) was carried out during 2003 and 2004 to identify all patients with chronic sputum production and suspected diagnosis of bronchiectasis who attended our Adult Pulmonary Outpatient Clinic. These patients are routinely investigated for Cystic Fibrosis (CF) immunodeficiency and ciliary motility disorders.

54 patients had CF (at least two elevated sweat chloride concentrations determined by the quantitative pilocarpine iontophoresis sweat test). 12 CF patients (22%) grew *Aspergillus* species in their sputum on at least one occasion; six patients had *Aspergillus fumigatus*, two had *Aspergillus niger* and four a non identified *Aspergillus*. Of these 12 patients, nine also had chronic *Pseudomonas* infection (evidence of positive sputum culture on two or more occasions, at least six months apart within a year) and nine had *S. aureus* (not exactly the same patients). *Stenotrophomonas maltophilia* was present in three patients: one with *Aspergillus fumigatus*, one with *Aspergillus niger* and one with *Aspergillus* sp.

Mean total serum IgE was $153,5 \text{ IU} \pm 167,2$ and the median value was 86 IU (range 9 IU-500 IU). None of the patients with *Aspergillus*-positive sputum had ABPA per consensus conference criteria, but three of them are dead by now and one received a double-lung transplantation.

Among 81 other patients with non-cystic fibrosis bronchiectasis (69 idiopathic bronchiectasis, 10 immotile cilia syndrome and one Young's Syndrome) 12 patients (16%) had positive sputum for *Aspergillus* (three patients *Aspergillus niger*, one patient *Aspergillus flavus*, one patient *Aspergillus fumigatus* and seven patients *Aspergillus* sp). Four of these patients were also chronically colonized with *Pseudomonas aeruginosa*. Mean total serum IgE in these patients was $331,8 \pm 738,4 \text{ IU}$ and the median was 117 (range 4,51 – 5030 IU). Three patients had total serum IgE greater than 1000 IU and fulfilled the criteria for the diagnosis of ABPA.

The prevalence of sputum positive cultures for *Aspergillus* in CF patients in our hospital seems to be similar to the reported prevalence in other countries. We did not find ABPA in our CF patients, but we were able to identify three ABPA cases in the population with non-CF bronchiectasis; in fact, this population had higher levels of total serum IgE than the CF patients.

Fungal colonization and/or sensitization may play a significant role in the progressive decline of pulmonary function in patients with bronchiectasis but we still do not know which signs of fungal “activity” are important to search in these patients.

THE IDENTITIES AND ANTIFUNGAL SUSCEPTIBILITIES OF FILAMENTOUS FUNGI ISOLATED FROM CF PATIENTS (2006-2009) AT THE UK MYCOLOGY REFERENCE LABORATORY

Andrew M. Borman, Michael D. Palmer, Christopher J. Linton and Elizabeth M. Johnson

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Bristol houses a major CF centre which serves patients from a large portion of the South-West UK. Sputum and BAL samples are first processed in the Bristol regional microbiology laboratory, which performs routine culture (but not microscopic examination of samples) and then refers a significant proportion of isolates to the Mycology Reference Laboratory (MRL) for formal identification and susceptibility testing. The MRL also identifies filamentous fungi isolated from CF patients nationwide and referred via the respective regional laboratories. Finally, the MRL also directly analyses sputum and BAL samples from those local CF patients of particular concern.

Here we present an epidemiological and mycological analysis of the filamentous fungi isolated from CF patients, predominantly from the Bristol CF centre. A total of 569 samples were analysed at the MRL from 218 patients (age range 1-54; average 18, median 21.1 years old). These correspond to 415 filamentous fungi submitted for identification +/- susceptibility testing, and 154 sputum/BAL samples (from 68 patients) which were examined microscopically and also cultured at the MRL. The relative prevalence and predominant filamentous fungi isolated from our cohort of CF patients were similar to those reported in various previous studies of CF populations worldwide, and included: *Aspergillus fumigatus* (74.6% of isolates); *Scedosporium apiospermum* (5.8%); *A. terreus* (3.2%); *Penicillium* sp (2.8%); *A. flavus* (2.4%); *Exophiala dermatitidis* (2.2%); *S. prolificans* (1.9%) and *Paceilomyces variotii* (1.7%). Indeed, *A. fumigatus* was isolated at least once from 67% of patients, followed by *S. apiospermum* (7.3% of patients), *Penicillium* sp (6.9% of patients), *A. terreus* (6.4%), *A. flavus* (5%), *E. dermatitidis* (3.2%) and *S. prolificans* (2.8%). A single fungal species was repeatedly isolated from 75 of the 111 patients with 3 or more positive cultures, while 25 and 10 patients harbored 2 and 3 species, respectively (with the fungal species isolated reflecting their general prevalence in the total population of CF patients examined in the study).

Almost 70% of the sputum/BAL samples submitted to the MRL contained no discernible filamentous fungal elements as judged by direct microscopic examination of fluorescently enhanced samples after centrifugation. However, filamentous fungi (with *A. fumigatus*, *A. terreus*, *A. flavus* and *S. apiospermum* again predominating) were cultured from almost 50% of these samples, including 29 /107 (27%) of the microscopy negative samples, raising the question as to whether culture alone is an accurate means of measuring colonisation in CF patients. Finally, the antifungal susceptibility profiles (MIC ranges, MIC₅₀ and MIC₉₀ values) measured at the MRL using the CLSI broth micro-dilution methodology were not significantly different between isolates from CF patients as opposed to non-CF patients for the majority of fungal species examined.

ASPERGILLUS SPP. AND OTHER MOULDS IN CYSTIC FIBROSIS PATIENTS IN DENMARK

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A six months survey was conducted in a joined collaboration between the Cystic Fibrosis Centre Copenhagen, Department of Clinical Microbiology at Rigshospitalet, Copenhagen and Mycology Unit at Statens Serum Institut in order to determine the incidence and susceptibility pattern of *Aspergillus* sp. from CF airway samples. CF airway samples received in July-December 2007 for routine microbiologic investigation was examined for growth of moulds following routine procedures with two to three days of incubation and an extended incubation with final examination day five.

Identification was performed by conventional criteria. We identified 294 mould isolates from 129 CF patients. *Aspergillus* sp. was isolated from 121 patients. Of the isolates 243 were *A. fumigatus* (81%), 15 *A. flavus* (5%), 15 *A. terreus* (5%), five *A. niger* and one *A. nidulans*. Thirteen patients had more than one *Aspergillus* sp. (10%) and five had *Aspergillus* sp. plus an other mould (4%). Of non-*Aspergillus* moulds we identified three each of *Scedopsporium* sp. and *Penicillium* sp., two each of *Paecilomyces* sp., *Scopulariopsis* sp. and *Trichoderma* sp., and one *Acremonium* sp. Two brown moulds were not identified.

Susceptibility testing following the EUCAST microbroth dilution method for azoles and e-test diffusion for amphotericin B was performed. Azole MICs were considered elevated if higher than 1 µg/ml for itraconazole and voriconazole and if higher than 0.25 µg/ml for posaconazole based on the recently described wildtype MIC distributions.¹ Repeated isolates obtained within 6 months excluded. Thus, 142 isolates was susceptibility tested. Five isolates had itraconazole MIC > 1 µg/ml (three *A. fumigatus*, one *A. niger*, one *A. terreus*. Two of the *A. fumigatus* isolates both had elevated posaconazole MIC of > 4 µg/ml and voriconazole MIC of > 4 and 2 µg/ml, respectively.

Isolates with elevated azole MIC to one or more azoles were thus detected in 4% of the isolates which were susceptibility tested and multi-azole resistant isolates were detected.

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FUNGAL ISOLATES IN PATIENTS WITH CYSTIC FIBROSIS: 14 YEARS OF EXPERIENCE IN A SINGLE TERTIARY CARE HOSPITAL

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Cystic fibrosis (CF) is an autosomal recessive disorder which results in dysfunction of the exocrine glands. Copious amounts of viscous respiratory mucus are secreted, which are difficult to clear and provide a breeding ground for microorganisms. The knowledge about the epidemiology of fungal colonization in CF patients is scarce. However, it has been reported that as the disease progresses, *Aspergillus fumigatus* is by far the filamentous fungus most frequently isolated from respiratory secretions of CF patients. In some series, nearly 60% of these patients were chronically colonized by it. We present our experience in a single tertiary care hospital (1156 beds) from January 1995 to May 2009. The results have been obtained from the microbiological data base of Cystic Fibrosis and Lung Transplant Units. Respiratory samples from sputum, bronchioalveolar lavage, bronchial aspirates or pharyngeal swab were sent to our laboratory as a result of a standard protocol or because of a medical request due to clinical suspicion of disease. Samples obtained from patients were routinely cultured on Sabouraud Agar for 7 days at 30 and 35 °C and fungal isolates were identified at the time of collection by their macroscopic and microscopic characteristics.

During the period of the study, isolates from a total of 302 patients (212 adults and 90 children less than 15 years-old) were collected. There were 82 adults and 20 children with a lung transplant. A total of 8552 respiratory samples were analyzed for fungal organisms; culture was positive in 5043 (59%) of them. Fungal isolates were more frequent in adult population (89% vs 73%). In both groups of patients, 66% of isolates were yeasts (mainly *Candida* spp). A total of 1697 filamentous fungi isolates were recovered, *Aspergillus* spp. was present in up to 91% of those. *Scedosporium* spp. was the second more frequent mould (6%). The species distribution of *Aspergillus* in the isolates was: *A. fumigatus* 63%, *A. flavus* 16%, *A. niger* 9% and *A. terreus* 8%. Isolation of *A. fumigatus* was more frequent in children (72%) than in adults (60%); in contrast *A. flavus* was more common in adults (18% vs 10%). In the samples where *Scedosporium* spp was isolated, *S. apiospermum* constituted 74% of them.

ASPERGILLUS INFECTIONS IN CYSTIC FIBROSIS LUNG TRANSPLANT RECIPIENTS

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Lung transplantation (LT) is recognized as a highly effective treatment for patients with end-stage cystic fibrosis (CF). This procedure has an excellent outcome, despite many potential problems of CF patients, and the high incidence of bacterial and fungal colonization prior LT. *Aspergillus* species are isolate before transplantation in 10–30% of patients with CF. Their presence does not influence outcome though indicate the need for prophylaxis. *Aspergillus* infections may manifest in CF lung transplantation recipients in four different forms: colonization, tracheobronchitis/anastomotic infections, invasive pulmonary / disseminated aspergillosis, and ABPA. The incidence of *Aspergillus* infection ranges from 6 to 16%. These wide ranges translate to the differences in definition criteria for *Aspergillus* infection, immunosuppressive therapy and antifungal prophylaxis existing in each Lung Transplant Programme. *Aspergillus* colonization usually occurs in almost 30% of patients during the first 6 months after transplantation and it is considered a risk factor for the development of anastomotic infections and invasive pulmonary aspergillosis (IPA). In relation to airways lesions, *Aspergillus* has a propensity for invading bronchial anastomoses, which leads to several degrees of endobronchial complications in up to 18% of CF the patients. Although the early mortality of patients with bronchial anastomotic infection does not differ significantly from that of patients without these infections. The incidence of IPA is less than 5% in CF LT receptors. Time of onset differs for various types of *Aspergillus* infection; IPA and disseminated aspergillosis occur significantly later than tracheobronchitis. Of the *Aspergillus* infections occurring within 3 months of transplantation, 75% are tracheobronchitis or bronchial anastomotic infections, 18% are invasive pulmonary infections and 7% are disseminated invasive infections. Nowadays, nearly one half of the invasive aspergillosis cases in transplant recipients are late-occurring. In fact, in our experience, the majority of invasive forms are late onset. These data have relevant implications for prophylactic strategies. With respect to mortality, whereas mortality rate is lower in patients with bronchial anastomotic infections, for patients with invasive aspergillosis it raises to 80%. Risk factors in CF for *Aspergillus* infection are well known: colonization prior to or after transplant, cytomegalovirus (CMV) infection, chronic rejection, renal insufficiency and the type of antifungal prophylaxis. Additionally, some studies have shown a direct relation between the use of some immunosuppressive drugs and invasive mycoses. Recently, risk factors from fungal infection during the first year after primary paediatric lung transplantation have been analysed.

The ABPA has been described in isolated case reports as an anecdotic and rare event that occurs in only CF LT recipients.

Regards to other filamentous fungal infections, there are few firm data on the impact of fungi such as *Scedosporium* on outcomes, though there are several short reports indicating difficulties in managing such infections post transplantation. In fact, invasive infections caused by *S. apiospermum* have been reported mainly in unilateral lung transplantation recipients and cystic fibrosis (CF) transplant patients. Early and accurate diagnosis is essential because these fungi can be confused with amphotericin B (AmB)- sensitive moulds. Colonization by *Scedosporium* in transplant recipients should not be ignored and prophylaxis or suppressive therapy should be considered in all cases.

CF lung transplant recipients have good outcomes after lung transplantation compared with those of other lung transplant recipients, and quality of life is dramatically improved. However, they are still prone to common complications including primary and chronic graft dysfunction, a variety of infections including fungal infections, and renal failure.

CLINICAL VALUE OF *ASPERGILLUS* DETECTION IN SPUTUM OBTAINED FROM 84 PATIENTS WITH CYSTIC FIBROSIS

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Aspergillus spp are filamentous fungi which colonize the respiratory tract of patients with cystic fibrosis (CF). As a consequence of colonization, sensitization to *Aspergillus* can induce allergic bronchopulmonary aspergillosis (ABPA). The objective of this study was to evaluate the clinical value of *Aspergillus* detection in the management of patients with CF.

Two hundred and eight sputum obtained from 84 patients with CF from the 2 CF Centers (pediatric and adult) of CHU, Rennes Brittany, France, were analyzed during a six month-prospective study. *Aspergillus* detection was performed both by mycological culture (Sabouraud Agar medium) and by quantitative PCR (ABI Prism 7000, Applied Biosystem) using 2 sets of primers targeting the rRNA 5.8s and *A. fumigatus* mitochondrial RNA.

Among the population of patients without ABPA, 51% were colonized by *A. fumigatus*, either by classic mycological culture or by PCR. Beside, 50% of patients with classic ABPA diagnostic criteria (clinical findings, immediate hypersensitivity skin test for *A. fumigatus*, total and *A. fumigatus* specific serum IgE, anti-*Aspergillus* precipitins, eosinophil count) showed a positive detection of *Aspergillus* positive. The correlation between mycological culture and quantitative PCR reached 91.8%. A lower sensitivity of mycological culture was observed in patients with ABPA receiving an antifungal treatment. *Aspergillus* detection in the sputum of patients suffering from CF without ABPA is a useful and non-invasive tool for the early characterization of patients at risk for sensitization. During ABPA, *Aspergillus* detection is an obvious marker of antifungal treatment failure when it is instituted and must incite practitioner to investigate several causes of failure.

PHARMACOLOGICAL ASPECTS OF ANTIFUNGAL DRUGS MANAGEMENT IN CYSTIC FIBROSIS TRANSPLANTATION

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Cystic fibrosis (CF) lung transplantation (LTx) is associated with **multi-factorial care management**, due to the immunosuppressive requirement of lung transplant, the high risk of infections, the frequent presence of GERD gastro-oesophageal reflux disease, hepatic alterations and pharmacokinetics (PK) specificities of the CF underlying background. CF patients are characterised by bioavailability changes due to decreased absorption, specially in case of bile-dependant drugs and increased clearances, enhanced by potential age related changes in pediatric context, and changes in distribution volumes, resulting in an important **pharmacokinetic variability** favoured by the frequent rate of lagtime in drug absorption.

The need for higher dosage are mostly representative of the lean body mass index but are partly balanced by the relatively low body weight (45 kg) of this population when expressed as total daily dose.

CF is associated with frequent fungi colonisation, specially aspergillosis (*A. fumigatus*) and subsequently prolonged post-transplantation antifungal treatment.

Antifungal therapeutic arsenal offers today several alternatives :

- the systemic reference parenteral amphotericin B. This drug is still nephrotoxic, despite the less toxic liposomal formulations, and therefore should be avoided when other alternatives are available
- the recent echinocandines class is well tolerated. Special caution is needed in case of severe hepatic insufficiency. Documentation of therapeutic drug level could be addressed in case of therapeutic failure
- azole drugs (itraconazole ITZ, voriconazole VRZ and posaconazole PSZ) are available for long-term oral therapy.

Azole profile is mainly hepatic, both due to a metabolic elimination pathway and a safety pattern of hepatotoxicity and PK metabolic **drug- drug interaction (DDI) as CYP3A4 metabolic inhibitors**.

Such DDI targets are numerous but **immunosuppressive drugs** (calcineurin inhibitors CNI and mTOR inhibitors SPI) are of major concern, justifying a joint therapeutic drug monitoring (TDM) of both azole (metabolic inhibitors) and immunosuppressants (targets) on an individualised patient basis to adjust the coprescription. ITZ is acting as a major inhibitor, possibly enhanced in case of high drug levels and VRZ and PSZ as moderate ones. SPI

concentration deviation are potentially higher as compared to those recorded with CNI, justifying a contra-indication between VRZ and sirolimus (SRL), whereas everolimus (RAD) DDI management is achievable under close monitoring. The most clinically relevant are tacrolimus (TRL) DDI, due to specific toxicity and the risks linked to excessive immunosuppression.

VRZ variability was higher than expected, including IV route, specially in CFLTx. VRZ is limited by both hepatotoxicity and neurotoxicity, even without obvious overdose in CFLTx receiving TRL and steroids (trough C0 VRZ concentration 2-4 mg/L). VRZ can be also a target for CYP3A4 interactions.

Target concentration range for C0 azole levels are > 0.5 mg referenced to MIC species, between 1-2 mg/L referenced to pivotal clinical trials PK studies, but difficult to reach in case of PSZ due to saturable absorption.

Fluconazole (FCZ), used in case of candidosis exhibits a DDI profile of metabolic inhibitor less severe than the 3 others, but renal failure frequent in LTx as a toxic effect of CNI must be taken in account.

The risk of long under-dosed period, frequently addressed in this population, could justify on a PK basis the need for **combination** with an exclusive parenteral antifungal waiting for azole relevant drug level.

The high PK variability, the risk of underdosed periods, therapeutic issues and DDI management in this complex underlying disease justify a close monitoring with **regular TDM** in case of azole administration together with immunosuppressive drugs in CFLTx.

DOES IGE BLOCKADE HAVE A ROLE IN THE TREATMENT OF ABPA?

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Allergic Bronchopulmonary Aspergillosis (ABPA) is a common complication in patients with CF, with a reported prevalence of 4.4% in the US [1]. 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 [2]. 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. The colonization of the airways of CF patients with *Aspergillus*, probably a consequence of disrupted mucociliary clearance, leads to heavy allergen load on the epithelial surface. This in turn will induce a strong allergic inflammatory response through activation and proliferation of the bronchoalveolar lymphoid tissue (BALT). Immunoblasts in the BALT produce large amounts of IgE directed against specific *Aspergillus* proteins [3]. For reasons that probably include individual genetic susceptibility, affected patients mount a hyperIgE response that is more noticeable in the acute flares of the disease. Thus the total serum IgE can also be used as monitoring parameter for disease activity [4]. Given the allergic inflammatory nature of this disease, systemic corticosteroids are considered the mainstay of therapy and many patients required prolonged treatment courses to control the disease [5]. The use of antifungal therapy has been recommended as a mean to decrease the fungal burden in the airway. However the evidence for its use in CF is limited to short case series [6]. The current recommended standard treatment is with the use of systemic corticosteroids and for prolonged periods of time [7]. However this has the potential to induce significant detrimental side effects in children, particularly in the context of CF [8]. Omalizumab is a humanized monoclonal antibody directed against IgE. Once complexed to circulating IgE it prevents binding to high- and low- affinity receptors on effector cells [9]. It has been shown to be effective in improving asthma control in patients with a strong allergic component [10, 11]. The experience with asthmatic patients elicited an interest in its applicability to CF patients severely affected with ABPA. It is hypothesized that the addition of Omalizumab to the regimen of CF children with ABPA could allow for disease control with freedom from systemic corticosteroids. Four case reports in the literature summarize the experience treating a total of 7 young patients with CF [12-15]. These children had a long standing diagnosis of ABPA, with recurrent flares and need for frequent and prolonged courses of oral corticosteroids. From the information available, these children were receiving recommended therapy and still showing difficulties with disease activity as well as serious side effects. Some failed to adjuvant therapy with Itraconazole and in spite of adequate serum levels of the drug. With variable treatment courses, all of the reported children have shown significant responses in their clinical status and some have been able to wean from systemic corticosteroids. In one of the case series a decreased occurrence of hospitalizations for

pulmonary exacerbation while on therapy was also noted [13]. Of note is that the fact that the use of Omalizumab led to significant improvements underscores the role that IgE plays in the pathogenesis of this disease. The limited experience reported so far suggests that Omalizumab has a potential role as adjuvant therapy for CF patients that are corticosteroid dependent to control ABPA. This has led to the design of a clinical trial to assess the effect of repeated doses of Omalizumab in teenage patients Cystic Fibrosis Complicated by ABPA and who are steroid dependent (ClinicalTrials.gov NCT00787917). The treatment will be randomized, placebo controlled and double-blinded for 6 months and followed by 6 months of open label treatment for both arms. This study is currently actively recruiting in several sites in Europe and the US.

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TRANSIENT COLONIZATION OF THE AIRWAYS BY UNUSUAL *ASPERGILLUS* SPECIES IN TWO CYSTIC FIBROSIS PATIENTS

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Patients with cystic fibrosis (CF) are at high risk of colonization of the airways by various microorganisms, mainly bacteria (such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Stenotrophomonas maltophilia*), but also fungi including *Candida albicans* and several filamentous fungal species, particularly some aspergilli, *Exophiala dermatitidis* and some *Scedosporium* species. Among the *Aspergillus* species associated with cystic fibrosis, *A. fumigatus* and *A. terreus* are the most common agents of chronic airway colonization with a prevalence rate of 16 to 56.7% and 1.9 to 6.2%, respectively, depending on the studies. Other *Aspergillus* species are rarely encountered in the context of CF, and for instance, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus nidulans* have been reported, but they are usually found transiently.

The aim of this study was to identify and characterize two unusual *Aspergillus* strains transiently colonizing two CF patients : morphological study including determination of growth rate at different temperatures, as well as DNA sequencing and antifungal susceptibility testing of these two isolates were performed.

The two strains were poorly sporulating, they presented few conidial heads and could not be identified only on basis of morphology. Growth curves showed that these two species have quite the same growth rate as *A. fumigatus* at 37°C, but none of them grew at 50°C, which excluded atypical *A. fumigatus* isolates. DNA sequencing (beta-tubulin and ITS regions of rDNA) allowed us to identify *A. lentulus* and *Neosartorya pseudofischeri*. For this last species, the use of different media and several subcultures were needed to observe ascogonia (beginning of the sexual state), but cleistothecia were never observed.

The isolate of *A. lentulus* had high MICs for the three common antifungal drugs amphotericin B (4 µg/ml), itraconazole (2 µg/ml) and voriconazole (VRZ; 8 µg/ml) and the isolate of *N. pseudofischeri* had a MIC of 4 µg/ml for VRZ. These data are in accordance with literature data for these two species.

This is the first case of isolation of *A. lentulus* in CF, while *A. thermomutatus* (anamorph of *N. pseudofischeri*) has been described from 2 CF cases in USA. The clinical significance of these transient agents of colonization is unknown, but their pathogenic potential, their thermotolerance and resistance to antifungal drugs must lead to be cautious, and identification at the species level is recommended.

SCEDOSPORIUM APIOSPERMUM SEROPREVALENCE STUDY IN A LARGE COHORT OF PATIENTS WITH CYSTIC FIBROSIS IN FRANCE

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Scedosporium apiospermum, the asexual anamorph of *Pseudallescheria boydii*, is an ubiquitous saprophytic filamentous fungus found in soil, decaying vegetation and polluted water. *S. apiospermum* infections range from localized mycetomas to disseminated infections especially in the immunocompromised hosts. This species also colonizes the respiratory tract of patients with cystic fibrosis and is currently the second most frequent fungus found in patients' sputum excretions after *Aspergillus fumigatus* in France. The significance of patients' respiratory tract colonisation by *S. apiospermum* is still not clear. Most patients seem to have an asymptomatic chronic colonisation, some suffer allergic bronchopulmonary disease and some others develop very serious invasive infections. Because of the ability of *S. apiospermum* to disseminate through the lungs, the respiratory tract colonisation should not be neglected, especially in patients immunocompromised by diabetes or lung transplantation.

To better know the epidemiology of *S. apiospermum*, we are planning to realise a retrospective seroprevalence study in a group of 450 pediatric and adult patients with cystic fibrosis in Lyon, France. The main objective of our work is to estimate the global prevalence of patients having a positive serology for this fungus and to study the age-prevalence profiles. We also want to consider the link between serology and findings of sputum excretions in this group of patients. Finally we want to look for potential risk factors associated with an increase probability of respiratory tract colonisation by *S. apiospermum*.

S. apiospermum serologies will be realised in the Groupe d'Etude des Interactions Hôte-Parasite in Angers. Total antibodies will be determined by counterimmunoelectrophoresis and clinical and paraclinical informations will be extracted from the electronic file of each patient.

This prevalence survey might be a preliminary work to a prospective cohort study of cystic fibrosis patients with respiratory tract colonisation and/or positive *S. apiospermum* serology. The aim of this ulterior study could be to better know the pathogenicity of this fungus and to draw guidelines for the management of patients with cystic fibrosis colonised by *S. apiospermum*.

DETECTION OF *PSEUDALLESCHERIA* / *SCEDOSPORIUM* SPECIES AND *EXOPHIALA DERMATITIDIS* IN THE UPPER RESPIRATORY TRACT OF PATIENTS WITH CYSTIC FIBROSIS

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Background. The respiratory tract of cystic fibrosis (CF) patients is colonised by opportunistic pathogenic bacteria and fungi. In the course of their disease, these patients suffer from recurrent infective exacerbations of their respiratory tract. Respiratory tract colonisation of CF patients by fungi such as *Pseudallescheria*, *Scedosporium* and *Exophiala* species is well known. Detection often fails, since these slowly growing fungi are overgrown by *Candida* and *Aspergillus* species on commonly used culture media. However, infections due to these fungi were recognised especially after lung transplantation.

Objectives. Monitoring of respiratory tract colonisation in CF patients is important for rapid initiation of adequate treatment and prior to lung transplantation. It is questionable however, whether conventional methods are sufficient to detect slowly growing hyphomycetes.

Methods. In total, 589 upper respiratory specimens from CF patients were examined to detect slowly growing hyphomycetes. In addition to conventional methods, erythritol-chloramphenicol agar (ECA) and SceSel+ agar were tested for selective isolation of *Exophiala*, *Pseudallescheria*, and *Scedosporium* species. Furthermore, two enrichment broths were used to detect these and other slowly growing hyphomycetes.

Results. Selective isolation techniques were superior in detecting these fungi as compared to conventional methods. Although liquid media detected fewer strains of *Exophiala*, *Pseudallescheria*, and *Scedosporium* species, additional some further hyphomycete species not detected by other methods were isolated.

Conclusions. In opposite to conventional methods, ECA and SceSel+ agar are suitable for the detection of *Exophiala*, *Pseudallescheria*, and *Scedosporium* species in upper respiratory samples of CF patients. Therefore, selective isolation media for the detection of fungi growing slower than *Aspergillus* and *Candida* species should be added to conventional methods to optimise the monitoring of respiratory tract colonisation in CF patients.

SCEDOSPORIUM AURANTIACUM – THE AUSTRALIAN PERSPECTIVE

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Scedosporium species are clinically important emerging pathogens with *Scedosporium prolificans* typically associated with the most serious infection noted. They are the second most frequent filamentous fungi after *Aspergillus* spp. isolated from cystic fibrosis (CF) patients. Following the recent taxonomic changes within the genus *Scedosporium* a re-evaluation of the microbiology and epidemiology of the species, using contemporary molecular tools associated with CF patients, is necessary. The new species *Scedosporium aurantiacum* comprises a substantial proportion of Australian clinical isolates (>16%) and causes a wide range of human infections. More recently a prospective study at the Westmead Hospital CF clinic investigated 218 sputum specimens from 69 patients. The most frequent pathogen was *A. fumigatus* (46 patients; 66.7%) followed by *Scedosporium* spp. (12 patients; 17.4%), *Penicillium* spp. (14 patients, 20.3%) and *A. flavus* (7 patients, 10.1%). Eleven of the 12 patients with *Scedosporium* colonization in their sputum were co-colonized with *Aspergillus* spp. ITS-RFLP analysis demonstrated that *S. aurantiacum* (n = 4 patients) and *S. prolificans* (n = 4) were most frequently isolated followed by *S. apiospermum* (n = 3); one isolate was speciated as *P. boydii* species complex. *Scedosporium* spp. and *A. flavus* were more frequently present in mixed cultures compared with *A. fumigatus* (p = 0.036 and 0.009 respectively). Environmental surveys conducted in the greater Sydney area revealed a high prevalence of *S. aurantiacum* in urban areas. The findings indicate that there may be species-specific associations with areas of high human activity and hint of a possible environmental link. PCR-fingerprinting using the microsatellite specific primer M13, and MLST analysis using 6 genes (*EF1α*, *SOD2*, *CAL*, *TUB*, *RPB2*, *ACT*) have identified different genotypes among the isolates. Based on these findings, we conducted preliminary virulence studies using a murine model on a range *S. aurantiacum* strains and compared the results using *S. prolificans*. Eight *S. aurantiacum* and two *S. prolificans* strains with an inoculum size of 1×10^6 conidia/ml were inoculated intravenously into 7-week old immunocompetent BALB/c mice. *S. aurantiacum* was noted to be as virulent as *S. prolificans*, causing death in 60%-100% of mice. There were significant differences in virulence between the different genotypes of *S. aurantiacum*. Further studies correlating genotype and colonization status are under way.

EXOPHIALA DERMATITIDIS IN CYSTIC FIBROSIS: PREVALENCE, RISK FACTORS AND CLINICAL RELEVANCE

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Aims: To assess the prevalence of *Exophiala dermatitidis* (ED) in respiratory secretions of patients with cystic fibrosis, to identify risk factors for its presence, to investigate its impact on the clinical course.

Methods: We conducted a retrospective case-control study among non lung-transplanted patients followed on a very regular basis at our centre. The results of all cultures performed over a 2 years period (from February 1, 2007 to January 31, 2009) were reviewed. To detect fungi, cultures were grown on Sabouraud Gentamicin-Chloramphenicol Agar medium (Becton-Dickinson) and incubated at 35°C for 2 days and then at ambient temperature (15-25°C) for 3 weeks. Index cases were defined as patients with one or more sputum cultures + for ED (Group A). Each ED+ patient was carefully matched with a single ED- control, taking into account age, gender, genotype, BCC and *Pseudomonas aeruginosa* status (Group B). The two groups were then compared at the end of the period in terms of FEV1, rate of FEV1 decline over the 3 past years, BMI (Z-score), IgG levels, *Aspergillus fumigatus* (Asp f) colonization, predominant respiratory pathogen, AB IV treatment (days), intermittent or continuous use of inhaled antibiotics, inhaled steroids, oral azithromycin, oral antifungal agents. Patients from Group A were also compared to all ED- patients ≥ 12 y (Group C).

Results: The study group included 154 patients (76M, mean age \pm SD: 18.49 y \pm 11.59, median number of cultures/patient/ 2 years: 12). Out of 2.065 cultures, ED was isolated from 58 specimens (2.8%), in 9 patients (5.8%). All ED+ patients were PI and ≥ 12 y of age ($p=0.056$). 8/9 were homozygous for the F508 del mutation. In this group, 45.4 % of cultures (mean number over 2 y: 13.3, range: 10-20) yielded ED. Six patients had at least 3 positive cultures over a period of 6 months. No significant difference of clinical status or previous treatment was found between Groups A and B. Comparison of Groups A and C (n=90) revealed that isolation of Asp f at the last culture of the study period was more frequent in patients from Group A (44.4 % vs 10%, $p= 0.0166$). In Group A, trends to a larger proportion of patients homozygous for the F508 del mutation (88.9% vs 48.9%, $p= 0.052$) and more frequent intermittent use of itraconazole (66% vs 31.1%, $p = 0.083$) were also observed.

In a single patient from group A, without ABPA, only fungi were isolated over the 2 years (ED: 14/20 \pm Asp f: 9/20) as well as over the 2 previous years (ED: 14/37 \pm Asp f: 34/37). Serologic studies showed high levels of antifungal specific antibodies (Asp F and ED: 4 and 2 precipitating lines respectively) and a continuous use of voriconazole resulted in a dramatic and sustained decrease of her bronchorrhea.

Conclusion: In our clinic, ED was isolated in 5.8 % of patients without lung transplant. It was not detected below 12 y and could result in chronic colonization of the airways. *Aspergillus* colonization might be a predisposing factor. A deleterious effect on the clinical course could not be demonstrated by this case-control study. Intermittent use of oral itraconazole failed to eradicate ED. One ED+ patient also colonized by Asp f clearly benefited from a prolonged use of voriconazole.

GEOSMITHIA ARGILLACEA: AN EMERGING PATHOGEN IN CYSTIC FIBROSIS PATIENTS ?

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The progressive deterioration of the lung function in patients with cystic fibrosis (CF) is closely associated with chronic airway infections. The defective mucociliary clearance and the thickening of the bronchial mucus are responsible for the entrapment of numerous microorganisms. Beside bacteria, a large variety of filamentous fungi are frequently recovered from respiratory secretions of CF patients. *Aspergillus fumigatus*, *Scedosporium apiospermum*, *Exophiala dermatitidis* and *Aspergillus terreus* are the most common species recovered from sputum samples. Less common species have also been described, including some thermophilic species such as *Penicillium emersonii* which was reported until now in a unique case of chronic colonization of the airways.

During the past few years, several isolates identified as *Penicillium* sp. or *Paecilomyces* sp. and collected from respiratory secretions of 6 distinct CF patients followed in Rouen or Giens hospitals, were addressed to our laboratory for species identification. Additionally, cultures from sputum samples from 2 CF patients followed in Angers hospital also revealed a filamentous fungus presenting morphological features of *P. emersonii*. However, sequencing of the ITS regions permitted the identification of all these isolates (as well as those from the case already published) as *Geosmithia argillacea*.

First isolation of the fungus in these patients arises in the mean age of 19 years (6-48). All patients were chronically colonized by the fungus (at least two positive samples during a 3- to 18-month period). More, all patients were also chronically colonized by *Staphylococcus aureus*, 6 by *Aspergillus fumigatus* and 2 by *Pseudomonas aeruginosa* and *Aspergillus flavus*. *In vitro* antifungal susceptibility testing revealed resistance to voriconazole and, for most of the isolates, to itraconazole, and variable susceptibility to amphotericin B and posaconazole. Conversely, all the isolates were susceptible to caspofungin.

Whereas a unique case was reported until 1999, the recovery of *G. argillacea* from 6 additional patients since 2005, together with the recent report of 8 cases from CF patients in the United Kingdom, suggests that this fungus is emerging. However, improvement of methods used for detection and identification of fungi associated with CF may explain at least in part this increased incidence. In addition, although no disease exacerbation seems associated with the appearance and persistence of *G. argillacea* in respiratory secretions of these patients, a concern remains about the pathogenic role of this thermophilic fungus (particularly in lung transplant recipients), since it has been reported this year a case of disseminated infection caused by *G. argillacea* in a susceptible dog.

***PNEUMOCYSTIS JIROVECI* COLONISATION AMONG CYSTIC FIBROSIS PATIENTS**

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Pneumocystis jirovecii is an atypical opportunistic fungus with lung tropism and worldwide distribution that causes pneumonia (PcP) in immunosuppressed individuals. The study of this fungus has been hampered by the lack of in vitro culture system and laboratory diagnosis of *Pneumocystis* infection has relied mainly upon microscopic visualization with conventional cytochemical or immunofluorescence staining of organisms in respiratory samples. These methods are useful when the organism burden is relatively high but they are insufficient for reliable detection when there is a small parasite load. The development of sensitive molecular techniques has led to the recognition of a colonization or carrier state of *Pneumocystis jirovecii*, in which low levels of the organism are detected in persons who do not have overt PcP. *Pneumocystis* colonization has been describe in subjects with various lung disease, and accumulating evidence suggests that it may be an important clinical phenomenon. Only a few published studies carried out in Europe have evaluated the prevalence of *Pneumocystis* colonization in patients with CF, reporting ranges from 7.4% to 22%. Recently, other unpublished study has described a high prevalence of 38% of *P. jirovecii* colonization among Brazilian CF patients. There is only one longitudinal study that identified the distribution and dynamic evolution of *P. jirovecii* genotypes in CF-patients. In this study, patients studied during a 1-year follow-up period showed a continuous colonization-clearance cycle involving *P. jirovecii* and frequent genotypes change with an accumulative tendency to be colonized with genotype 3 of the mitochondrial large-subunit rRNA gene. Interestingly, none of these colonized patients developed PcP during a 1-year follow-up period. Then again, in other study that included eight pairs of brothers there were a concordance in the *Pneumocystis* colonization status of siblings suggesting a common source of infection or person-to-person transmission. Since patients with CF could potentially act as major reservoirs and sources of infection for susceptible individuals further research is thus warranted to assess the true scope of the problem and to design rational preventive strategies if necessary. Moreover, it is necessary to elucidate the role of *P. jirovecii* infection in the natural history of CF in order to improve the clinical management of this disease.

This work is part of the project “Pneumocystis Pathogenomics: Unravelling the Colonization-to-Disease Shift” a Coordination Action supported by the European Commission (ERA-NET PathoGenoMics).

ASPERGILLUS FUMIGATUS: ITS EXTRACELLULAR MATRIX AND ITS INTERACTIONS WITH PSEUDOMONAS AERUGINOSA

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In ABPA, aspergilloma and in the first step of invasive aspergillosis (in the lung), the fungus is under static and aerial conditions. Growing the fungus in a Petri dish is closer to the *in vivo* situation than growth in shaken submerged conditions. We showed that *in vitro* the fungus has a higher growth rate than in shaken submerged conditions, was more resistant to antifungal drugs and that the hyphae were highly agglutinated and glued together by an electron-dense extracellular matrix (ECM). This ECM is not produced when the fungus was growing under shaken submerged conditions *in vitro*. ECM is composed of galactomannan, α 1,3 glucan, melanin, antigens hydrophobins and some monosaccharides. Transmission electron microscopy showed that ECM is also produced *in vivo* at the surface of the hyphae present in the aspergilloma or in lungs during aspergillosis. Polysaccharides galactomannan and galactosaminogalactan were found in ECM *in vivo* whatever the disease considered. However α 1,3 glucan was only detected in aspergilloma ECM. The major antigenic glycoproteins were not found *in vivo* in ECM, but Melanin is also an important component of the ECM *in vivo* particularly in aspergilloma.

In cystic fibrosis patients colonized by *A. fumigatus*, the presence of an ECM has never been investigated. Moreover, in these patients, *A. fumigatus* is currently found in association with *Pseudomonas aeruginosa*. The interactions between *P. aeruginosa* and *A. fumigatus* *in vivo* are unknown. Preliminary results showed that a chemotactism exists between the two microbes with the formation of a biofilm of *P. aeruginosa* on *A. fumigatus* hyphae which is mannan-dependent. The objective of our project is to study the molecules involved in the interaction between the two microorganisms *in vitro* in culture medium and on epithelial cell lines. The ultrastructure of the fungal- bacterial behaviour will be investigated in *in vivo* samples of the upper respiratory tract in infected *cfr*^{-/-} mice. The effect of the sputum of cystic fibrosis patients on the interaction and growth of the bacteria and the fungus, compared to the sputum from healthy patients, and the importance of the *A. fumigatus* cell wall composition during the bacterial-fungal interactions will be studied.

ASPERGILLUS BIOFILM FORMATION ON POLYSTYRENE AND CYSTIC FIBROSIS BRONCHIAL EPITHELIA

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Introduction: The preferred growth form of microorganisms is in a biofilm. The extracellular matrix (ECM) of the biofilm can protect against host defences and antimicrobials. *A. fumigatus* is a frequent colonizer of patients with asthma and chronic lung diseases (e.g. cystic fibrosis). The aim of this study was to investigate if isogenic strains of *A. fumigatus* can persist in the respiratory tract of CF patients, and if *A. fumigatus* is able to form a biofilm-like matrix *in vitro* on human bronchial epithelia cells.

Methods: *A. fumigatus* isolates from three CF centres were biotyped with a STRAf assay for strain relatedness. *A. fumigatus* ATCC #9197 was incubated on 16HBE and CFBE41o- in MEM +10% FCS. Dry weight measurement and antifungal drug susceptibility testing was performed. Scanning electron microscopy (SEM) and confocal scanning laser microscopy (CSLM) images were analyzed. *A. fumigatus* cDNA microarrays and 2D gelelectrophoresis of mature biofilm were analyzed.

Result: The STRAf assay displayed various infection patterns from 209 isolates from 36 CF-patients. 17% persisting strains were found in patients for up to 10 years. The dry weight of the produced biofilm exceeded 7.4 mg on 16HBE and 7.7 mg on CFBE41o- cells after 72 h of biofilm production. No significant difference in dry weight increase between the cell lines was observed. Planktonic *A. fumigatus* was susceptible to azoles, polyenes and echinocandins. All antifungals were less susceptible or resistant (> 8 µg/ml) to *Aspergillus* embedded in biofilm. The SEM pictures displayed a network of hyphal structures with packed strands glued together with ECM. CSLM images displayed attached conidia on the cells after 4h, conidia and hyphal structures at 24 h and matrix formations after 48 h. Three dimensional constructs of the CSLM pictures displayed biofilm on 16HBE and proofed viability of the cells after 48 h co-incubation. Proteins were regulated at 24 h involved in a developmental stage demanding energy. At 48 h the metabolic activity was reduced and proteins were significant upregulated involved in the biosynthesis of secondary metabolites. In *Aspergillus* biofilms genes that are involved in resistance, allergy and secondary metabolite production are upregulated.

Conclusions: *A. fumigatus* can persist in the CF respiratory tract for years and is able to form a biofilm structure *in vitro* on bronchial epithelia cells 16HBE and CFBE41o-. This may have potential clinical implications with regard to chronic infection and antifungal drug resistance of *A. fumigatus in vivo*. Nonetheless, further *in vivo* investigations are warranted.

PROTEOMIC APPROACH OF ABNORMAL PIGMENTED STRAINS OF *ASPERGILLUS FUMIGATUS* BY SELDI-TOF SPECTROMETRY

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The aim of this present study was to assess the usefulness and reliability of the SELDI-TOF method to analyse and discriminate global protein patterns of different *A. fumigatus* strains. Pre analytical studies allowed us to select the optimal fungal culture conditions and to optimize the SELDI-TOF process. Then, this proteomic approach method was used to discriminate atypical pigmented isolates of *A. fumigatus* from a reference wild strain (IHEM 18963) in order to point out target proteins for further studies.

The wild strain and four abnormal pigmented *A. fumigatus* strains (3 white strains: IHEM 2508, IHEM 9860, IHEM 13262 and a brown pigmented strain IHEM 15998) were grown on Sabouraud medium at 37°C under and without stirring. Metabolic and somatic protein extracts (5 µg of total proteins) were spotted on weak cationic (CM10) and hydrophobic (H50) chip arrays in 96-sample bio processors (Bio-Rad Laboratories, Hercules, CA, USA). Peak annotation was performed after base line subtraction; noise calculation and normalization by total ion current. Statistical analyses were performed using ProteinChipDataManager 3.0.

Four experiments were performed for each strain. The reproducibility was of 14.4%.

Two clusters were obtained for all the strains according to the oxygenation. When fructification occurred (stationary cultures), the extracts were perfectly discriminated in metabolic fraction and quite perfectly discriminated in somatic fractions whatever the protein chip arrays used. This method allows detecting differently expressed proteins as well by the wild strain than by the mutants.

SELDI-TOF is a powerful rapid and reproducible pre analytical tool of complex mixtures of proteins. It will be invaluable for the detection of proteins of interest particularly those secreted in low abundance by fungi. This proteomic analyses demonstrate the high capacity of the fungus to adapt its protein expression to the environmental modifications. If the parameters have been correctly chosen this methodology could be really promising in the analysis of differential protein expression patterns of fungal origin.

ANTIFUNGAL DRUG SUSCEPTIBILITY OF *ASPERGILLUS* SPP. ISOLATED FROM CYSTIC FIBROSIS PATIENTS AND IMMUNE RESPONSES AGAINST THEM

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Cystic fibrosis (CF) is an autosomal, recessive hereditary disease involving the lungs as a major target of the disease. *Aspergillus* species are among the organisms commonly isolated from cultures of sputum. Resident alveolar macrophages and recruited neutrophils have a major role in the innate immune response against *Aspergillus* by recognition and clearance of the organism in CF patients. The fungus possesses little pathogenicity in immunocompetent hosts, but in few individuals, hypersensitivity to *Aspergillus* allergens can lead to the development of allergic bronchopulmonary aspergillosis (ABPA). Furthermore, CF lung transplant recipients with evidence of airway colonization with *Aspergillus*, constitute a patient population at risk of developing aspergillosis due to postoperative immunosuppression. Although amphotericin B and itraconazole are the antifungals used in *Aspergillus* infections, antifungal resistance has resulted in the use of additional broad spectrum antifungals.

In this study we have analyzed the following properties of *Aspergillus* spp. isolated from sputa of 8 patients with CF: a) *in vitro* activities of posaconazole, voriconazole, caspofungin, anidulafungin, itraconazole and amphotericin B against the isolated species, b) temperature growth characteristics, c) *in vitro* antihyphal activity of neutrophils (PMN) and monocytes (MNC), d) determination of the amounts of superoxide anion released from PMN in response to aspergilli of CF patients, and e) intracellular conidiocidal activity effected by MNC. The aim of our study was to identify putative differences in drug susceptibilities, growth characteristics and induced immune effector cell responses among six isolates of *A. fumigatus*, and two isolates of *A. flavus*.

Culture-positive specimens were identified as *A. fumigatus* or *A. flavus* both macroscopically on Sabouraud agar plates and microscopically by observing the colony characteristics. Three samples were collected at three different time periods from the same patient (5A, 5B, 5C). Minimum inhibitory concentrations (MICs) for the azoles and AMB and minimum effective concentrations (MECs) for the echinocandins were determined by a broth microdilution method following the NCCLS guidelines for molds. Drug susceptibilities of aspergilli from CF patients were compared to those of *A. fumigatus* strain #4215 (control, ATCC MYA 1163). Temperature growth characteristics were analyzed at 45°, 48°, 50° and 53° C. Superoxide anion production of PMN and antihyphal activity of both PMN and MNC were assessed spectrophotometrically by the superoxide anion release assay and XTT reduction assay, respectively. For the conidiocidal activity, 2×10^5 conidia were mixed with 4×10^5 MNC for 4 h at 37°C in 96-well flat-bottomed microtiter plates and colony-forming units (cfu) were

counted. For the superoxide anion assay, 10^5 conidia were seeded in culture plates and incubated for 12h at 37°C. Resulting hyphae were opsonized with 50% pooled human serum and incubated with 10^5 PMN and 75 μ M cytochrome C for 1h at 37°C and 5% CO₂. The superoxide anion produced by PMN was then quantitated. For the XTT assay, 10^4 conidia were added to 96-well flat-bottomed microtiter plates and incubated for 12h at 37°C. Phagocytes were then added to aspergilli hyphae at 20:1 effector to target (E:T) ratio and incubated for 1h at 37°C and 5% CO₂. PMN and MNC were then lysed and 150 μ l of PBS containing 0.25 mg/ml XTT and 40 μ g/ml coenzyme Q₀ were added to the culture plates. Following 30 min incubation at 37°C and 5% CO₂, the percent hyphal damage was evaluated.

Among all clinical isolates, sample 3 was found to have high MEC value for anidulafungin (1 μ g/mL) compared to control sample (0.004 μ g/mL), whereas all isolates exhibited relatively low MICs/MECs for the other antifungal agents. Macroscopic examination of fungal growth at different temperatures showed samples 2 and 8, corresponding to *A. flavus*, to exhibit no growth at temperatures $\geq 45^\circ\text{C}$. Different growth profiles were also recorded between samples 5A, 5B and 5C with 5A showing the greatest growth even at 50° C. When PMN were incubated with hyphae of each clinical isolate at effector to target ratio (E:T) of 20:1, the highest antihyphal activity (>50%) observed was for samples 5B, 5C and 8 as compared to control (38%). In contrast, the antihyphal activity of MNC was less effective than that exhibited by PMN, since the percent hyphal damage for most samples ranged between 23%-26%. However, samples 4 and 8 seemed to be more susceptible than the remaining clinical isolates (46% and 62%, respectively). High amounts of superoxide anion were released from PMN (6 nM O₂⁻ / 10^5 PMN/h) when challenged with each clinical isolate. *A. flavus*, (samples 2 and 8) were found most susceptible to the conidiocidal activity of MNC, since the intracellular killing reached 66% and 75%, respectively. Among the three samples of patient 5, 5A was the least susceptible to the conidiocidal activity; % intracellular killing of 5A was 11% vs 63% and 54% for samples 5B and 5C. Samples of patients 3 and 7 exhibited similar susceptible profiles to those of 5A which were comparable to control (13%).

We present preliminary data showing that differences exist in susceptibility to antifungal drugs and immune response among samples isolated from CF patients. Further investigation is required however in order to determine the genotypic identity of *A. fumigatii* strains as well as *A. flavus* and verify these preliminary results.

DECREASED IL-8 SECRETION AND EXPRESSION BY FLUVASTATIN IN PRIMARY HUMAN MACROPHAGES AND IN THE WHOLE BLOOD FROM ADULT PATIENTS WITH CYSTIC FIBROSIS.

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Early in life, CF patients become infected with microorganisms including bacteria, particularly *Pseudomonas aeruginosa*, and fungi, *Aspergillus fumigatus*. Recent research has identified anti-inflammatory properties of statins beside their lipid-lowering effect. Therefore, we have investigated the effect of fluvastatin on IL-8 secretion, using ELISA, and gene expression, using quantitative PCR. Human primary macrophages were obtained by differentiation of peripheral blood mononuclear cells with GM-CSF. Besides whole blood from adult CF patients were collected at the Rennes Teaching Hospital (France) accordingly to the local ethical committee. Whole blood or macrophages were pretreated 1 h by fluvastatin and incubated 24 h with lipopolysaccharide from *Pseudomonas aeruginosa* and/or *Aspergillus fumigatus* antigens. In both cultures, IL-8 protein levels were dose-dependently increased when cells were stimulated by *Aspergillus* antigens or lipopolysaccharide. Additive effects were observed in case of co-stimulation. We also demonstrate that fluvastatin strongly decreases protein levels of IL-8 in a concentration-dependent manner. Similarly, in macrophages, fluvastatin induced potent down-regulation of IL-8 mRNA levels.

In conclusion the inhibitory effects of fluvastatin on systemic and local inflammation could reveal important therapeutic potential of statins in various pathological conditions associated with over-production of pro-inflammatory cytokines and chemokines like observed in cystic fibrosis.

FINANCIAL SUPPORT : « Vaincre la Mucoviscidose »

VNTR TYPING AND MLST FOR THE EPIDEMIOLOGICAL STUDY OF *ASPERGILLUS FUMIGATUS*

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As part of studies on the spread of infections, risk factors and prevention, various typing methods were developed to investigate the epidemiology of *Aspergillus fumigatus*. In the present study, 52 clinical isolates of *Aspergillus fumigatus* from 12 airway specimens taken from patients with invasive aspergillosis (hospitalized in three different centers) were typed by variable number of short tandem repeat (VNTR) typing and multilocus sequence typing (MLST). These isolates were previously typed by random amplified polymorphic DNA (RAPD), sequence-specific DNA polymorphism (SSDP), microsatellite polymorphism (MSP) and multilocus enzyme electrophoresis (MLEE). VNTR typing identified 30 genotypes and, for most patients all isolates were grouped in one cluster of the dendrogram. Using MLST, only 16 genotypes were identified among 50 isolates, while two isolates appeared untypable. RAPD, MSP, SSDP and MLEE identified 8, 14, 9 and 8 genotypes, respectively. Combining the results of these methods led to the delineation of 25 genotypes and a similar clustering pattern as with VNTR typing. In general, VNTR typing led to the same results as the combination of RAPD, SSDP, MSP and MLEE but had a higher resolution, while MLST was less discriminatory and resulted in a different clustering pattern. Our data strongly suggest that VNTR typing is a superior tool to study the local epidemiology of *Aspergillus fumigatus*, which requires a high discriminatory power.

Subsequently, we applied VNTR typing to 256 *Aspergillus fumigatus* isolates, recovered from eight patients with cystic fibrosis and 41 *Aspergillus fumigatus* isolates from nine patients with proven invasive aspergillosis, hospitalized in two different centers. Only a limited number of genotypes was shared between patients and co-colonisation of the lung with multiple strains was found for all patients. Additionally, some genotypes were isolated recurrently, indicating that they are capable of prolonged colonisation. For 8/9 patients with invasive aspergillosis, a single genotype was found for the isolates recovered from all anatomical sites involved.

GENOTYPIC DIVERSITY AND COLONIZATION PATTERNS OF *ASPERGILLUS FUMIGATUS* IN PATIENTS WITH CYSTIC FIBROSIS

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Aspergillus fumigatus is a chronic colonizer of the respiratory tract of patients with cystic fibrosis (CF). A total of 204 *A. fumigatus* isolates from 36 CF patients from three different centers and collected over a period of four months till 9 ½ years, were genotyped using the short tandem repeat panel for *A. fumigatus* (STRAf). The results showed the presence of four different colonization patterns. Colonization patterns with only unique genotypes were found in 36% of the patients indicating that the patients were able to clear the isolates but that they were continuously recolonized. In 17% of the patients a single genotype was obtained indicating chronic colonization and suggesting that the patient was not able to clear the *A. fumigatus* isolate from the respiratory tract. The remaining patients showed a predominant genotype or genotypes that succeed each other.

TRACKING THE EMERGING HUMAN PATHOGEN *PSEUDALLESCHERIA BOYDII* BY USING HIGHLY SPECIFIC MONOCLONAL ANTIBODIES

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Pseudallescheria boydii has long been known to cause white grain mycetoma in immunocompetent humans, but has recently emerged as an opportunistic pathogen of humans causing potentially fatal invasive infections in immunocompromised individuals. Diagnosis of *P. boydii* is problematic since it exhibits similar morphological characteristics to other hyaline fungi that cause infectious diseases such as *Aspergillus fumigatus* and *Scedosporium prolificans*. This presentation will describe the development of IgM and IgG1 k-light chain monoclonal antibodies (MAbs) specific to *P. boydii* and certain closely related fungi. The MAbs bind to an immunodominant carbohydrate epitope on an extracellular 120 kDa antigen present in the spore and hyphal cell walls of *P. boydii* and *Scedosporium apiospermum*. The MAbs do not react with *Scedosporium prolificans*, *S. dehoogii*, or with a large number of clinically relevant fungi including *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, *Exophiala dermatitidis*, *Fusarium solani* and *Rhizopus oryzae*. The MAbs were used in immunofluorescence and double-antibody-sandwich ELISA tests to accurately differentiate *P. boydii* from other infectious fungi and to track the pathogen in environmental samples. Specificity of the DAS-ELISA was confirmed by sequencing of the ITS1-5.8S-ITS2 rRNA-encoding regions of environmental isolates. This demonstrates the potential of the immunoassay as a diagnostic platform for *P. boydii*/*S. apiospermum* and may provide a useful tool for monitoring the pathogen in respiratory infections of cystic fibrosis patients.

RAPID QUANTIFICATION OF HUMAN-PATHOGENIC FUNGI IN VARIOUS SAMPLES USING SOLID-PHASE CYTOMETRY

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1. Rapid detection, quantification and susceptibility testing of *Aspergillus fumigatus* in air

A. fumigatus is an ubiquitous fungus causing serious infections such as aspergilloma, allergic bronchopulmonary aspergillosis and invasive aspergillosis in immunocompromised patients. Monitoring of the number of *A. fumigatus* spores in the air inhaled by these patients is crucial for infection control. In the present study, a new and rapid technique for the quantification and real-time susceptibility testing of *A. fumigatus*, based on solid-phase cytometry and immunofluorescence labelling, has been developed. Air samples were collected by impaction on a water soluble polymer that was subsequently dissolved. A part of the sample was filtered, the filter was placed on a growth medium and microcolonies were able to form for 18 hours at 47°C. By using a general growth medium or a growth medium supplemented with itraconazole, growth was allowed for all or only the resistant *A. fumigatus* conidia, respectively. Subsequently, labelling with a monoclonal anti-*Aspergillus* antibody in conjunction with tyramide signal amplification was used to detect the microcolonies with the aid of a solid phase cytometer (ChemScan RDI). The detected spots were microscopically validated using an epifluorescence microscope. The sensitivity and specificity of the assay were evaluated by testing pure cultures of 40 *A. fumigatus* strains, 12 other *Aspergillus* species, 14 different *Penicillium* species and 14 other filamentous fungi. All *A. fumigatus* strains yielded labelled microcolonies, which confirmed the sensitivity of the assay. Only *Rhizopus stolonifer* and *Paecilomyces variotii* were also labelled with the antibody and were able to form microcolonies at 47°C. These fungi, however, could be discriminated from *A. fumigatus* based on their morphology. Comparison with traditional culture-based methods indicated that our novel approach is a rapid (24h vs 96h), reliable and specific alternative with a high dynamic range. Quantification of air samples collected at 56 locations resulted in a total of 531 *A. fumigatus* microcolonies and 7 resistant *A. fumigatus* microcolonies, leading to an itraconazole resistance prevalence of 1.3 % among environmental isolates.

2. Rapid quantification of viable *Candida (albicans)* cells in whole blood

Candida spp. are a common source of nosocomial bloodstream infections in critically ill patients. Therefore, rapid isolation and identification of these pathogenic yeasts are crucial. Traditional diagnostic procedures based on blood cultures lack speed and a sufficiently low detection limit to ensure reliable and early diagnosis of invasive *Candida* infections. A two hour method based on immunomagnetic separation (IMS) and solid-phase cytometry (SPC) has been developed. In a first step, *Candida* cells present in a whole blood sample (max. 15 ml) are magnetically labelled with a primary anti-*Candida* FITC conjugated antibody and a secondary anti-FITC Microbead conjugated antibody. Subsequently, *Candida* cells are separated from the blood using the MACS technology. The obtained suspension is filtered and a double labelling procedure is used to discriminate between *Candida albicans* cells and other *Candida* spp. First, *C. albicans* cells are detected using a specific PNA FISH probe and the signal is amplified using tyramide signal amplification, leading to a red fluorescence. Additionally, all viable *Candida* cells are stained with the dye ChemChrome V6, resulting in green fluorescence. Finally, the membrane filter is scanned by a solid-phase cytometer and all detected, green cells are microscopically inspected for verification of red fluorescence. To evaluate the sensitivity of this approach, blood samples spiked with different numbers of *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis* or *C. tropicalis* were analysed. These tests confirmed that the detection limit for all *Candida* spp. was as low as 1 cell/10 ml of blood. Additionally, applying the assay to blood samples spiked with other fungi including *Aspergillus*, *Cryptococcus* and *Fusarium* spp. confirmed its specificity. In conclusion, we developed a rapid and highly sensitive method for the diagnosis of candidemia. The procedure has been validated on spiked blood samples and analysis of patient samples is ongoing.

3. Quantification of *Pseudallescheria boydii* complex spp. in river water

A new method for the detection of *Pseudallescheria boydii* complex spp. will be developed for application in the monitoring of long-term water quality. While existing methods for monitoring health risks of surface water usually provide data of short-term relevance only, inclusion of this fungus as bio-indicator might enable monitoring of long-term water quality. For the specific detection of *Pseudallescheria* strains, two specific FISH probes targetting the 18S and 28S rRNA respectively were designed. Currently, the development and optimisation of a FISH-SPC procedure is ongoing.

KEEPING AN EYE ON ENVIRONMENTAL SOURCES FOR SCEDOSPORIUM SPECIES

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Scedosporium and *Pseudallescheria* species became more and more important as an opportunistic fungal pathogen for patients infected during a near drowning event, by other traumata or those being predisposed by a hematological disorder. The risk of colonization in cystic fibrosis patients is still unclear. Nevertheless environmental sources for exposure of *Scedosporium* spec. are not sufficiently studied. Most of these isolates have not been identified according to the new taxonomy.

Based on the SceSel+ agar [Rainer *et al.*, 2008], environmental samples from Germany, Thailand, Israel and Italy have been cultivated.

Samples have been taken from wet areas like borders from ditches, streams, puddles und rain water barrels and from cow dung.

So far predominantly *S. apiospermum* and *P. boydii*, but also *S. aurantiacum*, *S. dehoogii*, *P. minutispora* and *S. prolificans* have been found in the environmental samples. The isolation of *S. apiospermum* from salty water in a wellness facility on Ischia / Italy was one of the most spectacular findings.

Rainer J, Kaltseis J, de Hoog SG, Summerbell RC (2008) Efficacy of a selective isolation procedure for members of the *Pseudallescheria boydii* complex. *Antonie van Leeuwenhoek* 93(3):315-322.

ABPA DIAGNOSIS IN CYSTIC FIBROSIS PATIENTS: THE CLINICAL UTILITY OF SPECIFIC IGE TO RECOMBINANT *ASPERGILLUS FUMIGATUS* ALLERGENS

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Objective: ABPA is a complicating factor of cystic fibrosis which can result in a devastating combination as lung disease progresses. The overlap between the signs and symptoms of the two conditions makes diagnosis problematic, even if standardized criteria are used. The objective of this study was to identify, in a group of cystic fibrosis patients, cases of ABPA by assaying specific IgE to recombinant *Aspergillus fumigatus* antigens.

Methods: Fifty-four patients, aged 2 to 20 years, had their: clinical data, chest TC scan, immediate hypersensitivity skin tests for *A. fumigatus*, total serum IgE assay, RAST for *A. fumigatus* and serum specific IgE for the recombinant allergens Asp f1, f2, f3, f4 and f6, systematically assessed.

Results: Thirty-nine patients, considered as risk group for ABPA, were eligible for the study. Thirty-two of these were investigated. Sensitization to *A. fumigatus* was observed in 34%. Using the Cystic Fibrosis Foundation criteria or the specific IgE to recombinant antigens, three patients were defined as suffering ABPA; however, only two of these patients were diagnosed by both methods.

Conclusions: Specific antibodies to recombinant *Aspergillus fumigatus* allergens were a useful tool for the early detection of sensitization and diagnosis of ABPA, especially during an early phase, when clinical symptoms are lacking.

ABPA IN CYSTIC FIBROSIS PATIENTS: VALUE OF BIOLOGICAL MARKERS

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Allergic bronchopulmonary aspergillosis (ABPA) is a common infectious complication in cystic fibrosis patients. The diagnosis remains difficult and required a combination of clinical, radiological, biological and mycological criteria. The aim of this study was to analyse the diagnosis value of two recombinant antigens: rAspf4 and rAspf6 associated to specific IgG and total IgE detection, and mycological data.

Thus we determined the IgE responses to these recombinants in sera of 68 cystic fibrosis patients by retrospective study. We selected 5 sera for each 18 patients with ABPA (15 proven ABPA and 3 probable ABPA) in order to determine the sensitivity and precocity of these markers in the course of the disease and one serum for patients without ABPA (50 patients). The sensitivity of rAspf4 IgE per patient was higher (80%) than those of the rAspf6 IgE recombinant (46.6%). Furthermore all the patients with positive IgE detection against rAspf6 gave also positive results with rAspf4. When rAspf4 IgE detection was associated with anti *Aspergillus* IgG-ELISA and precipitins, the sensitivity raised to 100%. The specificity was respectively of 94% and 92% for rAspf4 IgE and rAspf6 detection. It was slightly lower for the other diagnostic criteria (86% for anti *Aspergillus* IgG-ELISA, 88% for precipitins, 82.4% for total IgE and 85.4% for *Aspergillus fumigatus* positive culture in sputum).

In conclusion, this retrospective study underlined the importance of combination of biological markers (*Aspergillus* IgG-ELISA, precipitins, total IgE and rAspf4 IgE) for diagnosis accuracy and the weak help of rAspf6 IgE detection for ABPA diagnosis.

IMPROVING METHODS FOR IDENTIFICATION OF FUNGI IN CF SPUTUM.

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Fungal infections are increasing in prevalence in patients with cystic fibrosis. Factors which may explain this are better methods of detection, aggregation of patients in CF Centres and the increased median survival of patients with CF. Fungal infection may cause a number of problems including ABPA, semi-invasive colonisation and infection. We specifically have addressed the problem of identification of fungi using culture based methods and molecular methods to improve the diagnostic approach to fungal infection. We have experimentally developed a new media which has 100% ability to culture yeasts and filamentous fungi and suppresses all bacterial infection apart from *Burkholderia cepacia* complex organisms. In addition we have developed a PCR based assay using primers which amplify the 18sRNA gene and have identified a wide range of fungi from the sputum of adults with cystic fibrosis. Some initial suggestions will be made with regard to the potentially pathogenic nature of identified fungi.

MICROBIAL DIVERSITY IN CF LUNG DISEASE

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The use of culture independent molecular, profiling strategies to characterise microbial communities has revealed surprising levels of diversity in a range of different systems. In the context of infection, such findings have led to an interest in the relationship between microbial diversity and disease. For example, high bacterial diversity appears to confer greater resistance to antimicrobial treatment in certain contexts independent of the particular species present. In addition, where both bacterial and fungal pathogens are present, as is often the case in CF lung infections, the behaviour of each group of pathogens may significantly influence the behaviour of the other. Our investigations aim to determine whether levels of bacterial diversity determined in individual patients is mirrored by fungal diversity.

There is particular interest in examining the relationship between microbial diversity and periods of pulmonary exacerbation, during which significant loss of lung function occurs. Investigation of fungal diversity may prove particularly useful here, due to the impact of antibiotic therapy making relating bacterial diversity to clinical parameters difficult. However, before such a relationship can be investigated, it is first necessary to establish the degree to which total microbial diversity varies both between individual patients, and within given patients over time during periods of pulmonary stability.

More than 2000 sputum samples were obtained from 14 adult CF patients over the course of a year. Samples were retrospectively selected from each patient at approximately 4 month intervals, during periods of clinical stability. Clinical data, including temperature, lung function and antibiotic therapy, correlating to each sample were recorded. In addition, patient-derived measures of general well-being, cough-severity, sputum production and breathlessness were recorded using a visual analogue score. Bacterial and fungal diversity was then determined in this sample set through 16S and 18S ribosomal T-RFLP analysis respectively.

In order to avoid spurious amplification of human DNA template present in sputum samples when using 18S ribosomal primers, a nested PCR reaction was performed (Ott *et al.*, 2007). An initial step was used to amplify the full-length 18S rDNA sequence using the conserved primers NS0 and EF3. Inner PCR primers NS1 and FR1 were then used to amplify a fungal-specific amplicon of ~1650 bp for the use in T-RFLP analysis (Ott *et al.*, 2007). 16S ribosomal T-RFLP analysis of bacterial diversity in the sputum samples was performed as described previously (Rogers *et al.*, 2004).

The degree to which fungal diversity differed between patients, as well as over the course of a year within specific patients, was investigated. Further, the relationship between total microbial diversity in CF airways and disease severity was examined. The implications of these insights, both for achieving a greater understanding of underlying pathology, and for the design of effective treatments, will be discussed.

A METAGENOMIC APPROACH FOR DETERMINING THE MICROBIOTA ASSOCIATED WITH CYSTIC FIBROSIS

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The aim of this work was to identify the main features of microbiota associated with cystic fibrosis (CF) using a non-culture microbiological approach.

Material and Methods. Sputum samples (one per patient) were collected from 20 CF-patients. All patients presented a stable clinical status without acute exacerbations. Total DNA from each sputum sample was obtained manually using a phenol/chloroform protocol and diluted up to 50 ng/μl. PCR-DGGE technique was performed in all samples using universal primers for Bacterial Domain based on 16S rRNA conserved regions. Amplicons were separated in vertical electrophoresis polyacrylamide gels (8%) at 60°C; with a urea-formamide denaturing gel gradient of 30-45%. Gels were visualized with ethidium bromide; common and unique bands were excised, re-amplified and sequenced in order to assign bacterial species identification. Similarities among the electrophoretic band patterns were analyzed using the Phoretix 5.0 software® and dendrograms were constructed based on the Dice coefficient.

Results. All CF-patient samples presented a marked band of *Pseudomonas aeruginosa*. On the other hand, several band patterns were common to both groups. As expected, different species corresponded to bacteria habitually found in sputum samples (*Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Moraxella* sp., *Actinomyces odontolyticus*.) although several sequences corresponded to uncultured bacterium related with *Streptococcus*, *Actinobacterium* or *Neisseria* groups. Interestingly, environmental organisms such as *Pseudomonas synxantha*, *Ochrobactrum anthropi*, *Rothia amarae*, *Rothia mucilaginoso*, *Phycococcus dokdonensis*, or *Arthrobacter* sp. were detected.

Conclusion. Metagenomic tools are useful to identify the microbiota present in patients with CF. Moreover, we were able to detect uncultured and environmental bacteria in sputa that have not been previously described in this type of samples.

PATHOGENESIS OF *ASPERGILLUS* IN CF: A ROLE FOR MOLECULAR DIAGNOSTICS?

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Aspergillus fumigatus causes significant morbidity in Cystic fibrosis (CF). Adults with CF demonstrate a wide variety of immunopathological responses to *A. fumigatus* ranging from serological evidence of sensitization (60%) to allergic bronchopulmonary aspergillosis (ABPA) (15%). The diagnosis of ABPA in CF remains difficult due to overlapping and concomitant bacterial infection. The immunological pathway is triggered by *Aspergillus* spore germination, which releases allergens and proteases, but thereafter relies on continued antigen exposure to cause hypersensitivity. The prevalence of *A. fumigatus* in CF sputum samples varies between studies from 12-57% indicating a need to improve laboratory methods of detection.

The first aim of this research is, for the first time in CF, to use molecular techniques to detect and quantify *Aspergillus* in CF sputum. Precise quantification will be used to examine the relationship between *Aspergillus* load, patient clinical characteristics and the immunological markers of sensitization. Methods of optimal DNA extraction and purification from CF sputum have been tested and are presented. *Aspergillus* load is now being accurately quantified using a new, clinically validated, commercial Real-time PCR kit (FXG™: RESP (Asp+)) which utilises molecular beacon technology. PCR results will be compared to optimal standard sputum culture. This is being done in the context of a cross-sectional observation study of the frequency of sensitization to *Aspergillus* and other fungi in the Manchester CF cohort, using skin prick testing and serology. It is hypothesized that as fungal load increases this leads to a heightened inflammatory and clinical response. Early antifungal treatment may prevent progression to sensitization and ABPA, or may reduce inflammation in the lung directly.

The second aim of this research is to examine the frequency of azole resistance in a cross-sectional observation study of the same CF cohort. Preliminary data shows low voriconazole resistance in 2 of 9 CF isolates. All *Aspergillus* isolates cultured are being susceptibility tested to azoles and those resistant investigated for the genetic mechanism of resistance (CYP51A sequencing and up-regulation) and relatedness (microsatellites).

The third aim of this research is to investigate the changes in fungal diversity during CF pulmonary exacerbations. The impact of broad spectrum antibiotics on fungal diversity and load is unknown. Advanced parallel gene sequencing is being used on sputum samples collected during and after exacerbations to identify and quantify all fungal species present.

Through these three studies I aim to develop a clinical management strategy for *Aspergillus* in CF and improve knowledge of fungal diversity and resistance in CF.

IDENTIFICATION OF HERPOTRICHIELLACEAE USING A BARCODE-LIKE SEQUENCE OF THE ITS2

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Members of the herpotrichiellaceous black yeasts i.e. *Cladophialophora* spp., *Exophiala Fonsecaea* spp., *Phialophora* spp., *Ramichloridium* spp., and *Rhinocladiella* spp., are of medical importance because they can cause a variety of different mycoses whereas some of them could be life threatening. In case of patients with Cystic Fibrosis long-term colonization with *E. dermatitidis* and *E. phaeomuriformis* had been described.

The sequence of nuclear internal transcribed spacer (ITS2) turned out as a useful genetic marker for discrimination of members of the Herpotrichiellaceae. Nevertheless, inference of phylogeny using this gene is hampered by difficulties in obtaining reliable alignments was mainly due to lengths variation. Recently numerous algorithms had been proposed to derive putative secondary structures of non-coding RNAs.

We have analyzed the derived putative secondary structure of the ITS2 by applying such programs e.g. Mfold, FOLDALIGN in case of sequences of ex type strains of medically important Herpotrichiellaceae. Thereby it could be shown that the transcribed ITS2 RNA could be folded accordingly to universal 4 domain model recently proposed for Eukarya. Comparative analyses revealed that the highest degree of nucleotide variation was found in the external loop region of the second domain. Upon analyzes of 434 respective sequences belonging to 86 species of the Herpotrichiellaceae we could show that this region represent a species-specific region. Using this barcode-like region reliable species identification could be derived for 74 species whereas 12 species showed an identical signature sequence. All of them but one could be differentiated by taking the surrounding nucleotides (15 nucleotides on both sides) into account. The only exception was *E. dermatitidis* and *E. phaeomuriformis* showing and identical ITS2 sequence, but could be differentiated by the ITS1 sequence analyzes.

Our finding is in sharp contrast to *Candida* spp. where the 3rd domain showed the highest degree of nucleotide variation.

Our finding opens the possibility of an easy-to-perform species identification in case of pleoanamorphic black yeasts belonging to the Herpotrichiellaceae, which are otherwise difficult to specify.



METHYL COPROGEN B, A NEW POTENTIAL MARKER OF COLONIZATION OF THE AIRWAYS BY *SCEDOSPORIUM APIOSPERMUM*



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Scedosporium apiospermum is the second most common filamentous fungus colonising the airways of patients with cystic fibrosis (CF) [1]. Its detection from respiratory specimens is only achieved by mycological studies on semi-selective agar culture media. Unfortunately, mycological examination of sputum samples is not standardized and its detection and identification requires about one week.

To improve the detection of *S. apiospermum* from respiratory secretions of CF patients, some fungal components may be investigated as biological markers. Here, we investigated the potential usefulness of siderophores for rapid detection of the presence of *S. apiospermum* in sputum samples.

Siderophores are small molecules with a molecular mass usually comprised between 300 and 1300 Da, which exhibit a very high affinity for ferric iron. As part of the iron acquisition systems of micro-organisms, they are secreted under ferric stress conditions to scavenge iron from the environment [2]. In a previous study, we have identified two siderophores, namely dimerumic acid and methyl coprogen B, secreted by *S. apiospermum* in an iron-restricted culture medium (Figure 1). Additionally, it has been demonstrated the *in vivo* secretion of siderophores, especially in the airways of CF patients [3]

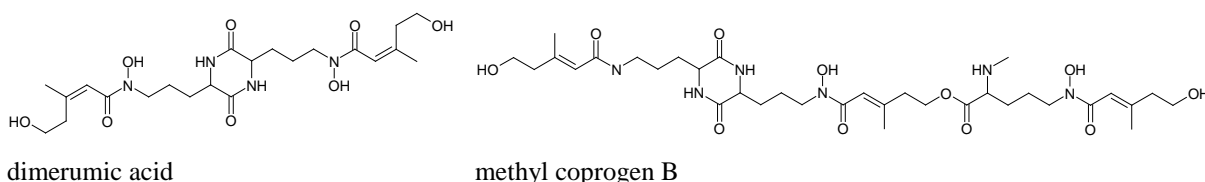


Figure 1: Siderophores produced by *S. apiospermum*

In this work, we adjusted a very simple and efficient method to detect the siderophores of *S. apiospermum* from sputum samples. This method consisted in XAD-4 extraction and HPLC analysis. The method was applied to various strains of the *S. apiospermum*/*P. boydii* complex grown under iron-restricted conditions, but also to some isolates belonging to the main other fungal species associated with CF such as *A. fumigatus* and *A. terreus*. Analysis of the obtained results showed that dimerumic acid and methyl coprogen B are specifically secreted by *S. apiospermum sensu lato*. This method was then applied to sputum samples from patients with CF colonized or not by *S. apiospermum*. Methyl coprogen B was never detected from culture-negative sputum samples, whereas three out of five culture-positive sputum samples revealed to be positive for methyl coprogen B.

In conclusion, methyl coprogen B may be considered as a biological marker of airway colonization by *S. apiospermum*.

This work was supported by “Le Conseil Général du Maine et Loire”.



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