Cystic Fibrosis AND fungi

3rd ISHAM workshop, Ljubljana, May 2010

Emerging Potential of Black Yeasts

Gerhard Haase
Institute of Medical Microbiology
University Hospital RWTH Aachen, Germany
Cystic fibrosis - mucoviscidosis

- autosomal recessive hereditary disease
- mutation in the CFTR gene *(more than 1,400 are known so far)*
- causing problems in electrolyte transports
- leading to mucus thickening mainly in lung
Mucus thickening leads to:

- chronic infection of the bronchial system
- insufficient enzyme release of the pancreas (malnutrient - poor growth)
- destruction of the pancreas – Diabetes mellitus
- destruction of biliary system (liver cirrhosis)
Cystic fibrosis - perspective

- life expectancy today approx. 30 years
- death is mainly due to lung destruction caused by chronic bacterial infection
- no definitive therapy available
- *Ultima ratio*
  bilateral lung transplantation
  (or lung & heart *en bloc*)
Cystic fibrosis is the most common life-limiting autosomal recessive disease in caucasians.

Canada ~ 3,000
Germany, GB ~ 6,000
USA ~ 40,000

CFTR gene mutation carriage rate:
Ashkenazi Jewish 1 in 22
caucasians 1 in 25
Hispanics 1 in 46
Africans 1 in 65
Asians 1 in 90
Genesis of chronic bacterial infections

Modifications of the electrolytic exchanges

Defect in the mucociliary clearance

thickness of the bronchial mucus

Entrapment of the inhaled bacteria and fungal spores
Chronic bacterial infections (2)

*Staphylococcus aureus, Pseudomonas aeruginosa, Burkholderia cepacia*

Bacterial proteases

Inflammatory reaction

Elastase

neutrophils

Fungal spores

Lm  Fn

Fg

Bouchara et al., Contrib Microbiol, 1999
Age-specific infection rates in CF-patients

Total Cultures (%)

Age (y)

Drugs are often been aerosolized for inhalative application via a nebulizer:

- bronchodilators
- mycolytic agents
- antibiotics (tobramycin, colistin)
- enzymes (DNAse)
Fungi recovered from CF patients mostly for a longer period of time

- yeasts (~ 70 %)
  - C. albicans
  - C. dubliniensis
- hyphomycetes (~ 45 %)
  - A. fumigatus
  - Scedosporium apiospermum
  - Exophiala dermatitidis
- Pneumocystis jirovecii (~ 20 %)
Candida spp.

- stable genotypes could be recovered for more than 9 months
- siblings showed comparable genotypes

Muthig et al., 2010
Fungi recovered from CF patients only occasionally OR rarely (< 5%)

- yeasts
  - *C. lusitaniae*, ....

- hyphomycetes
  - *Aspergillus thermomutatus*
  - *Aspergillus flavus*
  - *Penicillium spp.*
  - (chrysogenum, citreonigrum, corylophilum, emersonii)
  - *Scedosporium prolificans*
  - *Exophiala phaeomuriformis*
  - *Acrophialophora fusicpora*
<table>
<thead>
<tr>
<th>Reference, location</th>
<th>Patients, $n$</th>
<th>Age (where available), mean (range)</th>
<th>Aspergillus fumigatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nelson et al., 2 Rochester, USA</td>
<td>37</td>
<td>14.2 (5–46)</td>
<td>21 (57)</td>
</tr>
<tr>
<td>Laufer et al., 4 Wisconsin, USA</td>
<td>55</td>
<td>14.2 (2–34)</td>
<td>5 (9)</td>
</tr>
<tr>
<td>Schoenheyder et al., 5 Copenhagen, Denmark</td>
<td>150</td>
<td>13 (2–35)</td>
<td>75 (50)</td>
</tr>
<tr>
<td>Penketh et al., 3 London, UK</td>
<td>288</td>
<td>(12–51)</td>
<td>27 (9.4)</td>
</tr>
<tr>
<td>Bauemfeind et al., 1 Munich, Germany</td>
<td>102</td>
<td>16 (4–31)</td>
<td>6 (5.9)</td>
</tr>
<tr>
<td>Mroueh and Spock, 11 Durham, USA</td>
<td>236</td>
<td>14.5 (1–41)</td>
<td>60 (25)</td>
</tr>
<tr>
<td>Becker et al., 6 Seattle, USA</td>
<td>49</td>
<td>25.8 (18–50)</td>
<td>8 (16)</td>
</tr>
<tr>
<td>Milla et al., 15 Delaware, USA</td>
<td>370</td>
<td>17.2</td>
<td>45 (12.2)</td>
</tr>
<tr>
<td>Burns et al., 7 USA, different centres</td>
<td>465</td>
<td>21.2 (6–63)</td>
<td>108 (3.2)</td>
</tr>
</tbody>
</table>
obviously frequency of isolation varies due to

- *length of incubation time*
- *usage of selective media (Exophiala, Scedosporium)*

*Antonie Van Leeuwenhoek. 1993;64(1):17-26.*
Selective media

Piher et al., 2009
Observation (II)

despite long lasting colonization with well-known fungal species capable of causing invasive disease e.g.

A. fumigatus
S. apiospermum
E. dermatitidis

• such infections are rarely observed in case of CF patients

• also no PCP is observed in case of P. jirovecii colonization
Therefore there is **no indication for**
an "**empirical therapy**"
in **case** of fungal colonization only

**BUT**

in any case systemic **antifungals**
may be indicated **in colonized patients**
with **respiratory deterioration**
not responding to antibacterial chemotherapy,
Types of fungal disease in CF

- Allergic bronchopulmonary „disease“ (Aspergillus, Scedosporium)
- Candidemia due to indwelling catheters
- Invasive mycoses after lung transplantation
A. fumigatus & CF

The major causative agent of colonization of the airways (9 to 57% of the patients)

- Becker et al., Chest, 1996: 16% USA
- Milla et al., Pediatr Pulmonol, 1996: 21.2% USA
- Burns et al., Clin Infect Dis, 1998: 24.5% USA
- Cimon et al., EJMMID, 2000: 46.1% France
- Bakare et al., Mycoses, 2003: 45.7% Germany

Responsible for various diseases in the context of CF

- Asthma, bronchitis and aspergilloma
- Invasive pulmonary aspergillosis
- (after lung transplantation)
- Allergic broncho-pulmonary aspergillosis or ABPA
Genotype study of multiple and sequential isolates
Sequence-specific DNA primer and RAPD (primers NS3 and NS7)

Recently colonized patients:

Huge diversity of genotypes even in the same sample
(frequency and diversity of genotypes of *A. fumigatus* in the environment)

Associated with the presence of serum anti-*A. fumigatus* catalase antibodies
(marker of altered lung function)

ABPA is a long-term allergic response to *Aspergillus*, mainly observed in patients with severe, persistent asthma and in CF.

In patients with asthma clinical manifestations are episodic wheezing, expectoration of brown mucus plugs, low-grade fever, eosinophilia, and transient pulmonary infiltrates due to atelectasis. Central bronchiectasis occurs in some patients after several years of disease.
**Allergic Bronchopulmonary Aspergillosis**

- After colonization, *Aspergillus* germinates

**Inflammation** in the bronchial submucosa leads to:

- excessive mucin production,
- extravasation of eosinophils into the bronchial mucin,
- intermittent **bronchial obstruction with atelectasis**, and
- over time, to **bronchiectasis**
Risks factors for acquiring ABPA

- inhaled cumulative dose of corticosteroids
- years of colonization with *P. aeruginosa*
- concurrent colonization with *S. maltophilia*

Implanted CVC-related fungal infections

In patients with CF disease severity like

• frequent antibiotic usage
• corticosteroid therapy
• diabetes mellitus

have been associated with an increased risk of candidemia
Post transplant aspergillosis in CF

- Fungal infection developed in 44% (14/32) of patients
- tracheo-bronchial aspergillosis was observed in 9 patients
- isolated pneumonia was observed in 5 patients
- survival was (only) **21%** (3/14) !!!
PROBLEM:

colonization with *Scedosporium* sp. (as well as *Burkholderia cenocepacia*) is therefore a relative contraindication for lung transplantation.
### Scedosporium apiospermum & CF

<table>
<thead>
<tr>
<th></th>
<th>Transversal study</th>
<th>Longitudinal study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aspergillus fumigatus</strong></td>
<td>21.4</td>
<td>46.1</td>
</tr>
<tr>
<td><strong>Scedosporium apiospermum</strong></td>
<td>3.3</td>
<td>8.6</td>
</tr>
<tr>
<td><strong>Aspergillus terreus</strong></td>
<td>1.9</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Up to 10% in Australia *(Williamson et al., J Clin Microbiol, 2001)*

Usually associated with *A. fumigatus* (10 out of 11 patients)

Emerged subsequently to *A. fumigatus* in 9 patients (average delay: 14 months)

Improvement of its detection by the use of cycloheximide
Scedosporium prolificans & CF

(mainly reported in Spain)

Del Palacio *et al.*, Mycoses, 2001

Transient colonization

Fatal invasive infection after lung transplantation

(Vagefi *et al.*, Am J Ophthalmol, 2005)
Mainly reported in Germany

7.5% Haase et al., Lancet, 1990
9.1% Haase et al., Mycoses, 1991
15.7% Blaschke-Hellmessen et al., Mycoses, 1994
6.2% Horre et al., Respiration, 2004

Lack of standardization of the mycological examination of sputum samples (slow growth: 1-to 4-week incubation)?

Transient or chronic colonization, usually without obvious clinical signs of pneumonia or invasive pulmonary mycoses.

Kusenbach et al., Eur J Pediatr, 1992
Diemert et al., Scand J Infect Dis, 2001
Patient with bronchiectasis, and repeated episodes of bronchial infections requiring a lobectomy in June 1997

May 2003, hospitalization due to a new episode of hemoptysis

July 2003, sputum sample exclusive and profuse growth of *E. dermatitidis*

Similar results on successive samples (Dec 2003, Feb 2004, May 2004)

Retrospective serological study (somatic extract):

3 to 6 precipitin lines by CIE since June 2003 and no reactivity with *A. fumigatus* extract

Itraconazole treatment (200 mg/day from November 2003 to June 2004) Improvement of the patient which was cured in May 2004
The regular isolation of *E. dermatitidis* from this patient (65-year old) with bronchiectasis and repeated episodes of bronchial infections led us to search a CF-related disease

Sequencing of the gene *CFTR* revealed for both alleles minor mutations

L206W (exon 6A) / V754M (exon 13)

*Biomarkers of a CF-related disease ?*
In CF, transient colonization of the airways in a 11-year old patient
González-Escalada et al. Rev Iberoam Micol, 2000
Cimon et al., J Clin Microbiol, 2005

Detected in 4 patients

All patients were also colonized by A. fumigatus, and A. fusicpora was isolated repeatedly in one patient.
Prevalence low, maybe underestimated because of misidentifications with Scopulariopsis spp., Paecilomyces spp. or S. prolificans
Guarro & Gené, J Clin Microbiol, 2002;
Sigler & Sutton, J Clin Microbiol, 2002
Acrophiialophora fusispora & CF

Pale buff colonies of 5 to 6 cm in diameter at day 7
Basally inflated phialides, arising mostly singly on vegetative hyphae
Long chains of lemon-shaped or fusiform conidia, with ornamentations arranged in spiral bands
Factors associated with fungal colonization

- age of patient
- lengths of bacterial colonization
- treatment with inhalated antibiotics

Sudfeld et al., 2010
Fungal species: CF patients - air

Fungi isolated from sputum
- Thanatephorus cucumeris
- Malassezia sp.
- Trichosporon sp.
- Acremonium strictum
- Fusarium culmorum
- Fuscospora ferrea
- Scedosporium apiospermum
- Saccharomyces cerevisiae
- Exophiala dermatitidis
- Candida glabrata
- Candida parapsilosis
- Candida dubliniensis
- Candida albicans

Fungi isolated from the air
- Sporidiobolus salmonicolor*
- Phaeococcomyces chersonesos*
- Emericella sp.*
- Coniosporium sp.*
- Phoma herbarum*
- Blumeria sp.*
- Kondoa aeria*
- Trametes sp.*
- Rhexocercosporidium sp.†
- Sclerotinia sclerotiorum†
- Stereum annosum†
- Heterobasidion annosum†
- Paecilomyces sp.§
- Aspergillus sydowii§
- Cryptococcus sp.§
- Cryptococcus magnus§
- Engyodontium album§
- Yarrowia lipolytica§
# Categories of fungal pathogenicity

<table>
<thead>
<tr>
<th>I</th>
<th>II</th>
</tr>
</thead>
</table>
| *Aspergillus fumigatus*  
*Scedosporium apiospermum* | *Candida albicans*  
*Candida dubliniensis*  
*Candida parapsilosis*  
*Candida glabrata*  
*Exophiala dermatitidis*  
*Aspergillus versicolor.* |

Nagano et al., 2010

I: Very significant fungi related to CF. Widely reported as either an aetiological agent of invasive disease or associated with ABPA.

II: Found relatively frequently in CF sputum. Clinical significance unknown in CF but showing a high association with CF sputum.
### Categories of fungal pathogenicity

<table>
<thead>
<tr>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhodotorula</em> spp.</td>
<td><em>Fuscospora</em> ferrea</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td><em>Fusarium</em> culmorum</td>
</tr>
<tr>
<td><em>Trichosporon</em> sp.</td>
<td></td>
</tr>
<tr>
<td><em>Aureobasidium pullulans</em></td>
<td><em>Thanatephorus</em> cucumeris</td>
</tr>
<tr>
<td><em>Acremonium strictum</em></td>
<td></td>
</tr>
<tr>
<td><em>Malassezia</em> spp.</td>
<td></td>
</tr>
<tr>
<td><em>Cladosporium</em> spp.</td>
<td></td>
</tr>
<tr>
<td><em>Penicillium</em> spp.</td>
<td></td>
</tr>
</tbody>
</table>

**III:** Found relatively infrequently in CF sputum. Clinical significance unknown in CF. Have been described elsewhere in the literature as aetiological agents of mycological infections.

**IV:** Potentially not relevant to CF. Mainly environmental fungi not previously reported as human pathogens.

*Nagano et al., 2010*
Problems concerning fungi & CF

Proper & regular cleaning of nebulizer

Contribution of fungi to mortality unknown
  - polymicrobial infections
  - no autopsies performed

What is needed?
  - Implementation of standardized protocols (selective media)
  - Source of colonization
  - Robust data for incidence
Further research is done by:

**ISHAM working group:**

**Filamentous fungi & chronic respiratory diseases in cystic fibrosis**

*Coordinator: Prof. Dr. Jean-Philippe Bouchara*

[http://www.med.univ-angers.fr/GEIHP](http://www.med.univ-angers.fr/GEIHP)
Conclusions

• At present fungal infections do not seem to represent a major challenge in patients with CF

• ABPA and CVC-related candidemias are the most frequent clinical features, but invasive infections due to Aspergillus and Scedosporium are described with increasing frequency, especially after lung transplant

• This scenario may change in the (next) future because of the overall increase in patients survival and therefore it is possible that fungal pathogens become (soon) a challenge also in CF

• Considering the peculiarity of pharmacokinetics of drugs in CF and the great number of drugs administered to these patients, specific studies are needed in order to identify the correct schedules and the possible risk of adverse events due to drugs interactions
Thank you very much for your attention
Bronchiectasis, meconium ileus
The genetic defect underlying cystic fibrosis disrupts the functioning of several organs by causing ducts or other tubes to become clogged, usually by thick, sticky mucus or other secretions.

**AIRWAYS**
Clogging and infection of bronchial passages impede breathing. The infections progressively destroy the lungs. Lung disease accounts for most deaths from cystic fibrosis.

**LIVER**
Plugging of small bile ducts impedes digestion and disrupts liver function in perhaps 5 percent of patients.

**PANCREAS**
Oclusion of ducts prevents the pancreas from delivering critical digestive enzymes to the bowel in 85 percent of patients. Diabetes can result as well.

**SMALL INTESTINE**
Obstruction of the gut by thick stool necessitates surgery in about 10 percent of newborns.

**REPRODUCTIVE TRACT**
Absence of fine ducts, such as the vas deferens, renders 95 percent of males infertile. Occasionally, women are made infertile by a dense plug of mucus that blocks sperm from entering the uterus.

**SIGN**
Malfunctioning of sweat glands causes perspiration to contain excessive salt (sodium chloride). Measurement of chloride in sweat is a mainstay of diagnosis.
CFTR
Cystic Fibrosis Transmembrane Conductance Regulator

Gene Locus
Chromosome 7
Locus 7q31.2

The CFTR gene:

► is 250,000 bp long
► contains 27 exons
► the protein has 1,480 amino acids with a molecular mass of 168,138 Da
► nucleotide of ~ 6,500
The image provides a diagram of the CFTR protein, which is also known as the cystic fibrosis transmembrane conductance regulator (CFTIR) and an ATP-binding cassette (ABC) protein. The diagram illustrates the hydrophobic segments that span the membrane, with distinct domains labeled, including the ATP binding domain, NBF (N-terminal domain), and COOH (C-terminal domain). The most common site of CF mutation, ΔF508, is indicated. The protein kinase A site and protein kinase C site are also labeled.
CFTR

- Discovery of the CFTR gene
  - 1989 by Francis Collins and Lap-Chee Tsui

- What Causes CF?
  - Caused by a mutation in the gene that produces the protein responsible for moving the chloride ions through the cell membranes

- Mutations
  - Most common is the deletion of the phenylalanine (ΔF508)
  - Deletion of three consecutive base pairs in the ATP-binding, nucleotid-binding fold (NBF), cause of defective folding of CFTR in the endoplasmic reticulum which prevent it from moving efficiently through the Golgi apparatus
Summary pathophysiology CF

Defect
- Abnormal Gene
  - Abnormal CFTR
    - Abnormal Sodium Chloride & Water Movement Through Cell
      - Abnormally Thick and Dry Mucous
        - Bronchial Airway Obstruction
          - Infection
            - Inflammation
              - Progressive Lung Tissue Destruction
                - Respiratory Failure

Therapy
- Gene Replacement
- Protein Replacement
- Correction of Electrolytes
- Mucolytics
- Mucous Clearance Techniques
- Anti Inflammatory Agents
- Anti Microbials
- Lung Transplants
Alteration of CFTR leads to mucus thickening mainly in the lung

- exact mechanism unknown so far
- further (unknown) cofactors determine the progress of disease
Chronic bacterial infections (1)

*Staphylococcus aureus, Pseudomonas aeruginosa, Burkholderia cepacia*
Special type of bacteria in CF

- mucoid *P. aeruginosa* strain (alginate - biofilm)
- All *P. aeruginosa* CF isolates had lipid A with palmitate
- small colony variant *Staphylococcus aureus*
Antifungal therapy in APBA

Considering the risk of interactions, especially for the drugs with oral administration, or the need for daily i.v. infusion for the others

- nebulized liposomal \textbf{amphotericin B}
- aerosolized nanostructured \textbf{itraconazole} \\
  \textit{(produced by spray freezing into liquid)}

could represent interesting alternatives,

but presently they had not been clinically evaluated in these patients
Using these criteria in CF patients the incidence of ABPA is approximately 7% (ranging 2% - 30%), increasing after the 6th year of age.

Colonization with *A. fumigatus* has been shown to be age-related.
Sinusitis

- nasal obstruction
- chronic congestion or discharge
- headaches
- post-nasal drip with morning cough
- cough that is aggravated by lying down
- severe bad breath
- constant need to “clear one’s throat”
Aspergillus spp. is thought to colonize sinuses in up to 40% of adults, although its presence is unlikely to cause symptoms (???)