Aspergillus – *bystander or intruder?*

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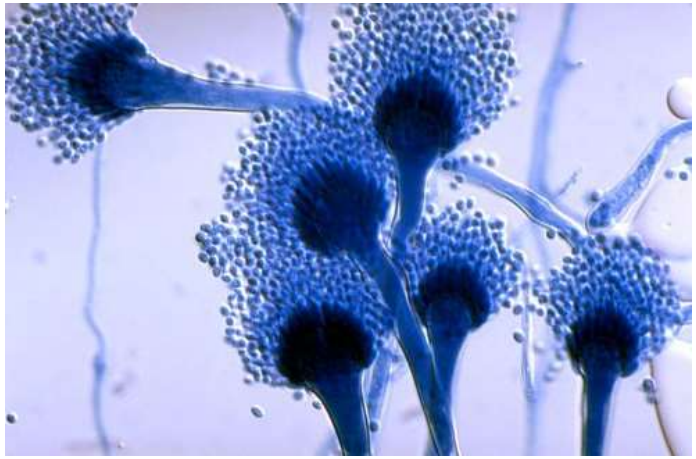
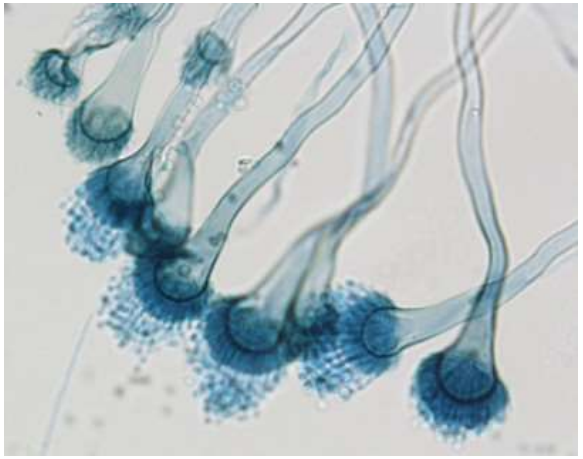
Background

- Over the last decade, a significant increase in the prevalence of fungi in CF respiratory cultures was reported

- *A. fumigatus* (Af) is the most common filamentous fungus involved in CF lung disease, with reported prevalence rates ranging from 6% to nearly 60%





Sudfeld, JCF 2010; Muthig Med Mycol. 2010; Maiz Chest 2002; Baxter Chest 2013

Why do CF patients acquire fungi?

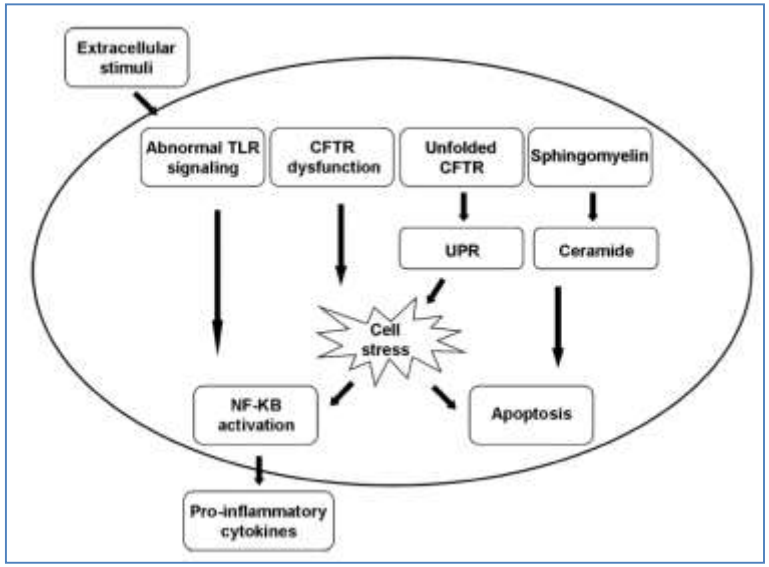
- Patients with CF are at increased risks of fungal acquisition, colonization and infection for similar reasons they acquire bacteria

- Abnormal mucociliary function and local immunogenic impairment promote fungal colonization whereas prolonged antibiotic and corticosteroid use may facilitate fungal growth

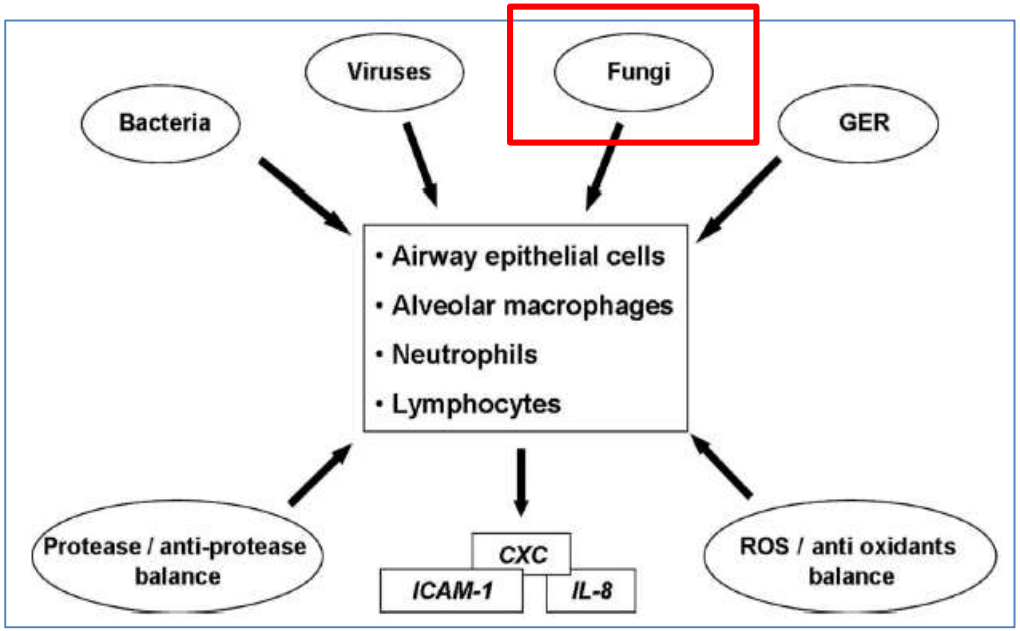
Cimon et al. J Mycol Med 1995; Sanjay et al, The International Journal of Biochemistry & Cell Biology 2014

Triggers of inflammation in CF airway cells

Intracellular triggers



Extracellular triggers



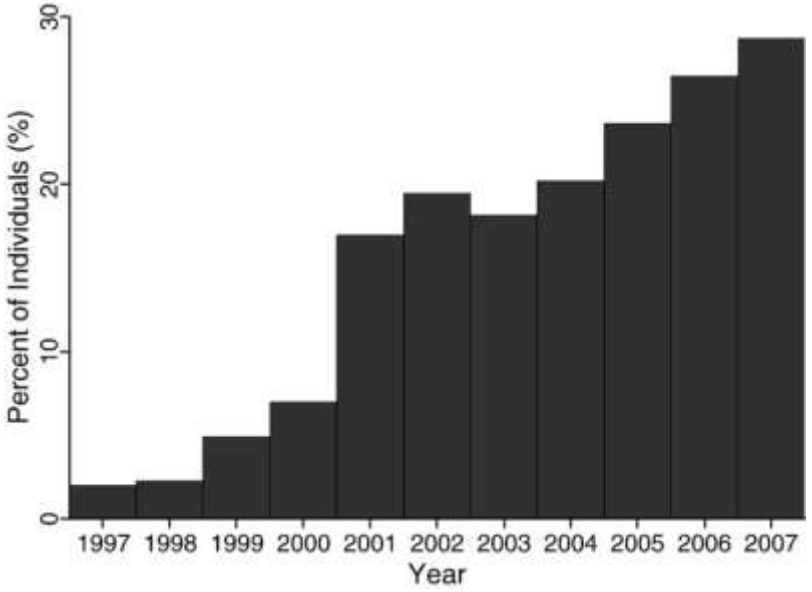
Cohen-Cymberknoh M et al. Thorax 2013

Original article

Prevalence and risk factors for recovery of filamentous fungi in individuals with cystic fibrosis

Christopher R. Sudfeld [a,*], Elliott C. Dasenbrook [b], William G. Merz [a,c], Karen C. Carroll [c], Michael P. Boyle [b]

- A CF cohort database from JHH (1997-2007)
- n=614 children and adults with CF
- Prevalence of filamentous fungal isolation increased from 2% in 1997 to 28.7% in 2007



Risk factors include older age, decreased lung function, and chronic oral antibiotics, while inhaled corticosteroids surprisingly decreased the likelihood

J Cyst fibrosis 2010

The dilemma:
Aspergillus fumigatus: bystander or intruder?

Innocuous…. the “result” of poor lung function and clearance?

or

It is the “cause” of poor outcomes?

Association of Chronic *Candida albicans* Respiratory Infection With a More Severe Lung Disease in Patients With Cystic Fibrosis

Alex Gileles-Hillel, MD,[1] David Shoseyov, MD,[1,2,3] Itzhack Polacheck, PhD,[4] Maya Korem, MD,[4] Eitan Kerem, MD,[1,2,3] and Malena Cohen-Cymberknoh, MD[1,2,3*]

4,244 sputum cultures (2003-2009) from 91 patients with CF (mean age 19.7 yrs.)

Chronic respiratory colonization of *C. albicans* is associated with worsening of FEV_1 and a higher annual rate of FEV_1 decline

Risk factors for chronic *C. Albicans* colonization

A multivariate logistic regression model

	OR (95% CI)	P value
Aspergillus spp. colonization	9.8 (2.5 – 38.2)	0.001
CFRD	3.5 (1.1 – 10.8)	0.02
PI	3.9 (1.3 – 11.6)	0.01
FEV_1 < 60%	3.1 (0.9 – 9.8)	0.02
Chronic P. aeruginosa	1.7 (0.8 – 4.6)	0.18

Ped Pulmonol 2015

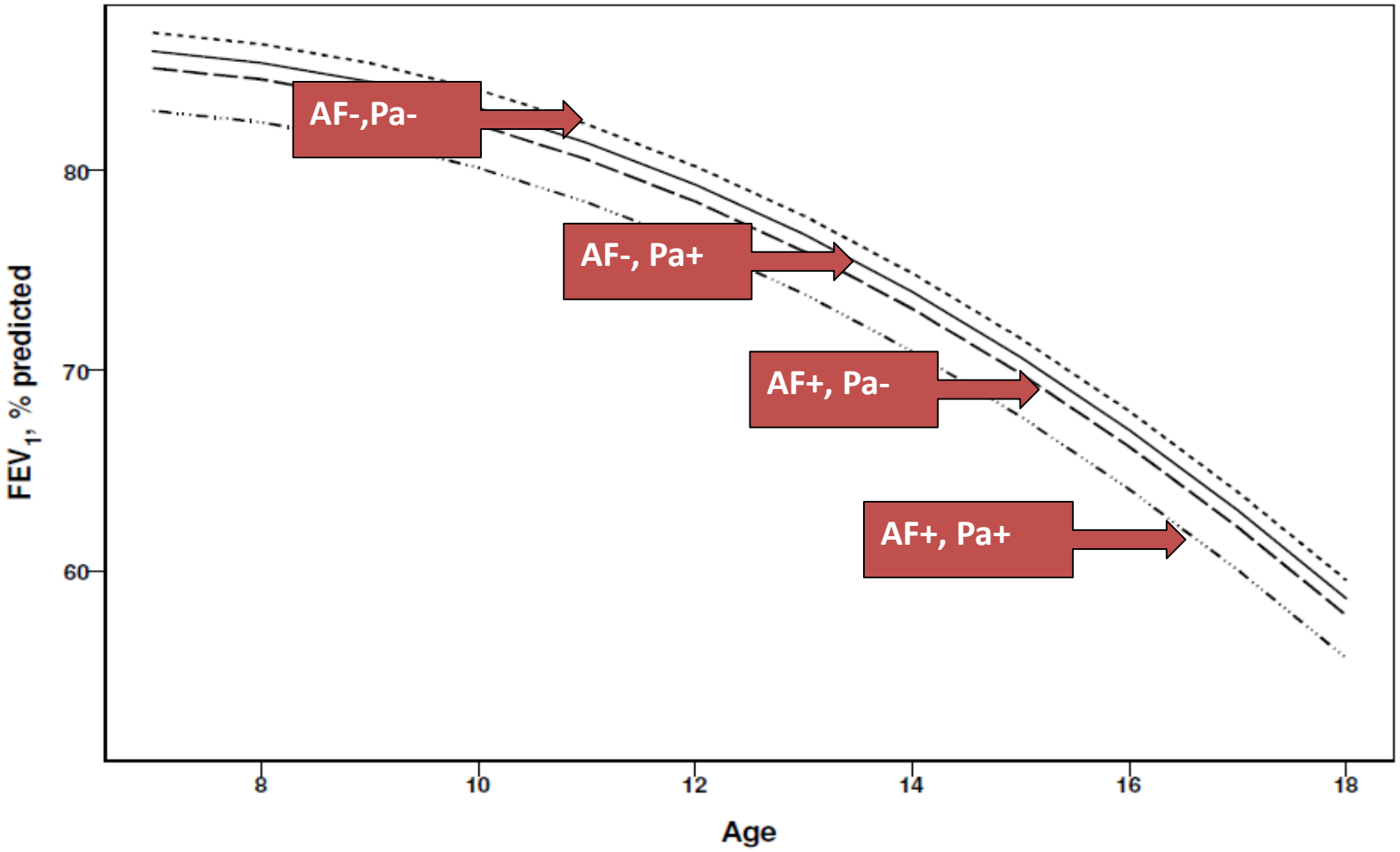
The Effect of Chronic Infection With *Aspergillus fumigatus* on Lung Function and Hospitalization in Cystic Fibrosis Patients

Reshma Amin, Annie Dupuis, Shawn D. Aaron and Felix Ratjen

- N= 230 CF patients, Hospital for Sick Children, Toronto, 1999-2006
- Primary outcome: annual number of hospitalizations for pulmonary exacerbations
- Persistent Af infection and CFRD were associated with an increased risk of pulmonary exacerbations
- No increased risk among patients with ABPA
- Significant interaction between Af and Pa on lung function *(p=0.0006)*

Chest 2010

Decline in FEV_1% over time for the *A. fumigatus* and *P. aeruginosa* interaction

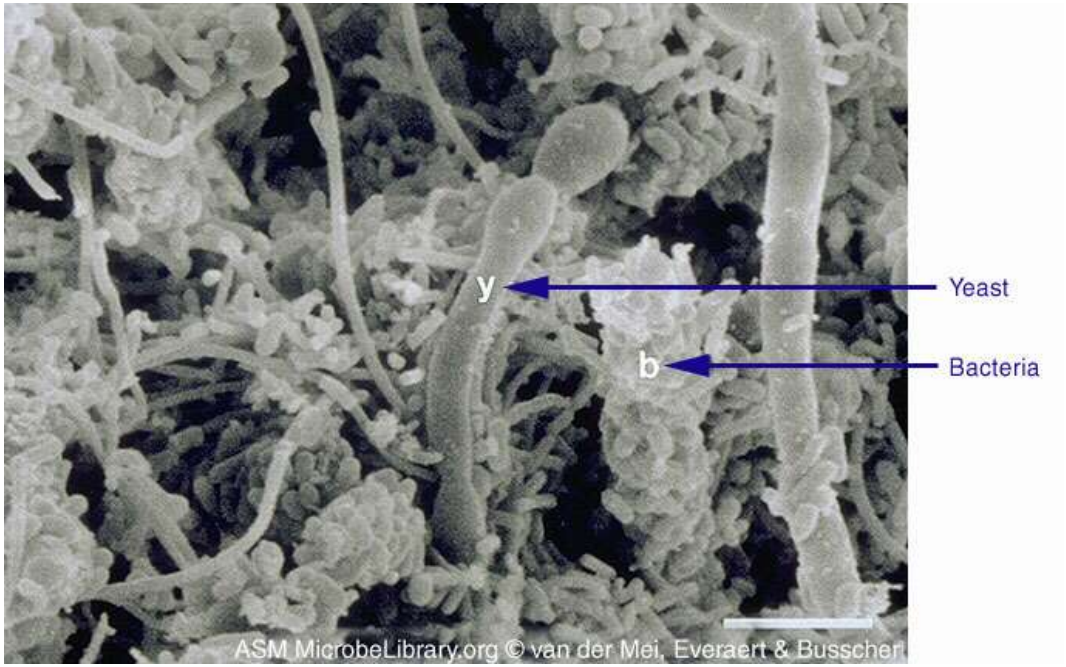


Adjusted for baseline FEV_1, only chronic *A. fumigatus* infection was associated with a significantly increased risk of pulmonary exacerbations *(RR 1.40, P= 0 .065)*

Amin et al, Chest 2010

Fungal - bacterial interactions

Co-species biofilm formation increases drug resistance of both



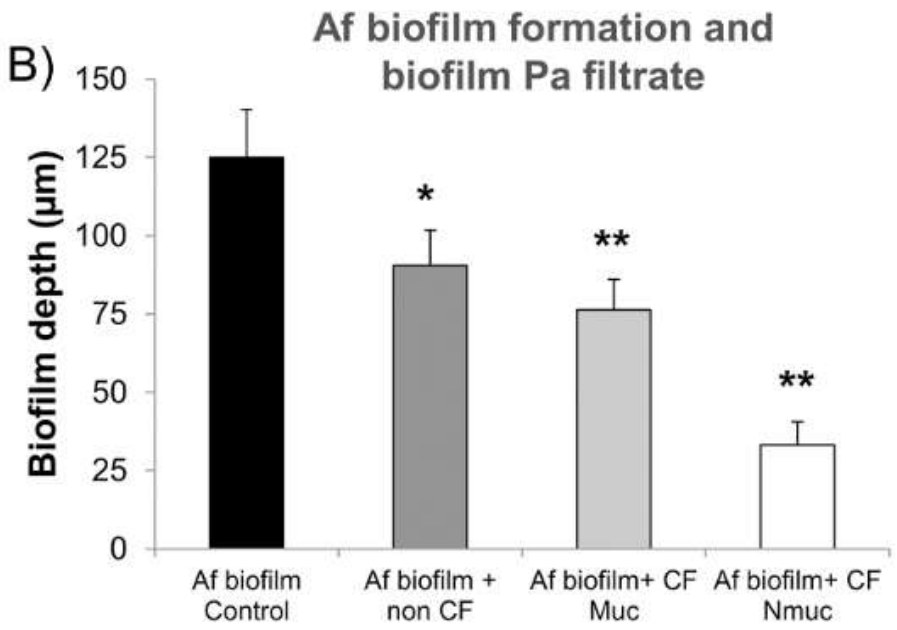
- Both *Pa* and *Af* possess the ability to form multicellular biofilm consortia, making it difficult to eradicate the infection

- *Pa* could significantly impede filamentous growth and biofilm formation in *Af*, by affecting the intercellular communication

Duan K, Mol Microbiol 2003; Amin R, Chest 2010; Mowat E, FEMS Microbiol Lett 2010

Inhibition of *Aspergillus fumigatus* and Its Biofilm by *Pseudomonas aeruginosa* Is Dependent on the Source, Phenotype and Growth Conditions of the Bacterium

Jose A. G. Ferreira[1,2¤a], John C. Penner[1¤b], Richard B. Moss[3], Janus A. J. Haagensen[4], Karl V. Clemons[1,2], Alfred M. Spormann[4], Hasan Nazik[1,2,5], Kevin Cohen[1], Niaz Banaei[6], Elisabete Carolino[7], David A. Stevens[1,2]*

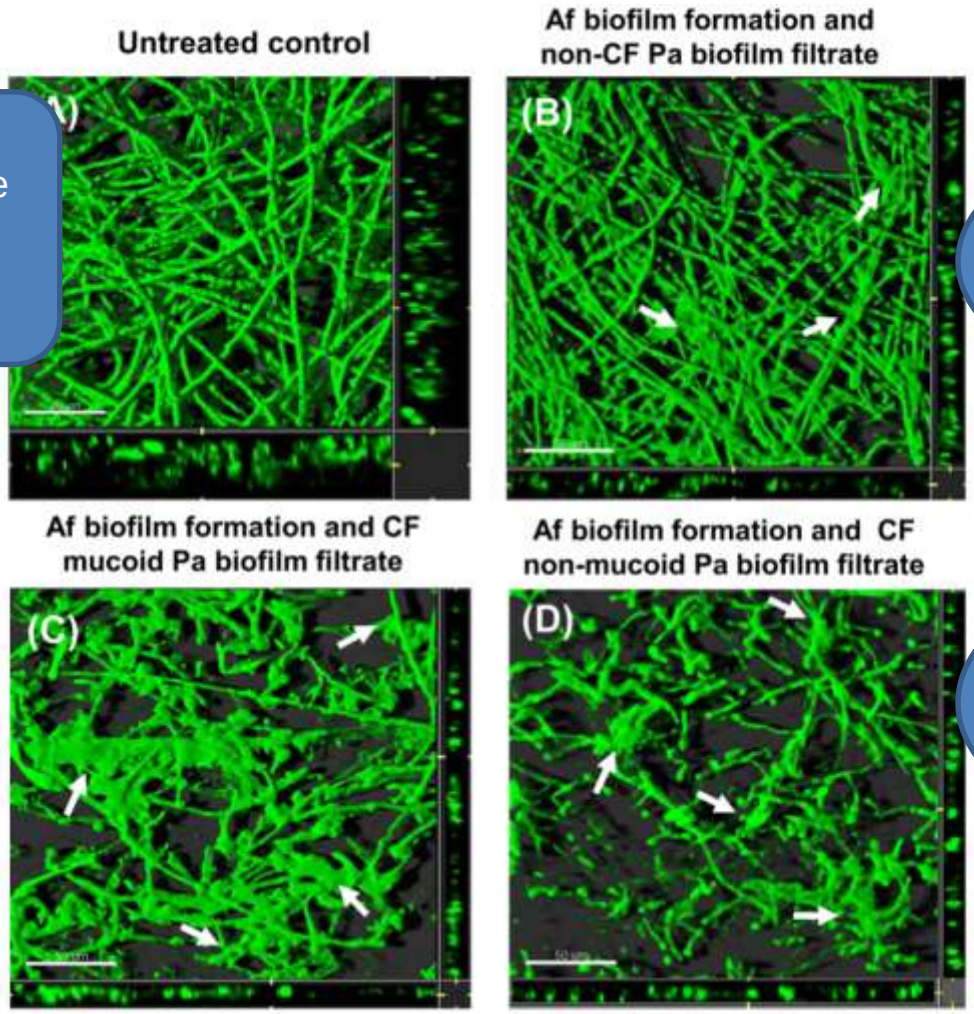


Non-mucoid CF isolates (NMuc-CF) are more inhibitory to formation of Af biofilms than mucoid isolates (Muc-CF)

PLoS One 2015

The effect of Pa culture on Af biofilm formation

Dense filamentous multicellular structure with acute-angle dichotomous branching



Decreased number of hyphae; presence of some "bulging" structures

Severe effects on hyphal structure, loss of filamentation, and *thin* hyphal tips

Ferreira JAG, PLoS One 2015

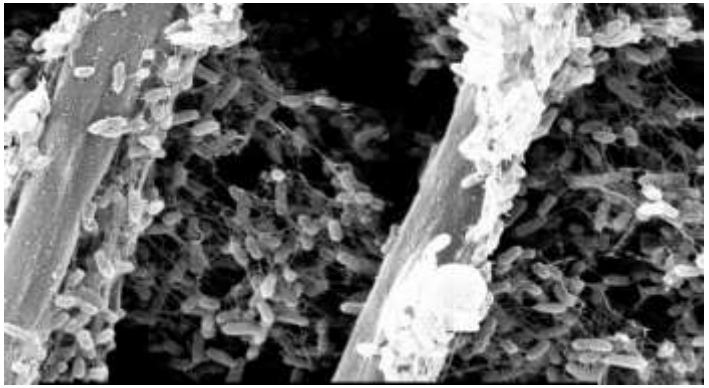
Aspergillus fumigatus enhances elastase production in *Pseudomonas aeruginosa* co-cultures

Karen Smith[1], Ranjith Rajendran[2], Stephen Kerr[2], David F. Lappin[2], William G. Mackay[1], Craig Williams[1] and Gordon Ramage[2,*]

- The presence of Af biofilm increases the production of elastase from Pa in a biofilm model

- This may have a role in the damaging pathology associated with the lung tissue

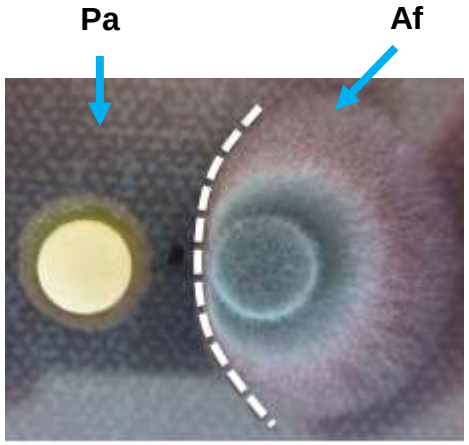
- Co-colonization with these two organisms may result in a poorer prognosis



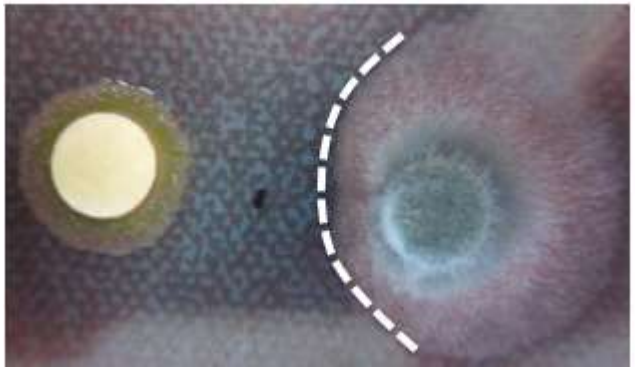
Medical Mycology, 2015

P. aeruginosa and *A. fumigatus* on solid media

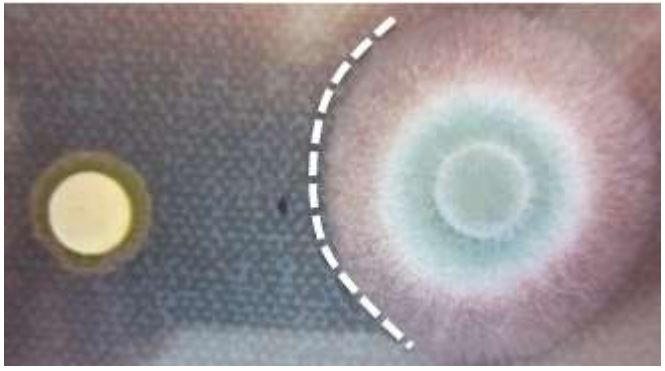
Pa inhibits Af filamentation in a non-contact distance dependent manner



5 mm spacing



10 mm spacing



20 mm spacing

The growth of Af for 5-mm and 10-mm spacing is inhibited in close proximity to *Pa*

Growth recovered when the discs were placed 20 mm apart

The growth *Pa* isolates also appeared to be slightly reduced by the presence of Af, suggesting the **complex interplay between these two species**

Smith K, Medical Mycology 2015

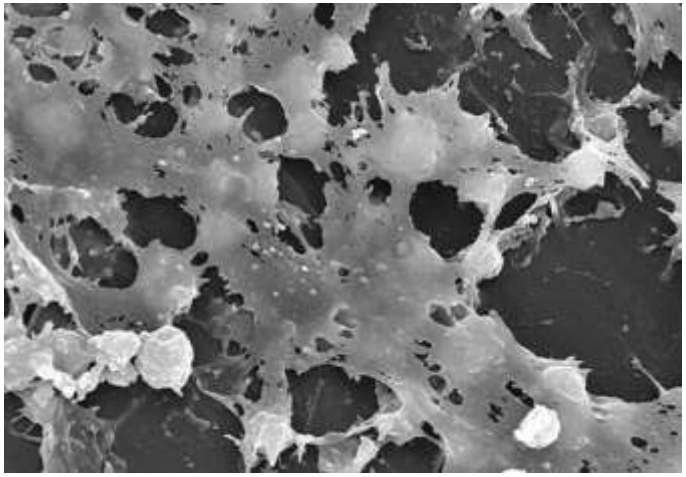
RESEARCH ARTICLE

Biofilm Filtrates of *Pseudomonas aeruginosa* Strains Isolated from Cystic Fibrosis Patients Inhibit Preformed *Aspergillus fumigatus* Biofilms via Apoptosis

Fazal Shirazi[1], Jose A. G. Ferreira[2,3], David A. Stevens[2,3], Karl V. Clemons[2,3], Dimitrios P. Kontoyiannis[1]*

- Pa culture filtrates depresses metabolism, damages membranes and inhibits biofilm Af formation

- Concurrent colonization with both Pa and Af in CF patients leads to further **decline in PFT's** compared to mono-colonization with either microbe



Mowat E, FEMS Microbiol Lett. 2010

PLoS One 2016

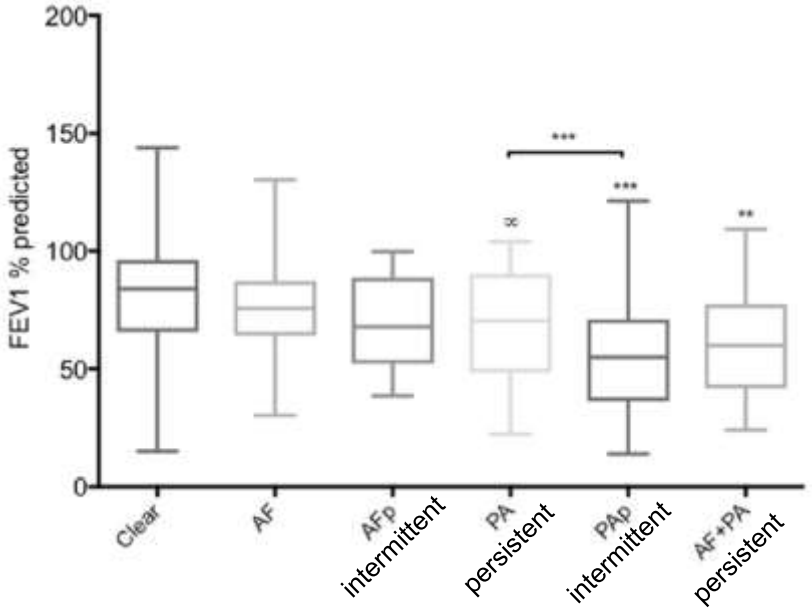
Reece *et al. BMC Pulmonary Medicine* (2017) 17:70
DOI 10.1186/s12890-017-0416-4

BMC Pulmonary Medicine

RESEARCH ARTICLE Open Access

Co-colonisation with *Aspergillus fumigatus* and *Pseudomonas aeruginosa* is associated with poorer health in cystic fibrosis patients: an Irish registry analysis

Emma Reece[1], Ricardo Segurado[2], Abaigeal Jackson[3], Siobhán McClean[4,5], Julie Renwick[1*] and Peter Greally[3,6]



Co-colonization Pa-Af was associated with:

- ✓14% reduction in FEV_1 *(p = 0.016)*
- ✓more exacerbations *(p = 0.042)*
- ✓more hospitalizations *(p = 0.023)*
- ✓more antimicrobial usage *(p = 0.014)*

Reece E et al, BMC 2017

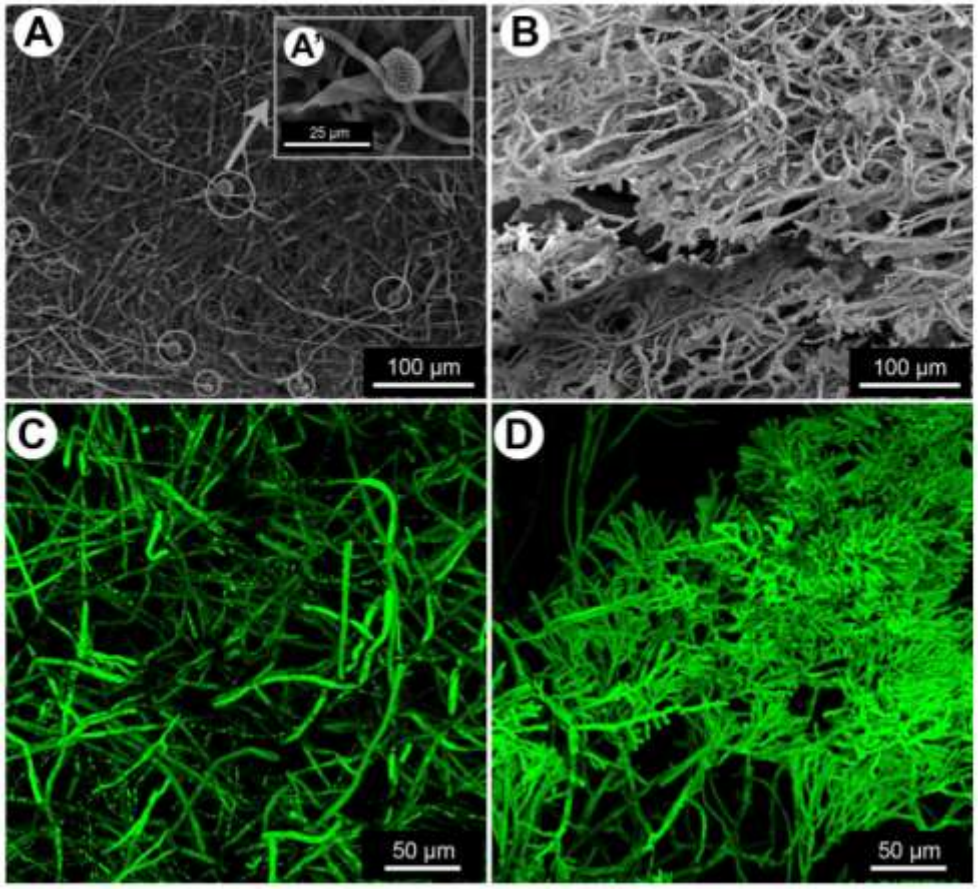
Characteristics of *A. fumigatus* in association with *S. maltophilia* in an *in vitro* model of mixed biofilm

24 h-old single Af biofilm

24 h-old mixed biofilm of Af and Sm

SEM

CLSM



Mixed- biofilm:

- The cell wall is thicker in the presence of the bacteria
- There is more significant chronic inflammation
- Understanding how this microenvironment operates may provide important insight to the development of effective anti-inflammatory therapies

Melloul, PLoS ONE 2016

Novel immunologic classification of aspergillosis in adult CF

	RT-PCR	Galacto-mannan	Asp. Specific IgE	Asp. Specific IgG
Class 1 No disease	**+/-**	**-**	**-**	**-**
Class 2 ABPA	**+**	**+**	↑	↑
Class 3 Asp. sensitized	**+/-**	**-**	↑	**-**
Class 4 Asp. bronchitis	**+**	**+**	**-**	↑

Baxter CG, J Allergy Clin Immunol. 2013

Aspergillus bronchitis in CF

- 6 patients with CF and *Af* in sputum culture but not meeting criteria for ABPA, with acute/subacute clinical deterioration
- No response to appropriate antibiotic treatment but good response to antifungal medications
- Antifungal therapy should be considered during respiratory exacerbations and *Af* isolation when there is no response to appropriate antimicrobial therapy

Shoseyov et al, Chest 2006

Aspergillus sensitization

- It has been shown to be associated with reduced FEV_1 in individuals with CF, but it is not clear if:
  - this is a causal relationship or an epiphenomenon
  - It is amenable to antifungal therapy or any immuno-modulatory treatment
  - It is a precursor to ABPA or a separate distinct aspergillosis phenotype within CF

Kanthan S et al, Pediatric Pulmonology 2007; Wojnarowski C et al, AJRCCM 1997; Fillaux J et al, Scandinavian Journal of Infectious Diseases 2012

Factors Effecting Impact of *Aspergillus fumigatus* Sensitization in Cystic Fibrosis

Senthooran Kathirgama Kanthan, BSc,[1] Andrew Bush, MD,[2]
Michael Kemp, MSc,[2] and Roger Buchdahl, MD[2]*

Methods and Aims:

- To examine the effect of Af sensitization* (Afs) on PFT's and growth using a retrospective cohort analysis over two 5-year study periods
  - 1996–2000 (19 Af cases and 19 controls)
  - 2001–2005 (24 Af cases and 23 controls)
- To examine the impact of changing treatment schedules over these periods

**defined as Af specific RAST 17.5 iu/ml + total serum IgE level 150 iu/ml*

Ped Pulmon 2007

Factors effecting impact of Af sensitization in CF

- Afs cases had lower FEV_1% compared to matched controls
- Afs cases in the 2001 cohort, compared to the 1996 cohort:
  - had a higher FEV_1%
  - were prescribed significantly more oral antifungal treatment
- The use of antifungal treatment is associated with better lung function

Kanthan et al, Ped Pulmon 2007

Antifungal therapy to treat *Aspergillus* colonization or infection in CF patients

Table 2
Summary of published studies using antifungal medication to treat *Aspergillus* spp. respiratory colonisation or infection in patients with CF.

Study	Patients	Intervention	Outcome
Hilliard et al. [32]	n=21; median 11.3 (5–16) years. Mixture of ABPA and non-ABPA subjects.	All children received voriconazole for a median of 22 (1–50) weeks.	Significant increase in FEV_1 and FVC over the study period in ABPA subjects only.
Shoseyov et al. [33]	n=6; median 14 (10–30) years.	Oral itraconazole for 4 months up to 2 years. One patient also received iv Ambisome.	All experienced some improvement in clinical parameters, FEV_1% predicted improved in all but one.
Kanthan et al. [31]	n=85; *1996 cohort*, *Af* cases, n=19, median age 13.66 (9.78–15.99) years; controls n=19, median age 12.44 (9.02–14.96) years. *2001 cohort*, *Af* cases n=24, median age 12.58 (9.61–16) years; controls n=23, median age 14.61 (12.68–15.29) years.	Observational cross-sectional cohort study using two cohorts of patients (1996–2000 and 2001–2005).	Patients in the 2001 cohort received more antifungal therapy and had a higher FEV1% predicted compared to the 1996 cohort.
Aaron et al. [30]	n=35; enrolled patients 6 years and older, mean age 25 years in both groups.	Oral itraconazole for 24 weeks or placebo.	No difference in exacerbation rates, FEV_1% predicted or QOL between the two groups.

OPEN ACCESS Freely available online PLoS one

Treatment of *Aspergillus fumigatus* in Patients with Cystic Fibrosis: A Randomized, Placebo-Controlled Pilot Study

Shawn D. Aaron[1*], Katherine L. Vandemheen[1], Andreas Freitag[2], Linda Pedder[2], William Cameron[1], Annick Lavoie[3], Nigel Paterson[4], Pearce Wilcox[5], Harvey Rabin[6], Elizabeth Tullis[7], Nancy Morrison[8], Felix Ratjen[9]

Objective: to determine whether treatment directed against Af improves PFT's and clinical outcomes in patients with CF

N= 35 CF patients, chronically positive for Af were centrally randomized to receive for 24 weeks

- oral itraconazole 5 mg/kg/d (n = 18)
- placebo (n = 17)

- Primary outcome: proportion of patients who experienced a respiratory exacerbation requiring IV Abx: **no difference**
- Secondary outcomes: changes in FEV_1 and QoL: **no difference**

Problematic:
1- Adequate plasma levels were achieved in only half of the patients
2- Itraconazole resistance was not tested
3- Small number of patients (17 and 18 patients!), short time

Aaron et al, PLoS One 2012


The dilemma continues…
Aspergillus fumigatus:
to treat or not treat…?