Cystic fibrosis and biofilms: clinical and experimental scenarios

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Clinical symptoms CF

- Lungsymptoms
  - Colonisation or chronic infection with
    - *S. aureus, H. influenzae*
    - Non-muc/muc *P. aeruginosa*
  - Chronic cough and sputum production
  - X-ray with atelektasis, infiltrates
  - Nasalpolyps

- The defect CFTR protein lead to sticky and dehydrated secretion in the airways and is the main reason for bacterial lung infections

- Gastrointestinal and nutritional problems
- Loss of salt in sweat

- Male infertility

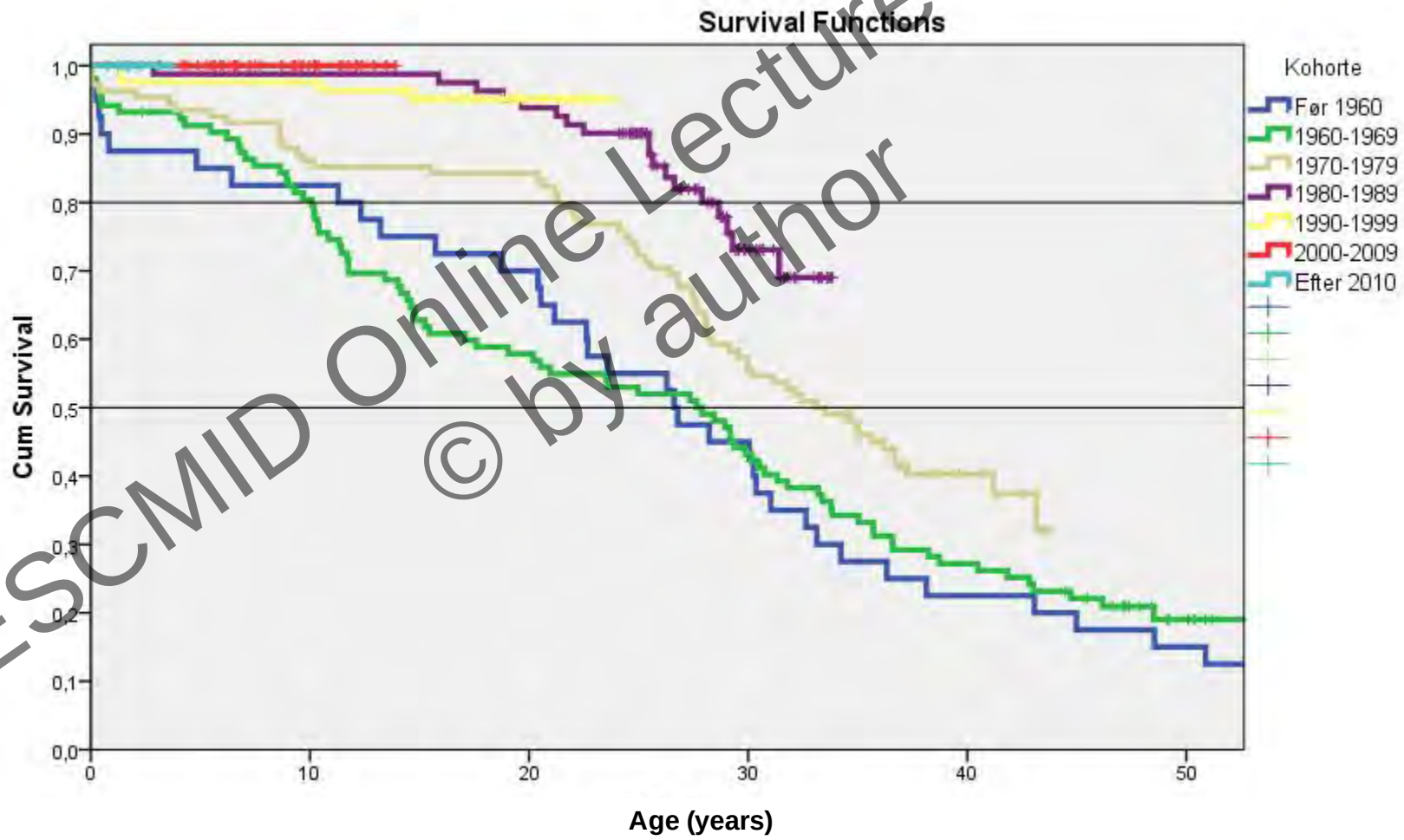
Treatment strategies

- 1968 The Copenhagen CF-centre is established
- 1971 Symptomatic antibiotic therapy
- 1976 Regular antibiotic therapy
- 1981 Cohort-isolation to avoid cross-infection
- 1983 High-calorie diet
- 1987 Antibiotic therapy between iv courses
  ”maintenance therapy”
- 1989 Early eradication therapy
  antibiotics for colonised patients: 3 months (3 weeks)
  oral ciprofloxacin and inhaled colistin
- 1994 Pulomzyme inhalation for chronically infected patients
- 2001 Azithromycin
- 2007 Sinus surgery

Johansen et al: Pediatr Pulmonol, 2004

Survival from birth illustrated by birth cohorts in the Copenhagen CF centre

80% possibility for a new-born with CF to reach the age of 50 years



Pressler T & Hansen CR

Environment/transmission Sinuses Lungs

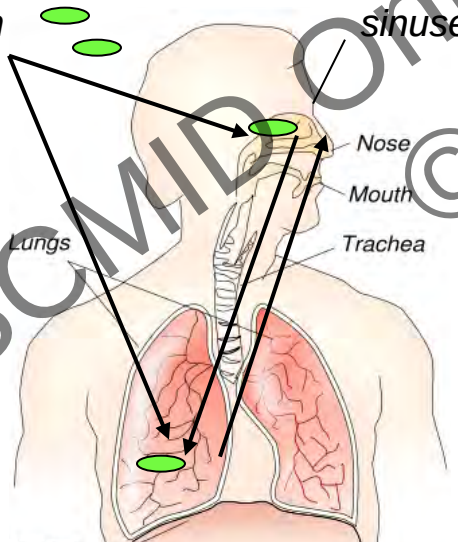
Unique genotypes, fast growth, non-mucoid, antibiotic-susceptible

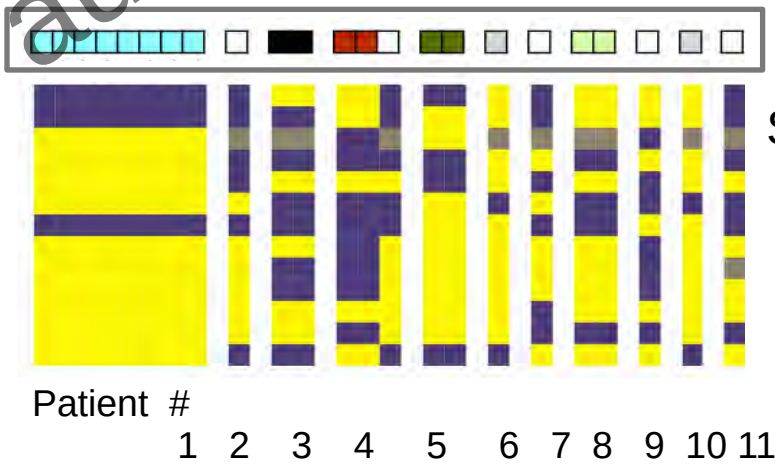
Antibiotic resistance, mucoid, loss of QS and motility, biofilm formation

Adaptation / evolution

Environmental strain

Paranasal sinuses

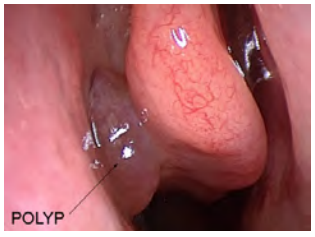


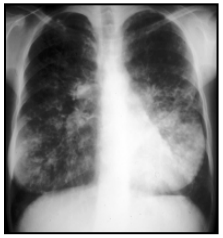
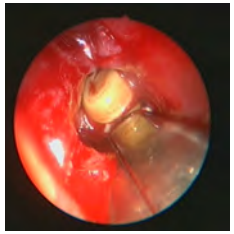


Jelsbak L et al. I&I 2007

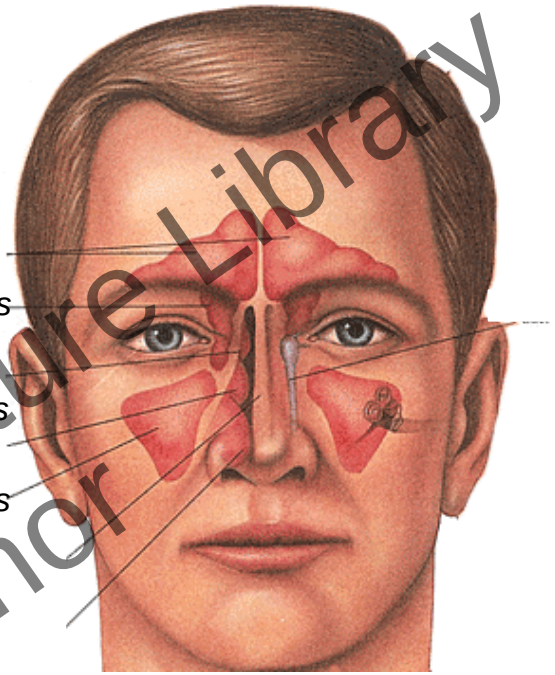
Early colonisation: sinuses versus lungs

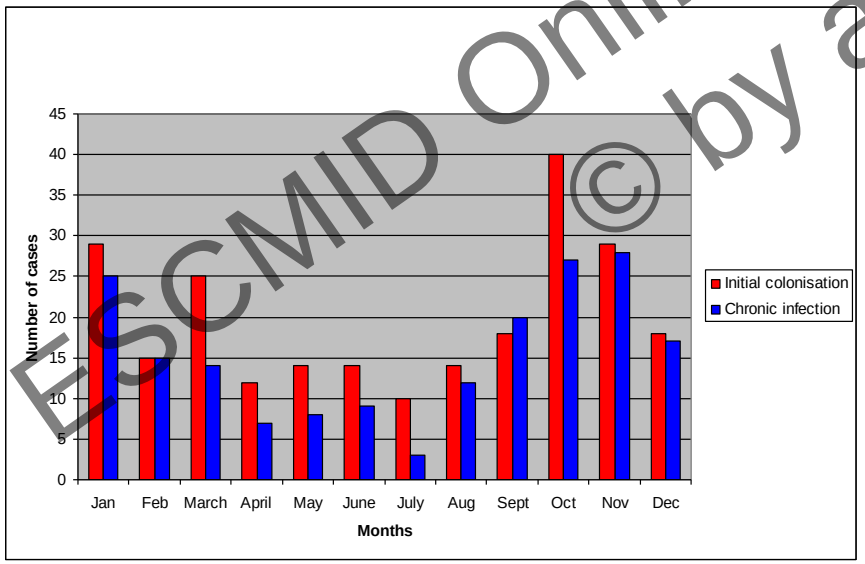
- Different types of colonisation
  - Every colonisation is most often with a new environmental *P. aeruginosa* strain
  - Several recurrent colonisations with the same genotype
- The sinuses may in the early colonisation periods provide opportunities for localised evolution
- Early antibiotic therapy removes *P. aeruginosa* from the lungs
- Obstructed sinus cavities lead to
  - Stationary bacterial population and reduced penetration of antibiotics leads to antibiotic resistance, changes in nutrient utilization and reduced growth rate

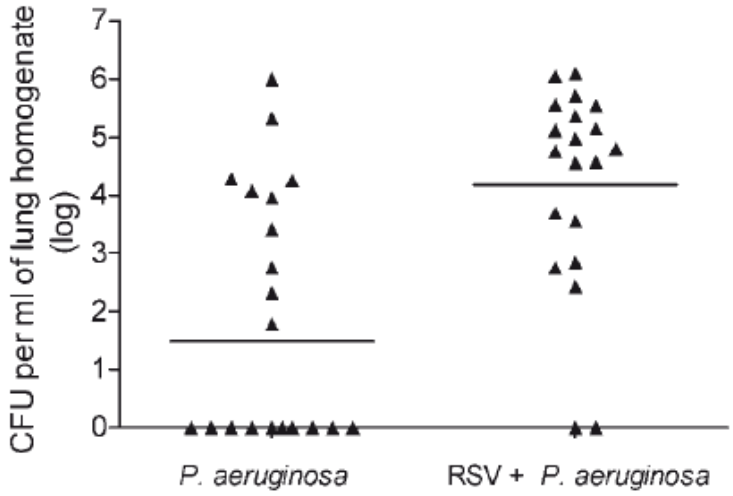


How do bacteria get from the sinuses to the lungs?







Johansen et al 1992, de Vrankrijker et al 2009

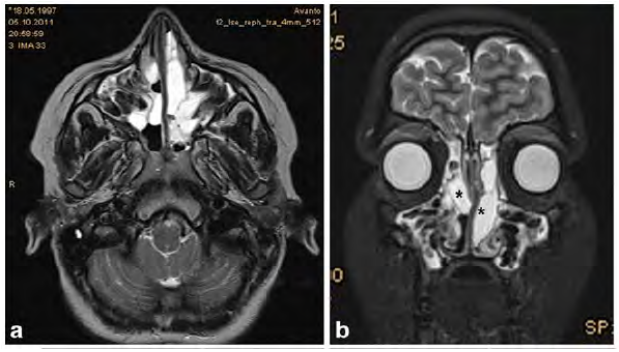
What do we know about the sinuses in CF?

- "United airways": sinuses and lungs have similar physico-chemical properties
- All CF patients have chronic rhinosinusitis (>12 w) - but it has been neglected for decades when compared to the lung symptoms
- Symptoms
  - Nasal obstruction and polyps (6-48%)
  - Facial pain, rhinorrhoea, loss of smell
  - Tiredness and fatigue
- Mechanisms leading to chronic rhinosinusitis:
  impaired mucociliary clearance and pus in the maxillary cavity

  oedema → bacterial overgrowth → obstructed sinus ostia

Copenhagen “principles” for sinus surgery in CF patients

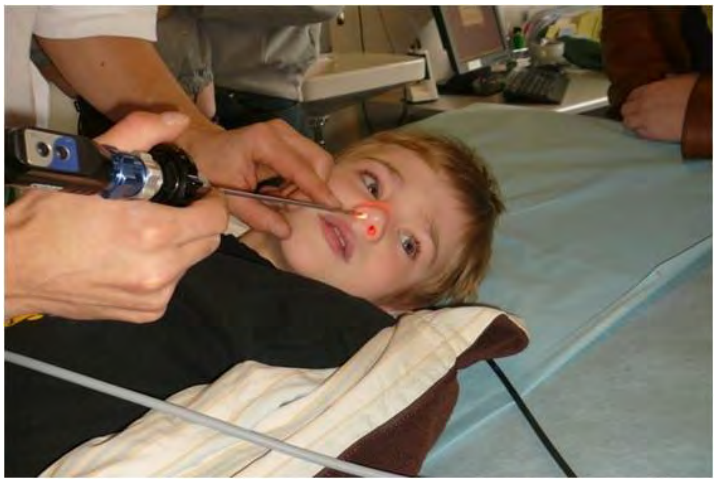
- Search for an infectious focus
  - increasing frequency of positive sputum cultures
  - declining lung function (>10%)
  - increasing antibodies against *P. aeruginosa, A. xylosoxidans* or *B. cepacia complex*
- Patients who have recently been lung transplanted (within a year after ltx)
- Patients with severe symptoms of chronic rhinosinusitis according to guidelines (EPOS) when conservative treatment was unsuccessful



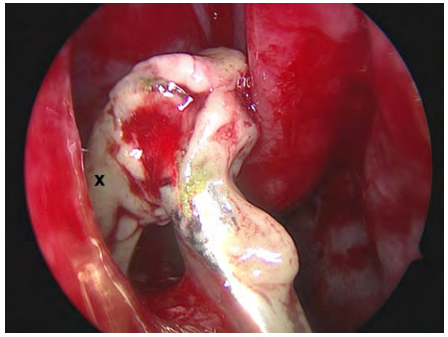
Sinus surgery: pre- and post surgery protocol

Monthly BMI, FVC, and FEV_1 measurements and samples from lower airway cultured

4 visits to the ENT-out-patient clinic



The SNOT-22 questionnaire focuses on sinonasal conditions
The CFQ-R questionnaire is CF disease specific



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Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Original Article

Colonisation and infection of the paranasal sinuses in cystic fibrosis patients is accompanied by a reduced PMN response☆

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78 CF patients included (FESS)

21 chronically infected with PA
18 growth of PA in sinuses and lungs, 100% genetically identical

31 intermittently colonised with PA
21 growth of PA in sinuses and lungs, 91% genetically identical

Concordant genotype of upper and lower airways

- Simultaneously nasal lavage and sputum culture (N=187)
- 86% (31/36) of *S. aureus* and 95% of *P. aeruginosa* strain pairs were genotypically identical (spa or SNPs)
- The UAW play a role as a reservoir of *S. aureus* and *P. aeruginosa*

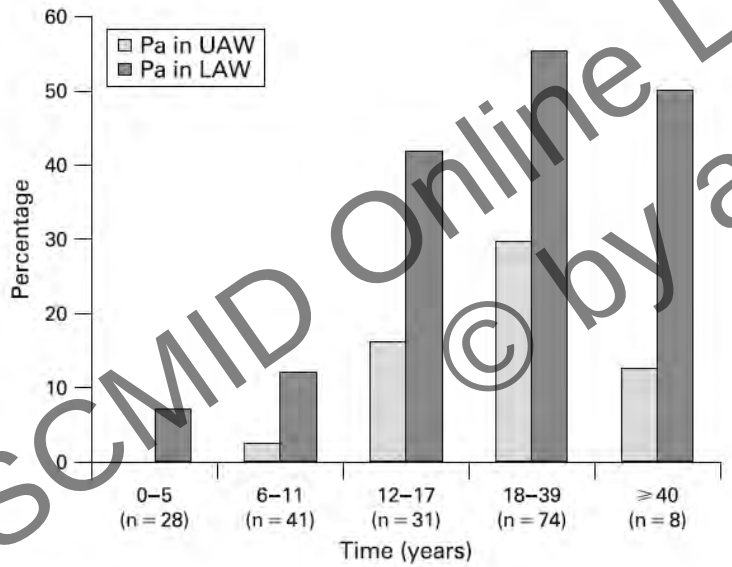
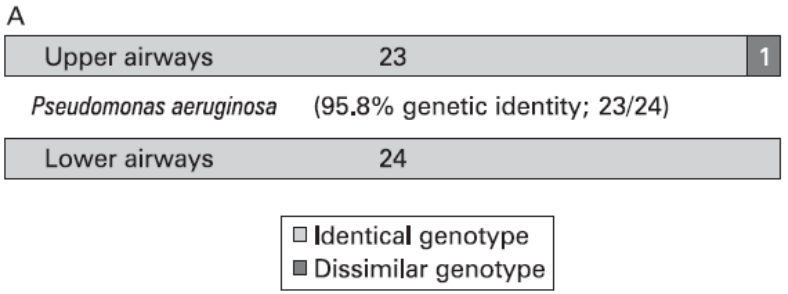
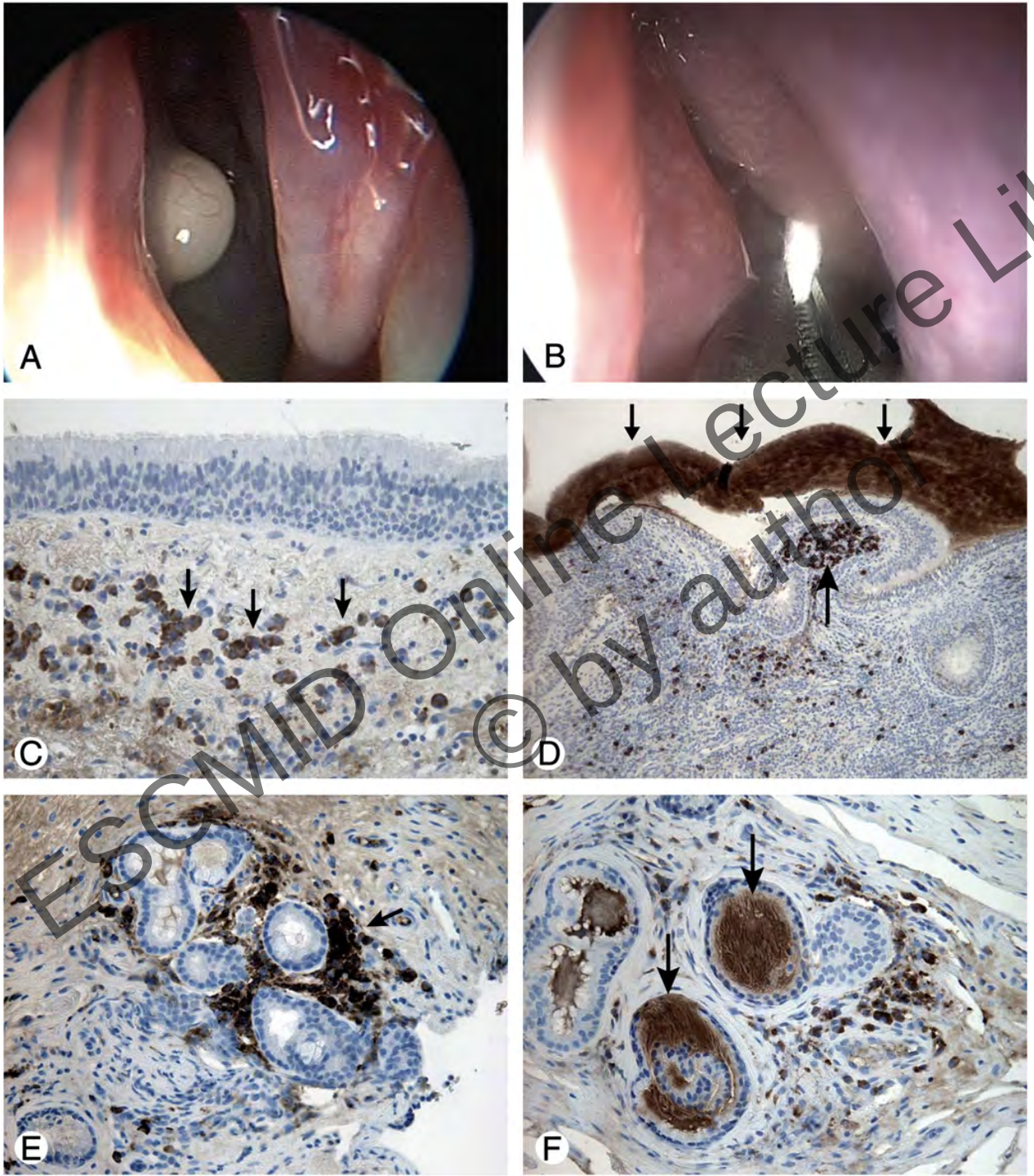


Figure 2 Fraction of patients with detection of *Pseudomonas aeruginosa* (Pa) in the upper (UAW) and lower airways (LAW) in relation to their age (n = 182).



Mainz JG et al. Thorax 2009;64:535-40

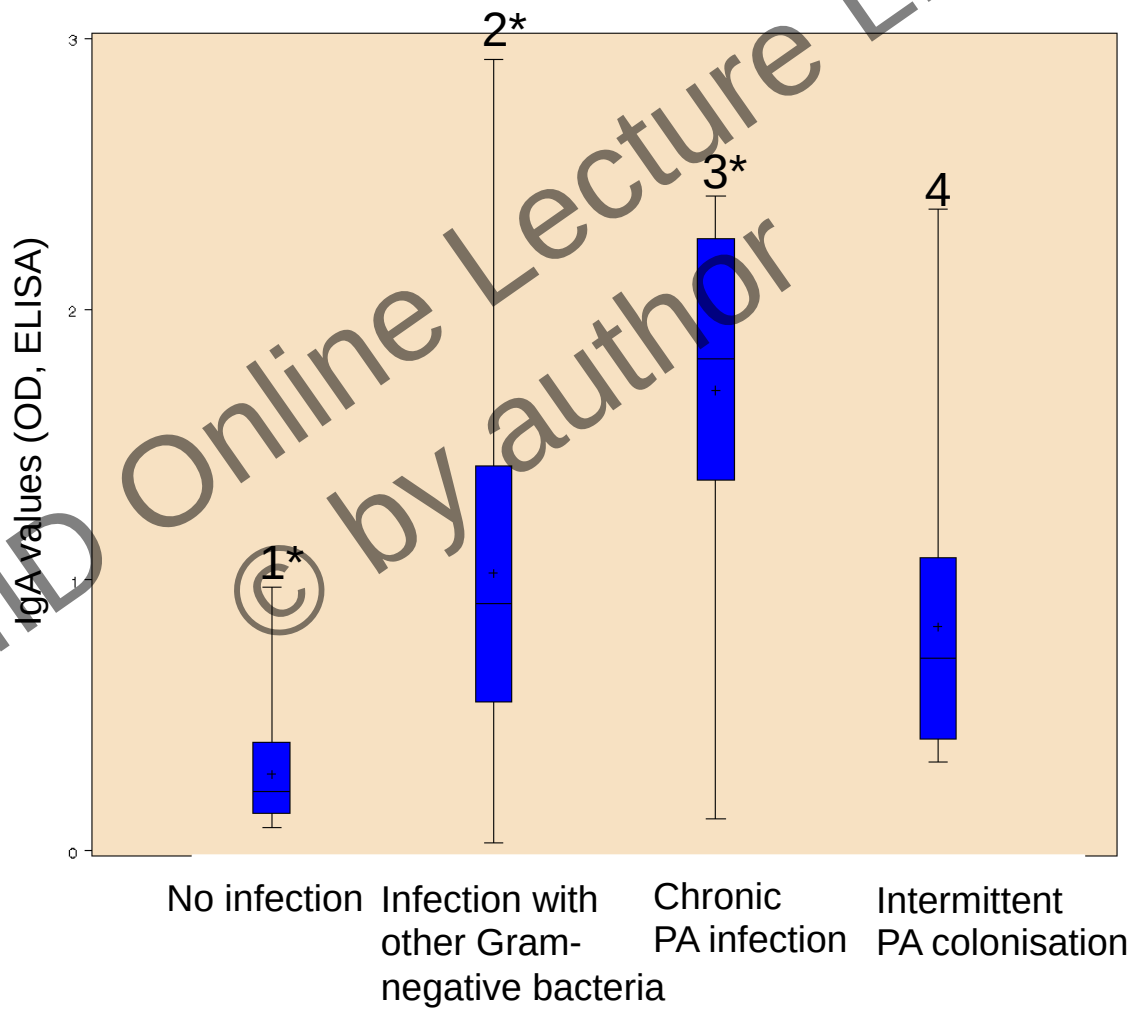


IgA containing plasma cells in lamina propria and secretion of excretory ducts

Polyclonal antibody to IgA

Aanaes et al. J Cyst Fibros 2013

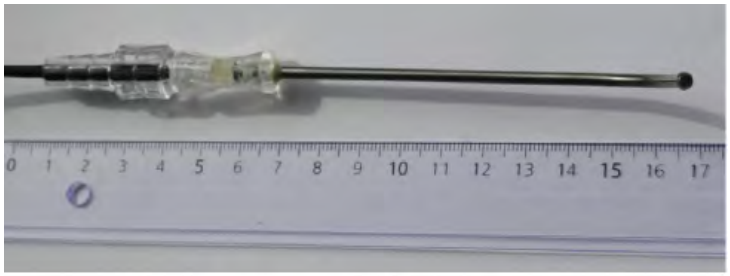
IgA against *P. aeruginosa* alginate in nasal secretions: four infection categories



*p<0.0001 when compared

Aanaes K. et al. JCF 2012

Reduced O_2 in CF sinuses



Catheter O_2 optode for measuring oxygen tension

- *P. aeruginosa* can adapt to the microaerophilic environment in the sinuses
- Less well functioning immune system in O_2 depleted areas

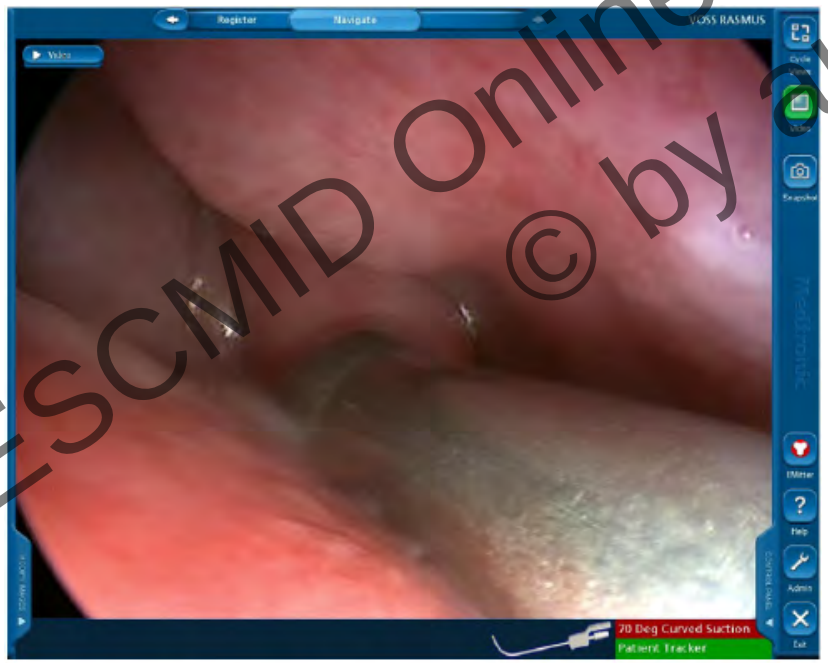
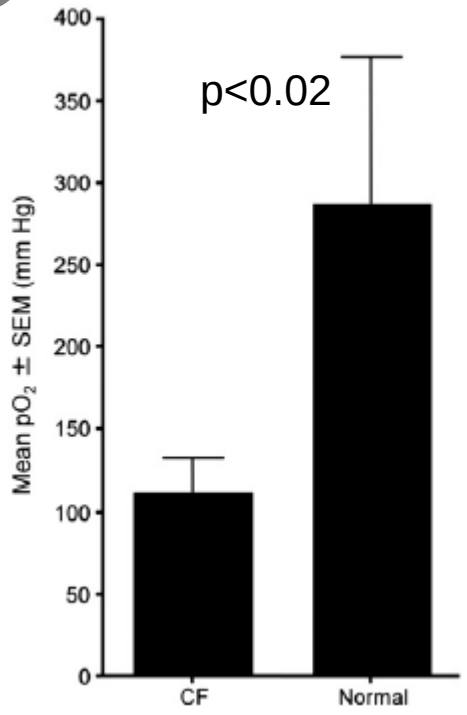
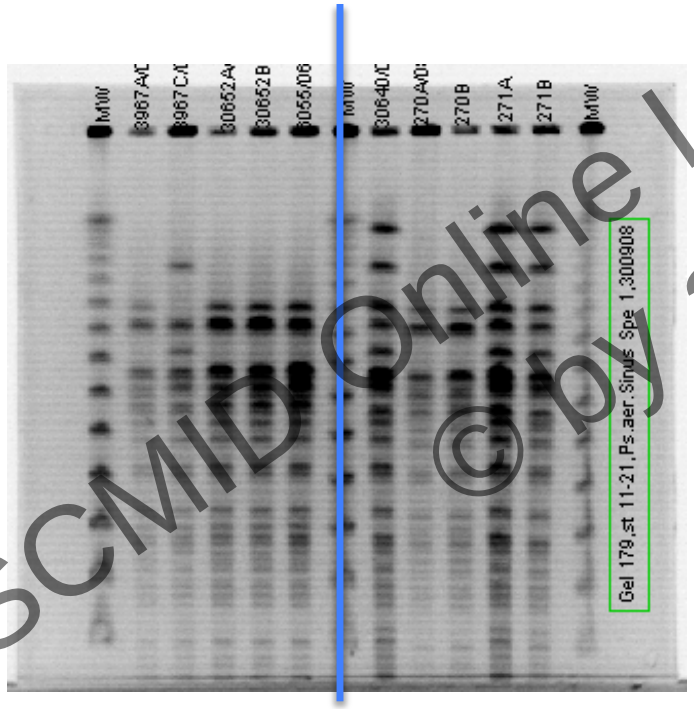


Fig. 2. The catheter on its way to the right maxillary sinus.



Aanaes K et al.: JCF 2011;10:114-20

Similar genotypes and colony phenotypes in the lungs and sinuses

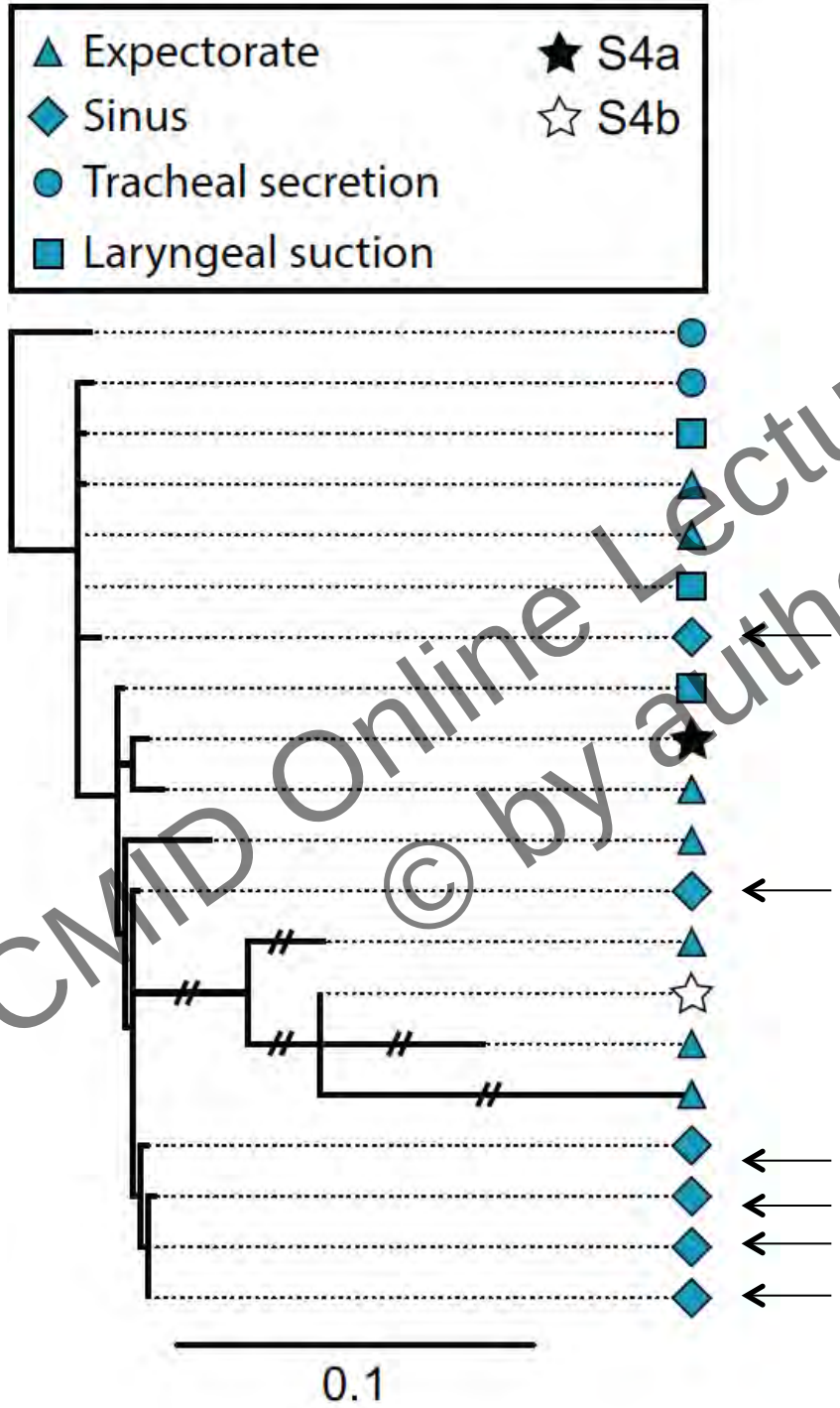


PFGE sputum (PA) sinus (PA)

Patient No.	No. of colony morpho-types in the lungs	No. of colony morpho-types in the sinuses	No. of colony morpho-types in both places
B11	6	11	4
B22	7	6	3
B28	4	5	2
B34	4	3	3
B42	5	6	2

Overlap, several *P. aeruginosa* phenotypes found in the lungs were also cultured in the sinuses

Hansen SK et al.: ISME J. 2012;1:31-45
Johansen HK et al.: JCF 2012

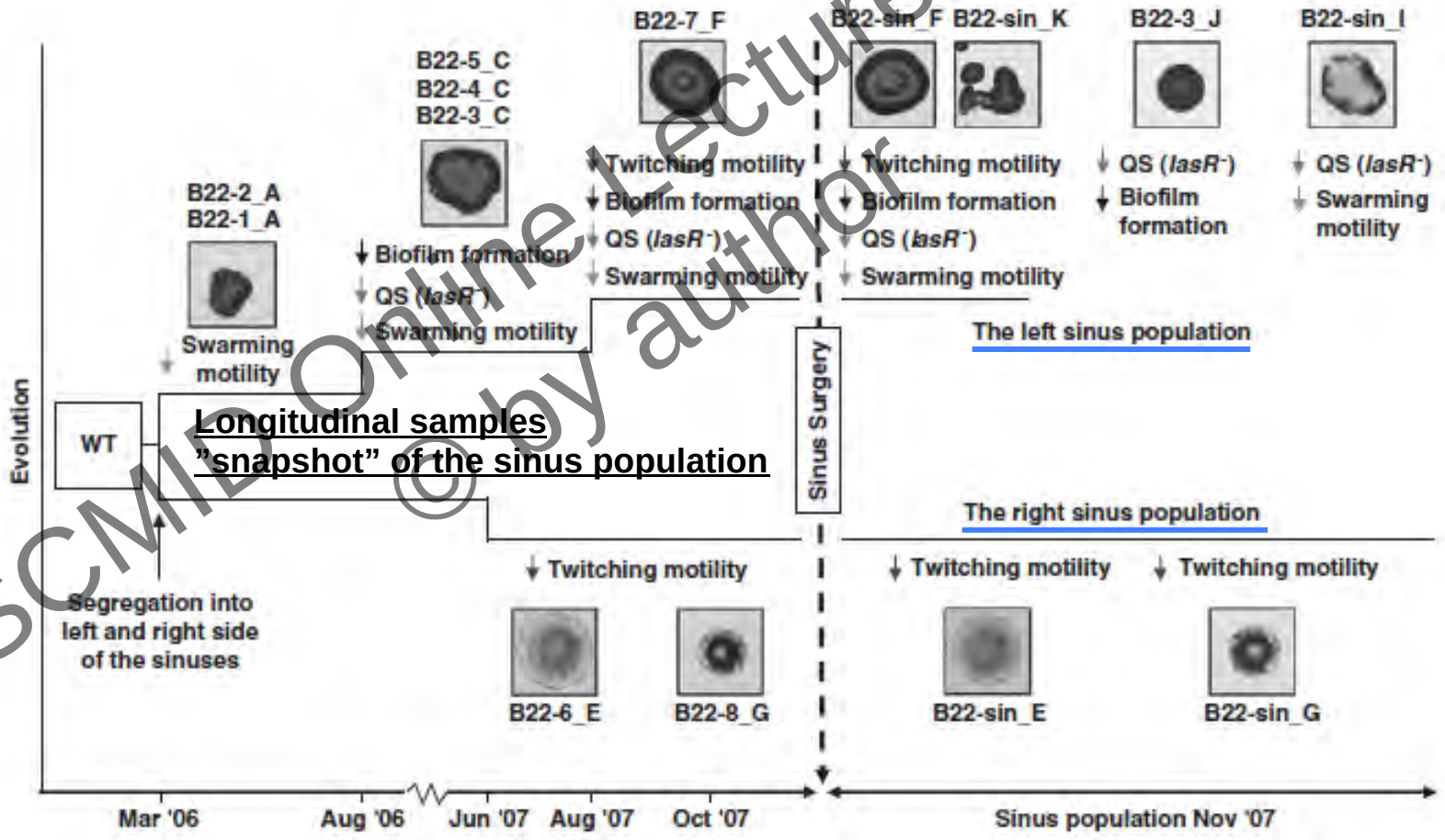


Phylogenetic three showing isolates from the upper and lower airways

Sommer et al. BMC genomics 2016

An evolutionary model of the sinus population in a CF child

Evolution of the lung population,
two different phenotypic lineages



Child intermittently colonised with *P. aeruginosa*: identity between late lung and sinus isolates

Hansen SK et al.: ISME J. 2012;1:31-45

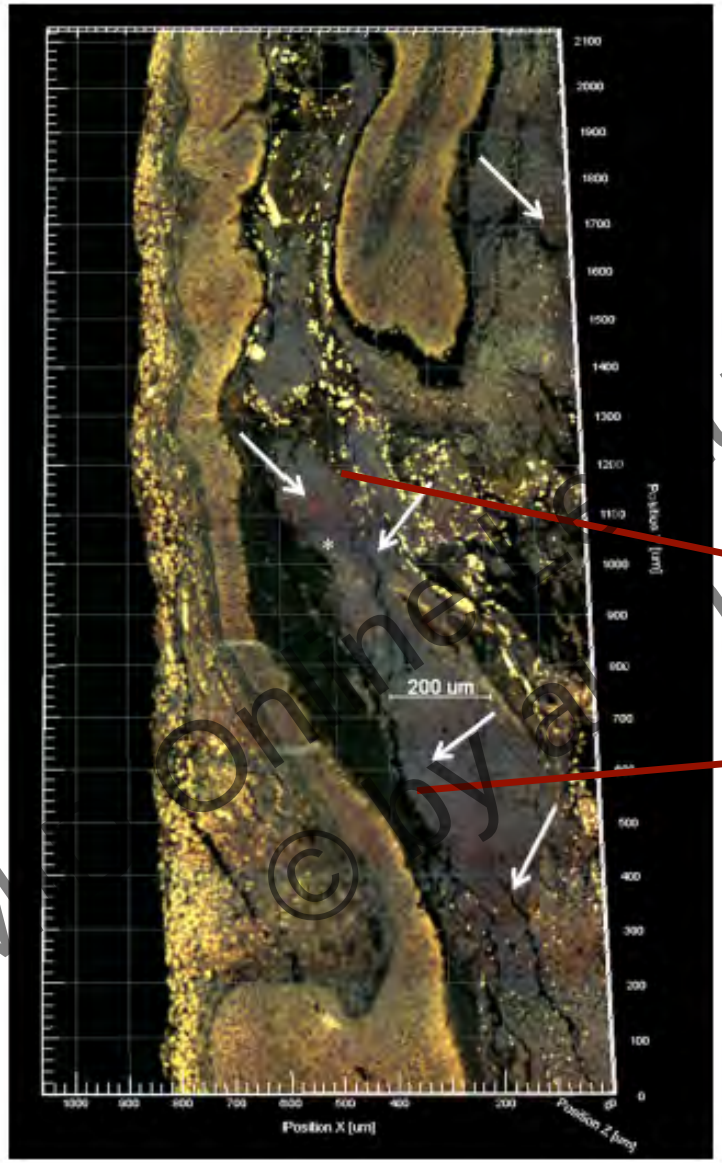


Fig. 2 Sinus mucosa and secretions stained with PNA-FISH containing a universal bacterial probe (FITC), a *P. aeruginosa* probe (*Texas Red*) and DAPI as a counter stain. The *blue stain* is DAPI, staining DNA within the nucleus of eukaryotic cells. The *golden colour* is autofluorescence from erythrocytes and their breakdown products. Overview of a sinus mucosa sample. The *arrows* denote areas with biofilm while *asterisk* denotes the area presented in Fig. 1

Aanaes at al. Eur Arc Oto-Rhin-Laryngol. 2014

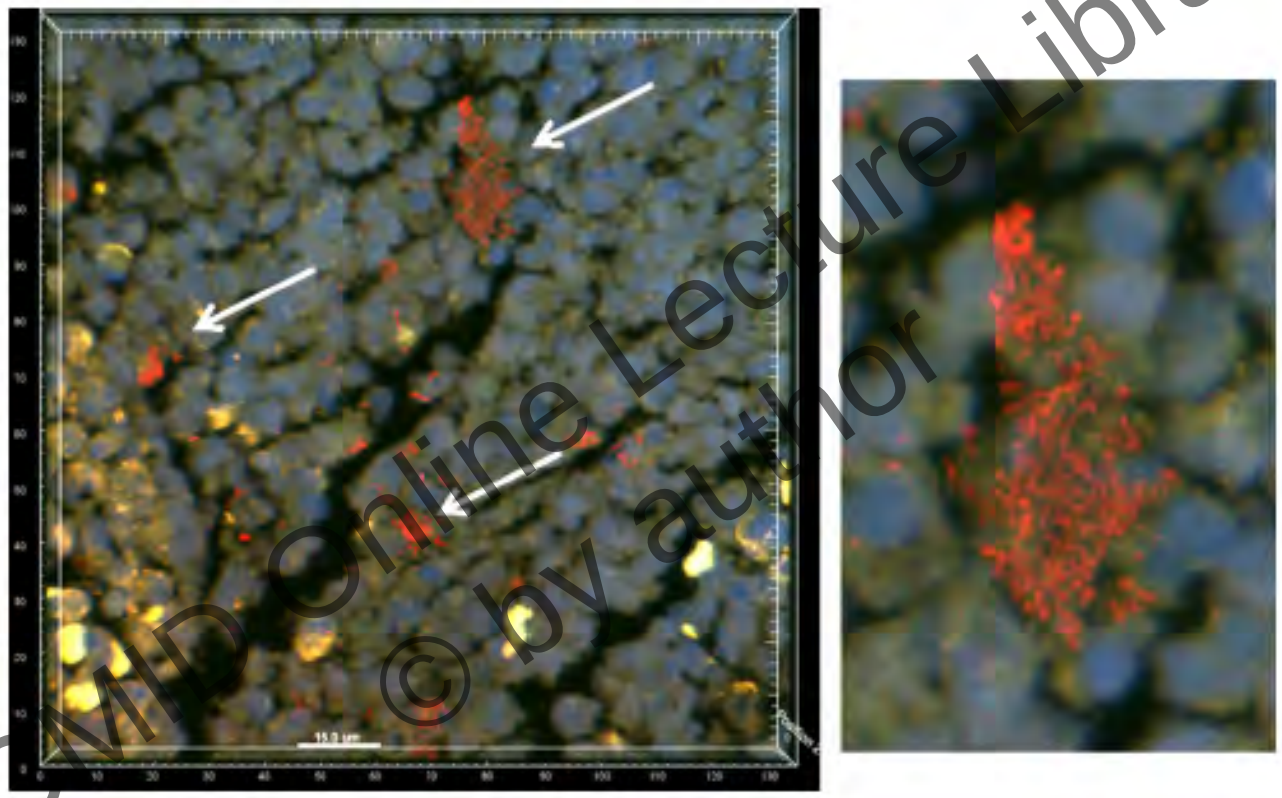


Fig. 1 Sinus mucosa and secretions stained with PNA-FISH containing a universal bacterial probe (FITC), a *P. aeruginosa* probe (*Texas Red*) and DAPI as a counter stain. The *blue stain* is DAPI, staining DNA within the nucleus of eukaryotic cells. The *golden colour* is autofluorescence from erythrocytes and their breakdown products. The micrograph shows several smaller *P. aeruginosa* biofilms (denoted with *arrows*) with varying density and size, and planktonic *P. aeruginosa* cells intermingled among a pronounced inflammatory response. The biofilm pointed out by the *top arrow* in the left picture is magnified to the right

Aanaes at al. Eur Arc Oto-Rhin-Laryngol. 2014

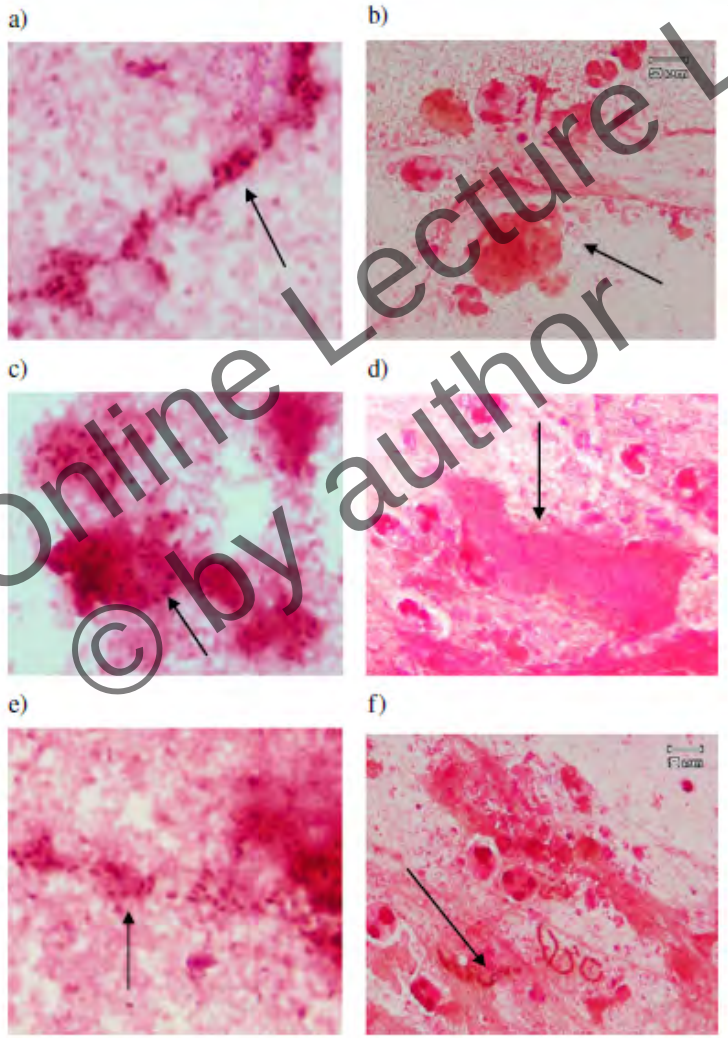
Gender, age at surgery, lung infection status	Genes with delta F508	Sinus bacteria at surgery	Biofilm	Biofilm PA	Biofilm SA	Biofilm other
♀ 21, LTX with AX	2	AX, SA	N			
♂ 45, LTX with PA	2	Muc PA, CNS	Y	N	N	Y
♀ 34, LTX with PA	1	Muc PA, NM PA	N			
♀ 34, LTX with SA	1	CNS	N			
♂ 38, Chronic PA	2	NM PA	Y	N	Y	N
♀ 26, Chronic AX	2	AX	Y	N	Y	Y
♂ 13, Intermittent PA	2	(Left): NM PA, SA, CNS	Y	Y	N	Y
		(Right): SA, CNS	Y	N	Y	N
♀ 7, Intermittent PA	1	(Left) No growth	N			
		(Right) No growth	N			
♂ 11, Intermittent PA	1	NM PA, Steno	N			
♂ 22, Intermittent PA	2	Muc PA, NM PA, SA	N			
♂ 7, Intermittent PA	2	NM PA, CNS	Y	N	N	Y
♂ 21, Intermittent AX	1	AX, SA	Y	N	Y	N
♂ 13, Intermittent AX	0	AX	Y	N	N	Y
♂ 9, Intermittent PA	2	Steno, Serratia	N			
♂ 28, Intermittent PA	1	Serratia	Y	Y	N	N
♂ 13, Intermittent PA	2	CNS	Y	N	N	Y

Small biopsies, PNA-FISH may not be the optimal diagnostic tool to detect biofilm

Aanaes at al. Eur Arc Oto-Rhin-Laryngol. 2014

P. aeruginosa biofilms (aggregates) different location - different immune response

Biofilms in <u>sinuses</u>
no PMNs



Biofilms in the <u>lungs</u>
many PMNs

Fig. 1. a–f. Microscopic investigation of Gram-stained smears of pus from the sinuses (a, c and e) and corresponding sputum (b, d and f) obtained from three patients chronically infected with *P. aeruginosa* at the time of sinus surgery, magnification ×1000. Arrows indicate biofilms.

Johansen HK et al. JCF 2012

Clinical effect of sinus surgery in 106 Copenhagen CF patients

Prospective, non-randomized, cohort study

	Non-infected	Intermittently colonized	Chronically infected	Total
Male/female	8 / 8	29 / 32	12 / 17	49 / 57
Median age in years at FESS (range)	18 (6–44)	15 (6–50)	30 (11–46)	19 (6–50)
F508del homozygous / heterozygous/ other mutations	13 / 3 / 0	41 / 16 / 4	17 / 12 / 0	71 / 31 / 4

Aanaes, Johansen et al. Rhinology 2013

Lung infection status after FESS (N=106)

	Non-colonized before/after FESS	Intermittently colonized before/ after FESS	Chronically infected before/ after FESS
P. aeruginosa	-	50/31	20/20
A. xylosoxidans	-	9/6	7/5
B. cepacia complex	-	2/1	2/3
Total	**16/40**	**61/38**	**29/28**

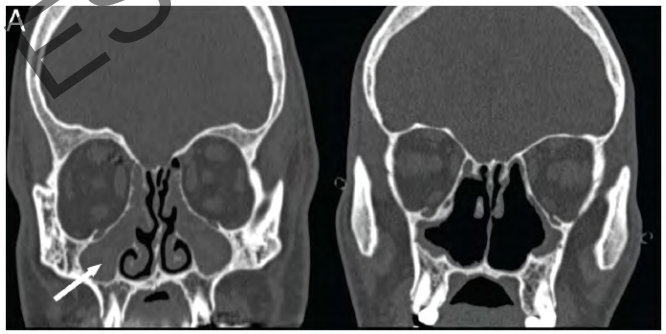
*The one-year prevalence of intermittent colonization **decreased** by 38% (CI: 24%–51%) after FESS (58% at surgery compared with 36% one year postoperatively; p<0.01)*

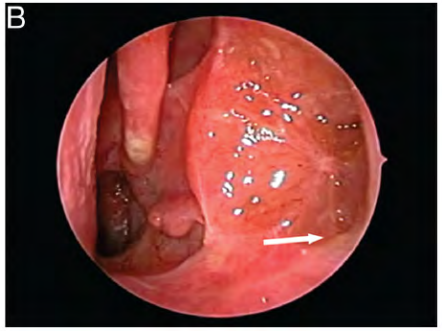
*The one-year prevalence of non-colonized patients **increased** by 150% (CI: 71%–310%) after FESS (15% at surgery compared with 38% one year postoperatively; p<0.01)*

Aanaes JCF 2013, Aanaes, Johansen Rhinology 2013

Case-stories of long-term follow-up after FESS

CF-patient	Lung and sinus bacteriology	Lower airway samples with PA or AX one year prior to FESS	Days free of bacteria
11-year-old girl	*P. aeruginosa*	62%	523
16-year-old boy	*P. aeruginosa*	60%	593
22-year-old man (ltx)	*A. xylosoxidans*	77%	>982
31-year-old woman (ltx)	*P. aeruginosa*	75%	508





Aanaes, Johansen et al. Rhinology 2013

Lung infection status at at sinus surgery	One year later*	Three years later*
No PA: 16 (15%)	**No PA: 14 (13%)** Intermittent: 2 Chronic: -	**No PA: 12 (11%)** Intermittent: 2 Chronic: 2
Intermittent PA: 61 (58%)	**No PA: 22 (21%)** Intermittent: 35 Chronic: 4	**No PA: 22 (21%)** Intermittent: 28 Chronic: 11
Chronic PA: 29 (27%)	**No PA: 4 (4%)** Intermittent: 1 Chronic: 24	**No PA: 1 (1%)** Intermittent: 4 Chronic:24

*= sinus surgery + adjuvant therapy

Alanin MC et al. Rhinology 2016

SNOT-22 and CFQ-R before and after FESS

	Pre-operatively	Three months	Six months	Twelve months
Patients who completed SNOT-22, (response rate), [mean time after FESS]	86	79 (92%) [103 days]		64 (80%) [367 days]
Mean SNOT-22	20	11*		14*
Patients who completed CFQ-R, (response rate), [mean time after FESS]	67		53 (79%) [168 days]	42 (69%) [369 days]
Mean (CFQ-R) 6–13 years: More than 13 years: Total:	 143 165 156		 145 170* 162*	 144 163 157

*: $p < 0.05$.

SNOT-22 focuses on sinonasal conditions:
contains 22 items graded from 0 (no problems) to 5 (as bad as it can be)

CFQ-R: two questionnaires for patients >13 years (max score 201) and one for patients 6-13 years (max score 177)

Conclusions

- Same environment in the sinuses and lungs
- Similar bacterial genotypes and phenotypes in sinuses and lungs
- Different immune responses
  - In the sinuses IgA prevents attraction of PMNs, complement is not activated and the oxidative burst is diminished
  - In the lungs *P. aeruginosa* elicits stimulation of the innate immune system and inflammation leading to high IgG response
- Aggregates have same 'phenotype' in upper and lower airways
- In early stage infection, FESS and intensive local antibiotic therapy can suppress the number of *P. aeruginosa* in the sinuses, which may delay transition to chronic lung infection
- The number of non-colonised CF patients increased from 16 to 35 three years after surgery + intensive antibiotic therapy
- After FESS: decrease in sinonasal symptoms / improvement in QoL