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Original Article

Bacterial sinusitis can be a focus for initial lung colonisation and chronic lung infection in patients with cystic fibrosis

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Abstract

A major purpose of treating patients with cystic fibrosis (CF) is to prevent or delay chronic lung infections with CF-pathogenic Gram-negative bacteria. In the intermittent stage, bacteria can usually be eradicated from the lungs with antibiotics, but following eradication, the next lung colonisations often occur with bacteria of identical genotype. This may be due to re-colonisation from the patient's paranasal sinuses. In our study, we found that approximately two-thirds of CF patients having sinus surgery (FESS) had growth of CF-lung-pathogenic Gram-negative bacteria in their sinuses (*Pseudomonas aeruginosa*, *Achromobacter xylosoxidans*, *Burkholderia cepacia* complex).

The environment in the sinuses is in many ways similar to that of the lower respiratory tract, e.g. low oxygen concentration in secretions. Sinus bacteria are more difficult to eradicate than in the lungs, thus, having good conditions for adapting to the environment in the lungs. In the presence of bacteria, the environment of the sinuses differs from that of the lower respiratory tract by having a higher immunoglobulin A (IgA):IgG ratio, and reduced inflammation. We found a significant correlation between the concentration of IgA against *P. aeruginosa* (standard antigen and alginate) in nasal secretions and saliva and CF patients' infection status (not lung colonised, intermittently colonised or chronically lung-infected with *P. aeruginosa*). This supports the hypothesis that infections often originate in the sinuses and can be a focus for initial lung colonisation or for maintaining lung infections in CF patients. We are confident that anti-*P. aeruginosa* IgA can be used as an early supplementary tool to diagnose *P. aeruginosa* colonisation; *P. aeruginosa* being the microorganism causing most morbidity and mortality in CF patients. This is important since urgent treatment reduces morbidity when CF patients are early colonised with *P. aeruginosa*, however, there is a lack of diagnostic tools for detecting the early colonisation in the lungs and in the sinuses.

We initiated a treatment strategy for CF patients to prevent sino-nasal bacteria being seeded into the lower airways: we recommended extensive functional endoscopic FESS with creation of sufficient drainage from all involved sinuses with subsequent i.v. antibiotics and at least 6 months of twice daily nasal irrigation with saline and antibiotics. By this strategy, sinus bacteria could be eradicated in a large proportion of patients. Essentially, growth of CF-pathogenic bacteria from the lower respiratory tract was decreased following the treatment. Furthermore, a number of patients have been free from CF-pathogenic bacteria for more than one year after FESS, and thus re-classified as "not lung colonised". We also corroborated that CF patients obtain an improved quality of life and reduction in their symptoms of chronic rhinosinusitis after FESS.

It is primarily intermittently lung colonised CF patients with CF-pathogenic bacteria in their sinuses that seem to benefit from the treatment strategy. This is in accordance with the fact that we did not see a significant increase in lung function and only a small decrease in specific antibodies after FESS; a high systemic immune and inflammatory response and a decreasing lung function is generally not present in patients who primarily have sinus CF-pathogenic bacteria.

It is important that guidelines are created for how CF patients with CF-pathogenic bacteria in the sinuses are to be treated, including criteria for who may likely benefit from FESS, and who may be treated exclusively with conservative therapy, e.g. saline and antibiotic irrigations.

Keywords: Paranasal sinuses; Chronic rhinosinusitis; s-IgA; Upper airway colonisation (*Pseudomonas aeruginosa, Burkholderia cepacia, Achromobacter xylosoxidans*); Sinus treatments (sinus surgery, nasal irrigation); United airways; Immune response; Quality of life

Abbreviations

BAL:	Bronchoalveolar lavage
CF:	Cystic fibrosis
CIE:	Crossed immunoelectrophoresis
CFQ-R:	Cystic fibrosis questionnaire-revised
CRS:	Chronic rhinosinusitis
CT:	Computed tomography scan
ELISA:	Enzyme-linked immunosorbent assay
EPOS:	European position paper on rhinosinusitis
FESS:	Functional endoscopic sinus surgery
HRQOL:	Health-related quality of life
Ig:	Immunoglobulin
LTX:	Lung transplanted
MRI:	Magnetic resonance imaging
ORL:	Oto-rhino-laryngologist
PFT:	Pulmonary function test
PMNs:	Polymorphonuclear leukocytes (neutrophil granulocytes)
RSOM-31:	Rhinosinusitis outcome measure-31
SN-5:	Sinus and nasal quality of life survey
SNOT-22:	Sinonasal outcome test-22
St-Ag:	Standard antigen

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1. Introduction

There has been little focus on the paranasal sinuses in patients with cystic fibrosis (CF). However, our multidisciplinary group has contributed to creating awareness about this important issue during recent years and has published four studies on the topic with a common thread combining basic, paraclinical and clinical research [1–4].

1.1. Aims

I have focused on elucidating whether the paranasal sinuses can be a focus for initial lung colonisations and whether it is plausible that they also may serve as a focus for re-infections in CF-patients. In detail, the aims are:

(a) Discuss the prevalence of bacteria found in the CF sinuses [1–4].
(b) Put the sinus mucosal inflammation into perspective [1,2].
(c) Describe a potential new method of diagnosing CF pathogen sinusitis; this will be a platform to discuss how CF sinusitis may be identified and treated [2,3].
(d) Put forward a treatment strategy for CF patients with pathogen sinusitis and discuss pros and cons for different treatments of CF sinuses [3,4].
(e) Present and discuss results on how our treatment addressing the sinuses influences lung colonisations and re-infections and relate these to further studies [3,4].

2. Background

2.1. Cystic fibrosis (CF)

CF is a severe recessive genetic disease, which is common among the Caucasian population. In Denmark, the incidence is 1:4,700 [5]; the Faroe Islands having one of the highest incidences in the world. Currently, approximately 450 patients with CF are living in Denmark; one-third of the patients are followed at the Cystic Fibrosis Centre in Århus while two-thirds are followed at the Cystic Fibrosis Centre in Copenhagen Rigshospitalet.

The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator protein (CFTR) located on chromosome 7 [6]. The gene encodes the cAMP-dependent chloride channel, and as a consequence of the defect, abnormal transport of chloride and sodium across the cell epithelium is seen, leading to reduced volume of epithelial lining fluid compared with non-CF individuals [7]. Thus, all secretions contain a higher concentration of salt, which more than doubles the viscosity compared with non-CF individuals [8].

The main clinical characteristics of CF are increased salt loss in sweat, malabsorption, diabetes, male infertility, chronic rhinosinusitis and increased fungal and viral airway infections; most severe is the increased susceptibility to bacterial infections of the lower airways.

2.2. Lower airways

Due to the viscous secretions, the mucocilliary clearance of inhaled microbes is impaired making CF patients very susceptible to lower-airway infections [8,9]. From early childhood, the infections are mostly caused by *Haemophilus influenzae* and *Staphylococcus aureus.* When older, the CF-pathogenic Gram-negative bacteria *Achromobacter xylosoxidans*, *Burkholderia cepacia* complex and especially *Pseudomonas aeruginosa* are more frequently seen. When reaching adulthood, the vast majority of patients have been colonised or infected with one or more of the three mentioned CF-pathogenic Gram-negative bacteria responsible for most of the morbidity and mortality in CF [10].

The initial stage of "never been lung colonised" with CF-pathogenic Gram-negative bacteria is often replaced by a stage of intermittent colonisation before entering the final stage of chronic infection. In spite of a frequently and regularly intensive antibiotic treatment [11], the bacteria are presumably constantly present in some pulmonary segments when chronically infected. The chronic stage is paraclinically characterised by constantly high serum levels of immunoglobulin G (IgG) antibodies and numerous polymorphonuclear leukocytes (PMNs) in the lower airways. Elevated serum levels of specific antibodies are also seen and are used as a supplementary diagnostic tool (mentioned in subsections 2.2.1 and 2.5.1). Likewise, increased numbers of PMNs are strongly correlated to poor lung function; the imbalance between PMN-proteinases and their inhibitors leads to impaired phagocytosis, T-cell and B-cell imbalance, and lung tissue damage [12]. Thus, the onset age of chronic lung infection with *P. aeruginosa* is correlated with the life expectancy in CF patients [13].

Clinically, CF-patients with chronic bacterial lung infections tend to have lower quality of life, lower body mass index (BMI) and declining lung function measured by FEV_1 (forced expiratory volume in 1 second %-predicted) and FVC (forced vital capacity %-predicted). The major purposes of treating patients with CF are to prevent or delay chronic lung infections and keep the lung function at a steady state. This goal is difficult to achieve, consequently, CF is the second largest group of lung-transplanted recipients in Europe [14].

2.2.1. Grading of pulmonary infection

Years ago, there was no international consensus about the consequences of Gram-negative chronic lung infections for the progression and prognosis of CF lung disease. By an epidemiological study of the respiratory tract microbiology, the definition of different infection categories was introduced [15]. It was shown that high serum levels of precipitating antibodies against *P. aeruginosa* were characteristic in chronically infected patients and in patients harbouring mucoid strains [16], and that high and rapidly increasing levels of antibodies correlated with poor prognosis [13]. The antibody response was shown to have high sensitivity and specificity for the early detection of chronic *P. aeruginosa* lung infection and was included in the following definition of chronic infection: persistent presence of bacteria in six consecutive months, or less when combined with the presence of elevated precipitating antibodies [7,13].

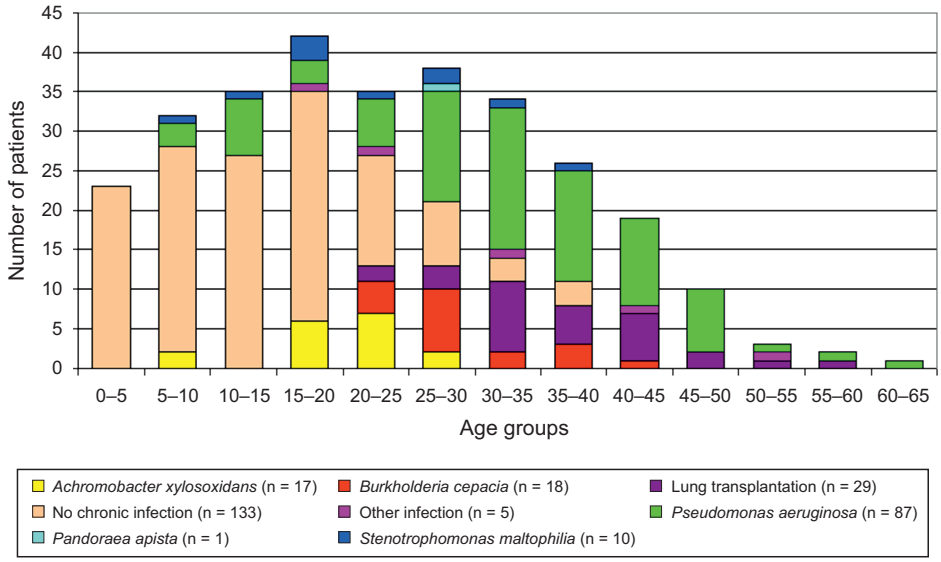

Fig. 1. Bacteria causing chronic lung infection in CF patients at the Copenhagen CF Centre in 2008 (with permission from Christine Rønne Hansen).

Since 1974, our centre has used the Copenhagen criteria [13,16], which grades pulmonary infection into three categories based on having 10–12 lower airway samples cultured a year: (1) never colonised, (2) intermittently colonised, and (3) chronically infected. These criteria cannot be applied in most CF centres outside Denmark, because the patients are not seen as regularly as in Copenhagen and as only a few centres have access to the antibody tests. Consequently, the Leeds criteria were developed [17] and were shown to correlate well with the Copenhagen criteria [18–20]. The advantage of using the Copenhagen criteria when patients are seen on a monthly basis is that they allow an earlier initiation of eradication or maintenance therapy, which improves lung function in both intermittently colonised and chronically infected CF patients [19,21].

Based on the fact that most CF centres outside Denmark only see their patients every third month, the following Leeds criteria are used:

(1) *Never infected*: there has never been growth of any CF related Gram-negative bacteria.
(2) *Non-infected*: no growth of any CF related Gram-negative bacteria over 12 months.
(3) *Intermittent colonisation*: growth in $> 0\%$ and $\leqslant 50\%$ of samples.
(4) *Chronic infection*: growth in $> 50\%$ of a patient's monthly lower-airway samples.

2.3. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative rod-shaped bacterium frequently found in soil, water and man-made environments (e.g. water pipes). It is an opportunistic pathogen of immune-compromised individuals. It thrives not only in normal atmospheres, but can adjust to hypoxic conditions as in the sputum and sinus secretions of CF patients [22,23]. Partly induced by oxygen radicals from the PMNs, some *P. aeruginosa* mutate during the initial colonisation making them more suitable for a chronic infection. The most important bacterial gene is *muc*A, which causes *P. aeruginosa* transition from a non-mucoid to a mucoid-phenotype producing alginate and biofilms. Other important mutations or changes of phenotypes cause: down-regulation of the cell-to-cell communication (quorum-sensing; *las* and *rhl* genes); increase antibiotic resistance; change colony morphology; reduce swimming, swarming and twitching motility; growth advantages; modify immune system tolerance; and increased protease production [24,25].

Lung infections with *P. aeruginosa* cause inflammation resulting in a systemic increase of IgG antibodies against polyvalent *P. aeruginosa* antigen (Standard Antigen (St-Ag)) [26] and the mucoid exopolysaccharide alginate (a biofilm-matrix component), which are highly characteristic of *P. aeruginosa* [27,28]. In addition to serum, specific antibodies are present in tears and in the upper airways as saliva and sputum; IgA being the dominant antibody at mucosal surfaces [28,29]. Prior to our studies [1,2], research on IgA in nasal secretions from CF patients has, to our knowledge, never been investigated.

2.4. *Achromobacter xylosoxidans, Burkholderia cepacia complex*

In CF, most research has been done on *P. aeruginosa*, being the bacteria causing the majority of chronic infections (Fig. 1). *A. xylosoxidans* and *B. cepacia* complex are less prevalent but have similar negative impact on pulmonary disease progression. They are expected to have similar adaptive mechanisms as *P. aeruginosa* causing similar inflammation and lung destruction [30]. These bacteria are also Gram-negative rods. In contrast to *P. aeruginosa*, *B. cepacia* complex is resistant to colistimethate sodium; *P. aeruginosa* is seldom pan-resistant and can often be treated with oral antibiotics, which is in contrast to *A. xylosoxidans*, which is difficult to treat with oral antibiotics and rapidly develops multi-resistance [31,32]. Patients can be infected with more than one Gram-negative bacteria but the outcome of bacterium–bacterium interactions are unknown.

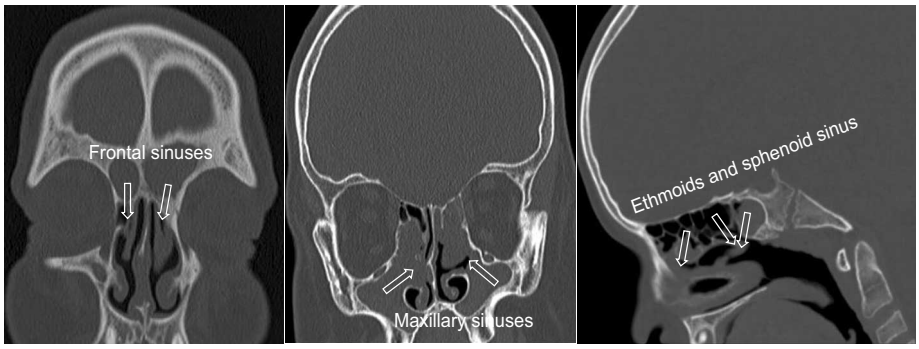

Fig. 2. The arrows show the supposed drainage pathways of the paranasal sinuses. In these CF patients the drainage seems to be occluded and the sinuses are partly opacified.

2.5. Detection of CF-pathogenic Gram-negative bacteria

Early aggressive antibiotic treatment of the first *P. aeruginosa* colonisation is crucial in order to prevent or postpone chronic lung infections and is also cost-beneficial [11,33]. Thus, this fact has increased the necessity of rapid and sensitive detection techniques [34,35]. Serum antibody titres against alkaline protease, elastase and exotoxin A are on average low when *P. aeruginosa* is isolated from the respiratory tract for the first time [36] and early diagnosis is challenging [37]. In our clinic, specific IgG and precipitating serum antibodies are used as a supplementary tool for monitoring lung colonisations and infections. Clinical and paraclinical outcomes, e.g. pulmonary function tests, are also used in the detection of pulmonary bacteria. Culturing lower airway samples is the one of the most important tools in the detection. These samples are obtained by coughed sputum, endolaryngeal suction in non-sputum producers, induced sputum that increases the recovery rate of *P. aeruginosa* [38], or by bronchoalveolar lavage (BAL) having a lower degree of upper respiratory tract contamination [39]. Detection of CF-pathogenic bacteria from the upper airways is discussed below.

2.5.1. Immune responses

Elevated levels of specific anti-Pseudomonas IgG antibodies, measured by enzyme-linked immunosorbent assay (ELISA), is a risk-indicator for developing chronic *P. aeruginosa* infection [40]. Precipitating antibodies measured by crossed immunoelectrophoresisis (CIE) is used as a supplementary tool for diagnosing and predicting the outcome of lung infections [41]. Precipitating antibodies remain within the normal range (0–1) in most cases during intermittent lung colonisation but rise during chronic infection. The antibody response has previously been shown to be helpful in distinguishing between intermittently colonised and chronically infected patients using the Copenhagen criteria mentioned in subsection 2.2.1 [7,40,41].

2.6. Upper airways

2.6.1. Sinus anatomy

The paranasal sinuses are a group of air-filled-spaces: the maxillary sinuses surrounding the nasal cavity, the frontal sinuses placed in the forehead above the eyes, the ethmoidal sinuses are many small sinuses between the orbits, and the sphenoid sinuses are deep and posterior to the ethmoids. The sinuses are lined by mucosa and produce mucus. The drainage pathways are shown in Fig. 2. Unexplained, CF sinonasal anatomy very often divagates from non-CF patients. Common findings are nasal congestion, polyposis, mucoceles, mucopurulent material, medial bulging of the maxillary walls, ostitis and hypoplasia or aplasia of the paranasal sinuses especially of the frontal sinus [42–45].

2.6.2. Chronic rhinosinusitis

The hallmark of CF in the head and neck region is chronic rhinosinusitis (CRS) and nasal polyps. There is no specific definition on CRS in CF patients, so they follow the general definition stated in the European position paper on rhinosinusitis (EPOS) [46] shown in Table 1. However, nasal and sinus mucosal disease is by definition present in patients with CF because of defective CFTR-channels in the sinonasal mucosa, as found in the lower CF airways [46]. The inflamed tissue and viscous mucus results in a mechanical obstruction of the sinus ostia [45,47]. Further, the vast majority of CF patients have radiologic evidence of sinus disease [42,45,48–53], and nasal polyposis becomes more common with age that has been reported in varying prevalence with up to 50% of all CF patients [48,52,54,55]. There are inconsistent results on whether CF patients with nasal polyps and symptoms of CRS can be correlated with a better lung function [56–59].

CF patients are likely to under-report their symptoms of CRS, giving a false low share of CF patients with CRS by the definitions in Table 1; approximately two-thirds of all CF patients have impaired olfactory function [9]), and 81–86% of CF patients fulfil the EPOS criteria for CRS (ref. [60] and unpublished material by Berkhout *et al*.), which is in contrast to the low 10–15% who complain about CRS without specific questioning [45,48,61–63]. It is unknown whether the CF patients who do not complain about CRS always were asymptomatic, if they have adapted to their symptoms, or if their CRS symptoms are overshadowed by more troublesome symptoms from e.g. the lungs [45].

In general, non-CF patients with nasal polyposis being otherwise healthy have been shown to score worse on quality of life than patients with chronic obstructive pulmonary disease

Table 1
CRS definition in non-CF patients [a]

Inflammation of the nose and the paranasal sinuses with two or more symptoms for ⩾12 weeks;
One should be

- nasal blockage
- obstruction
- congestion
- nasal discharge (anterior/posterior nasal drip)

Others

- facial pain/pressure
- reduction of smell

Furthermore, demonstrable disease; at least one of following:

- nasal polyps
- mucopurulent discharge
- oedema/mucosal obstruction
- CT changes
- mucosal changes within the ostiomeatal complex and/or sinuses

[a] Adapted from the European position paper on rhinosinusitis and nasal polyps 2012 [46].

and patients with coronary artery disease [64,65]. This ought to give food for thought as to why symptoms of CRS should not be neglected in CF patients. RSOM-31 and SNOT-22 [64,66] are both questionnaires that are recommended outcome tools for adult CRS and can easily be used, while the SN-5 questionnaire [67] is recommended for paediatric CRS [46].

2.6.3. Bacteriology of the upper airways

In non-CF patients, the paranasal sinuses are regarded as sterile though they may be frequently and transiently contaminated by bacteria from neighbouring surfaces [68]. CRS in otherwise healthy individuals predominantly has viruses as a part of the aetiology. When bacteria are involved, the following species are most frequently cultured: *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes* gr. A*, Haemophilus influenzae* and *Moraxella catarrhalis* [69,70]. In CF patients, the picture is somewhat different. Sinus bacteria are more frequently present, *P. aeruginosa* being the most common as in the lungs. Other frequently found bacteria are *S. aureus, H. influenzae* and coagulase-negative staphylococci; anaerobes and other bacteria found in the lower airways such as *A. xylosoxidans* and *B. cepacia* complex are also found in the CF sinuses [44,53,70–76]. Presence of sinus bacteria is reported in 44–95% [62,73,76]. Two articles have described fungal sinusitis among North American CF patients but disagree on the prevalence (0–33%) [74,76].

Though it has not been addressed, it is likely that the sinus bacteria in CF patients also produce biofilms that further increase antibiotic resistance in the same way as in the non-CF patients [24,70,77,78]. As in the lungs, the sinus bacteria develop phenotypes that are resistant to the host immune response and antibiotic treatment.

2.7. United airways in CF patients

A marked association exists between upper and lower airway cultures in patients with CF [1,24,46,71,75,76,79–86] due to the paranasal sinuses often being colonised with concordant CF-lung-pathogenic Gram-negative bacteria of the same genotype [1,24,53]. Varying predictive values of CF-pathogenic bacteria in the upper airways have been reported when diagnosing lower airway pathogens [62,71,75,83].

A CF patient's initial lung colonisation with *P. aeruginosa* reflects the great diversity of genotypes in the environment, being in contrast to some patients who, after antibiotic eradication, subsequently are re-colonised with bacteria that are clonally related [71,87–89]. This indicates that the initial bacteria come from environmental sources rather from transmission between patients [90] and an existence of a bacterial reservoir in the patients' close environment after the initial colonisation. This reservoir is likely to be the sinuses where the bacteria can drain/migrate/be aspirated to the lower airways as seen with viruses [24,91].

The environment of the sinuses and the lower airways are similar in many ways [22,23,46], thus the sinuses may be colonised with bacteria before the lungs and be an evolutionary 'nest' in early airway colonisations, where the bacteria are diversifying, evolving antibiotic resistance and other phenotypes associated with adaptation to the CF airways in general; from there, the bacteria intermittently migrate and colonise the lungs and may ultimately cause chronic lung infections [1,23,24,71,76]. When bacteria colonise the lungs they are then pre-adapted to the environment and are therefore less virulent and more resistant compared with environmental *P. aeruginosa* isolates [24]. This also accounts for lung transplanted (LTX) CF patients, where molecular epidemiology studies have shown that CF lung-transplant recipients become re-colonised in their lung grafts with the same bacterial clones as those cultured before transplantation [86].

One study has shown that CF-lung-pathogenic bacteria potentially can be eradicated from the sinuses with extensive functional endonasal endoscopic sinus surgery (FESS) and postoperative local antibiotic treatment [92]. Nevertheless, large-scale prospective studies investigating the effects of FESS on lung colonisation and infection in CF are lacking [46], and data on surgical therapy for CF patients with CRS is primarily based on level III evidence (Table 2) [46,93].

Several studies have described the effect of FESS on the lower airways using various parameters and treatment modalities, thus showing inconsistent results (Table 3). One prospective study has performed extensive FESS intending to eradicate sinus bacteria in a group of 82 LTX patients, which is described in three papers [92,94,95], showing that *P. aeruginosa* and *A. xylosoxidans* could be eradicated from the sinuses resulting in reduced lung allograft infections. Shatz [96] found decreased antibiotic use, a lower hospitalisation rate and an increase in FEV_1 six months after FESS among 15 CF non-LTX patients. Lewiston *et al.* [97] postoperatively installed tobramycin directly into the sinuses and reported a lower rate of *P. aeruginosa*

Table 2
Categories of evidence [98]

Category	Definition
Ia	Evidence from meta-analysis of randomised controlled trials.
Ib	Evidence from at least one randomised controlled trial.
IIa	Evidence from at least one controlled study without randomisation.
IIb	Evidence from at least one other type of quasi-experimental study.
III	Evidence from non-experimental descriptive studies, such as comparative studies, correlation studies and case control studies.
IV	Evidence from expert committees' reports or opinions and/or clinical experience of respected authorities.

in the lungs among 11 LTX patients. The other retrospective studies were all based on moderate sinus surgery, and did not focus on lung infection status or a protocol for postoperative treatment. These studies found inconsistent postoperative reduction in lung colonisation, lower hospitalisation rates, reduced use of antibiotics and improvement of the pulmonary function tests (PFT). Table 3 shows an overview of the published papers on sinus surgery in relation to the lungs; none of the studies are level I evidence (Table 2).

2.8. Assessment of the upper CF airways

2.8.1. Imaging

The radiation dose of one CT scan of the paranasal sinuses is now reduced to only 0.5–1.0 mSv (in comparison, the Danish annual radiation dose varies from 2–20 mSv). Imaging of the sinuses is mandatory for planning surgical interventions but should not be performed abundantly, thus, CT scans have a low diagnostic value in CF patients [53,61,109]. Magnetic resonance imaging (MRI) allows better differentiation of mucosa, polyps and retained secretions but does not display osseous structures bordering the orbit and brain [50,110]. Imaging is mandatory prior to FESS due to the altered and varying anatomy of the sinuses with relation to the orbit, brain and major vessels (see subsection 2.6.1). The capacity for doing MRI is restricted at our institution, which is why solely CT scans are used.

2.8.2. Culture

Although sinus aspiration is the gold standard for the diagnosis of bacterial sinusitis, it is an invasive, time-consuming and potentially painful procedure [68,111]. The diagnostic accuracy of oropharyngeal swab cultures is low in predicting *P. aeruginosa* sinusitis, particularly at younger ages (positive and negative predictive values: 73% and 72%) [62]. Cultures of endoscopically collected middle meatus secretions is reported as effective in identifying microorganisms in non-CF CRS patients and in CF patients [112]. However, nasal irrigations are also suggested as a preferable technique over nasal swabs to obtain samples from the upper airways in CF patients [71].

2.8.3. Steroids

A Cochrane review states that oral corticosteroids appear to slow progression of lung disease in CF [113]. However, no research is published on oral steroids' effect on CRS symptoms in CF patients, while one study recommends intrapolyp steroid injection [114]. Another Cochrane review states that: "Overall, there is no clear evidence for using topical steroids in people with CF and nasal polyposis". This is due to the neutrophilic domination in CF polyposis compared with the eosinophil domination in non-CF patients [115]. However, it should be mentioned that some studies report a positive effect of using nasal steroids on CRS and nasal polyps in CF [116–118].

2.8.4. Surgery

In 2006, a Cochrane review concluded that more randomize controlled trials comparing FESS with other treatments were required, thus it could not be confirmed that CF patients with CRS symptoms could benefit from FESS [93]. The newest edition of EPOS 2012 and other recent studies state that symptoms of nasal airway obstruction, nasal discharge, facial pain, snoring, olfactory dysfunction, frequency of sinus infections and activity level are parameters that can significantly be improved after FESS in CF patients [46,107,119–124]. When evaluating the different studies, it is important to note the criteria for FESS and what FESS and postoperative treatment comprise. In spite of postoperative instrumental debridement and saline irrigations [121,125], it is accepted that the effect of FESS on CRS symptoms, in general, lasts a shorter time in CF patients than in non-CF patients [57,104,107,121,122,124], which is why a more extensive approach has been suggested [82,126–130] combined with antibiotic sinus irrigations [72]. As in any other surgery, FESS involves risks. Though they are rare, situations where the optic nerve or brain is damaged and extensive bleeding can occur. Nevertheless, when surgeons are aware of the altered anatomy in CF patients (described in subsection 2.6.1), reports show that FESS is well tolerated and that the complication rate in CF patients is similar to that of the non-CF population [46,104,131].

2.8.5. Local treatment

Nasal saline irrigations are well tolerated and the beneficial effect appears to outweigh the minor side effects, thus they can be included as a treatment adjunct for the symptoms of CRS in CF-patients [46,132]. Hypertonic saline 7% may have mucolytic effects and improve mucociliary clearance in the sinuses as seen in the lungs of CF patients and may be used for nasal irrigations [133–135]. Baby shampoo is also introduced as a supplement to the saline [136,137], as is nasal inhalation of dornase alfa used in the treatment of CRS in CF patients [138–140]. Studies of nasal irrigations with antibiotics (tobramycin or aminoglycosides) show decreased bacterial colonisation and nasal inflammation and a positive effect on recurrence rate of CRS in non-CF patients [141]. However, there is low-level evidence for the use of topical anti-bacterials in CF patients [46,142]. Several devices including nebulizers have been developed for nasal irrigations and distribution of medicine [139,143], which are all better than delivering it by nasal spray [46]. Finally, low-frequency ultrasound has recently been suggested as a supplementary method for biofilm disruption in patients with CRS [144].

Table 3
Overview of studies correlating sinus surgery to lower airway conditions

Author(s)	No. of CF patients	Study design	Extent of FESS	Post-operative treatment	Outcome
Holzmann et al. [92,94,95]	82 LTX	Prospective	Fronto-spheno-ethmoidectomy and maxillary antrostomy	Nebulized colistin and irrigations; i.v. AB	Decrease in colonisation of the lower airways
Jarret et al. [99]	17 Non-LTX	Retrospective	Ethmoidal and maxillary sinuses	Nasal saline irrigation; oral AB	PFT and BMI – non-significant
Leung et al. [100]	87 LTX	Retrospective, case-control	Ethmoidal and maxillary sinuses	ND	Lung re-colonisation – non-significant
Lewiston et al. [97]	11 LTX	Retrospective	Ethmoidal and maxillary sinuses	Tobramycin in the sinuses	Low hospitalization rate after surgery. Reduced PA in the lungs
Madonna et al. [101]	14 Non-LTX	Retrospective	Ethmoidal and maxillary sinuses	i.v. AB	PFT – non-significant
Osborn et al. [102]	41 Non-LTX	Retrospective	Ethmoidal and maxillary sinuses	ND	Sparse improvement in FVC, none in FEV1. No effect on microbes
Rosbe et al. [103]	66 Mixed	Retrospective	ND	Some had i.v. AB	Decrease in IHD. Steroid use and LFT – non-significant
Triglia et al. [104]	27 Non-LTX	Retrospective	ND	ND	Decrease in AB treatment, not in LFT
Umetsu et al. [105]	4 (ND)	Prospective	Ethmoidal and maxillary sinuses; AB flushing	i.v. AB	IHD reduced postoperatively, not in PFT
Kempainen et al. [106]	32 Mixed	Retrospective	Fronto-spheno-ethmoidectomy and maxillary antrostomy	ND	PFT and IHD – non-significant
Shatz [96]	15 Non-LTX	Retrospective	Frontal, ethmoidal and maxillary sinuses	Nasal irrigations	Decrease in AB and IHD. Increase in FEV1 after 6 months
Halvorson et al. [107]	8 Non-LTX	Retrospective, case-control	Ethmoidal and maxillary sinuses	ND	Increased exercise tolerance/ increased PFT after 3 months
Kovell et al. [108]	21 Non-LTX	Retrospective, case-control	ND	ND	Increase in PFT

LTX: Lung transplanted; PFT: Pulmonary Function Test; IHD: In-hospital-days; AB: antibiotics; BMI: Body Mass Index; FEV1: Forced expiratory volume in one sec.; FVC: Forced Vital Capacity; ND: not described

3. Material

3.1. Study population

In our four studies [1–4], patients were recruited among the 300 CF patients treated at the CF Centre in Copenhagen. The diagnosis of CF was based on clinical characteristics, abnormal sweat electrolytes, and genotype. CF patients followed a routine protocol with monthly medical examinations including lung function tests and lower airway samples taken for microbiological culture. Additional lower airway samples were taken whenever patients were hospitalised or when clinical and/or paraclinical parameters indicated a risk of lung colonisation or infection. Approximately every third month, blood samples were taken for analyses including specific antibodies against relevant Gram-negative bacteria [41]. LTX patients followed a different outpatient setting with fewer routine samples taken.
All CF pathogens were treated with antibiotics regardless of clinical symptoms according to the Copenhagen CF Centre's treatment protocols [11].

3.2. Usage of different grading of pulmonary infections

As mentioned in subsection 2.2.1, there are at least two different ways of grading pulmonary infections. In our studies reported in refs. [1] and [2], we applied our standard Copenhagen criteria for defining lung infection status and LTX patients were categorised as chronically infected.
Modified Leeds criteria [17] were used for defining lung infection status in our studies [3,4]; as lung infection status was the main outcome in the study reported in ref. [4], it was important to use simple criteria here that are known and can be used among other CF centres. This facilitates an international comparison in the future. Secondly, the use of the Leeds criteria allowed us to put our findings into perspective because intermittently colonised patients could be re-classified as non-infected. Thirdly, a rise in antibodies was, in some cases, a part of the reason for setting patients up for surgery (described in subsection 4.1.1), and so it would be a circular argument if antibodies were also used to define the outcome of lung infection status.

4. Methods

4.1. Functional endoscopic sinus surgery (FESS)

4.1.1. Criteria for FESS

FESS (Fig. 3) was a part of the studies reported in refs. [1], [3] and [4]. CF patients were selected for FESS based on the following criteria:

(1) Search for an infectious focus: Intermittently colonised patients with increasing frequency of positive lower airway cultures or repeatedly declining lung function (> 10%), despite intensive antibiotic chemotherapy. Patients with an unknown infectious focus and increasing antibodies against *P. aeruginosa, A. xylosoxidans* or *B. cepacia* complex were given the highest priority.

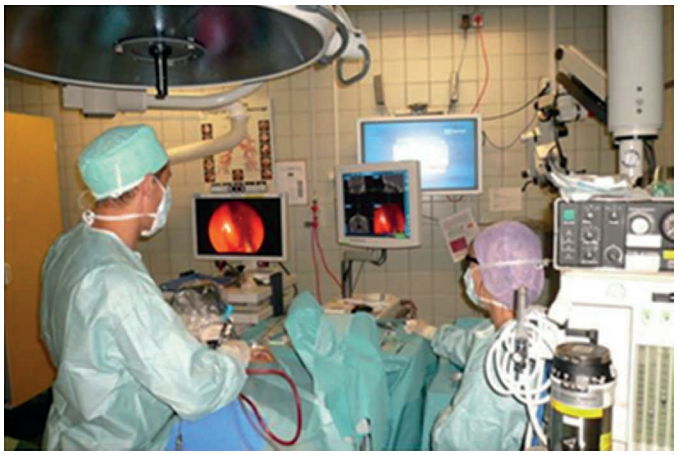

Fig. 3. Set-up for FESS.

(2) Patients who had recently been LTX. The ambition was to perform FESS within the first postoperative year.
(3) Patients with severe symptoms of chronic rhinosinusitis (CRS) according to the EPOS [46].

4.1.2. FESS procedure

A BAL was performed under general anaesthetic. The subsequent FESS was to ventilate and drain the paranasal sinuses and to make these accessible for postoperative instrumental cleansing and irrigation with saline and topical antibiotics. Each patient was evaluated for symptoms of chronic rhinosinusitis [46] followed by a clinical examination. The extension of surgery (e.g., exploration of the frontal or sphenoid sinuses) was undertaken based on the preoperative CT scan and perioperative findings. As a standard, we applied FESS with an uncinectomy, an anterior ethmoidectomy and a medial antrostomy, leaving a significantly enlarged maxillary ostium comprising more than half the medial maxillary wall as recommended [46]. Visible intramucosal abscess-like structures (especially found in the maxillary sinuses) were resected along with other inflamed mucosal tissue when accessible. Finally, the opened and now accessible sinuses were irrigated with saline and colistimethate sodium.
To optimize culture results, no patients received i.v. antibiotics within two weeks prior to FESS, and different anatomic

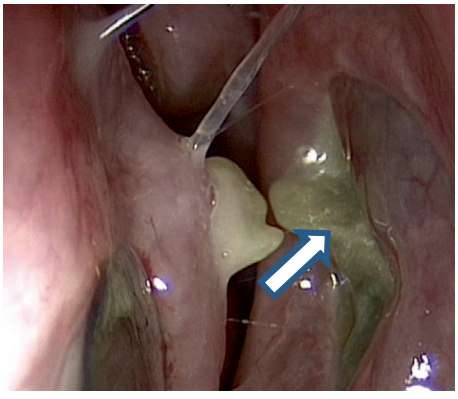

Fig. 4. Pus containing *P. aeruginosa* exiting the left maxillary sinus before FESS.

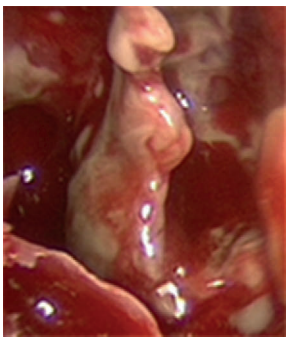

Fig. 5. A view in the maxillary sinus.

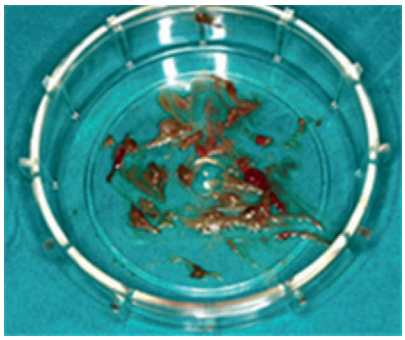

Fig. 6. Material obtained for culture.

sampling locations and multiple samples for culture were prioritized during surgery, including: nasal secretions, pus, mucosa, polyps, and bone (Figs. 4–6). Samples taken for culture were collected with sharp instruments or by suction tubes. The material obtained was immediately cultured at the Department of Clinical Microbiology at Rigshospitalet.

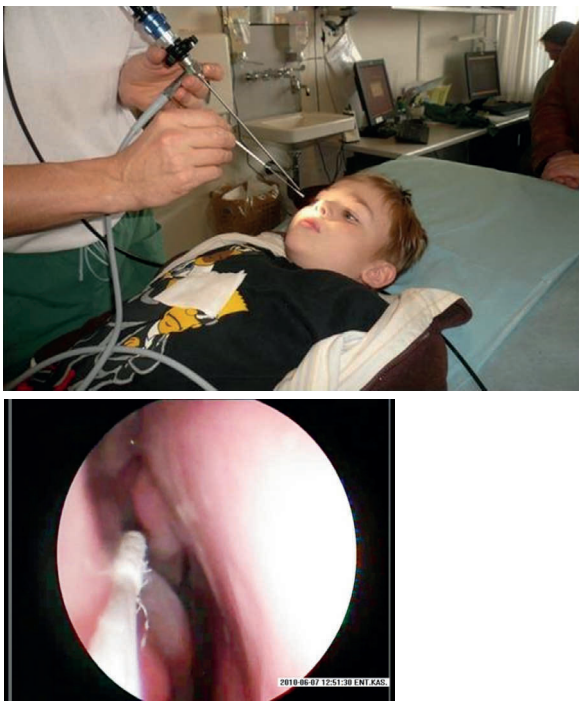

Fig. 7. Postoperative cleansing and culturing without use of local anaesthesia. (Thanks to 7-year-old Jonas; a fantastic young man.)

4.1.3. Postoperative treatment

Postoperative adjuvant therapy included: two weeks of i.v. antibiotics if there was the slightest suspicion that the lungs or sinuses contained CF-pathogens [11], at least 6 months of twice daily nasal irrigation with saline and antibiotics (starting Day 1 with colistimethate sodium but could be adjusted according to susceptibility), and 12 months of topical nasal steroids (mometasonfuroate). As a standard each patient had four postoperative visits to the oto-rhino-laryngologist (ORL) outpatient clinic: one week and one, three and twelve months postoperatively, where crusts and secretions were endoscopically cleansed from the nasal cavities and sinuses (Fig. 7). At each follow-up, under endoscopic guidance, the patients were bilaterally cultured.

4.2. Culture methods

In our four studies reported in [1–4] the bacteriology of the lungs and sinuses play a major role, thus the method of culture is described in detail:

Gram-stained smears and aerobic cultures on selective media were performed on all samples (Figs. 8, 9). These media included a Sabouraud plate (for fungal growth), a 7% NaCl plate, a *B. cepacia* plate containing Colistin and Gentamicin, a "blue plate" (modified Conradi Drigalski's medium) selective for Gram-negative rods, and two non-selective media including 5% Danish blood agar and chocolate agar. In order to avoid sampling bias, bacteria with different susceptibility patterns and different colony morphologies were chosen and identified as previously described [145,146]. In our study reported in ref. [1], Gram-stained smears were used for biofilm detection and Pulsed Field Gel Electrophoresis (PFGE) was used for genotyping *P. aeruginosa* isolates from the sinuses and the lungs [146].

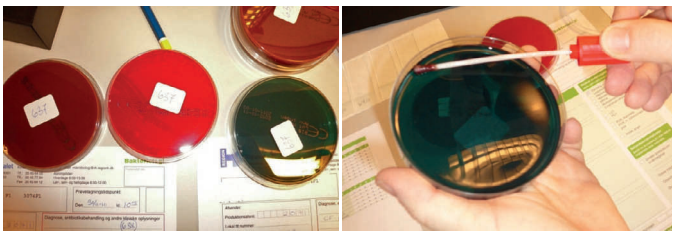

Fig. 8. Smears on selective media for culturing.

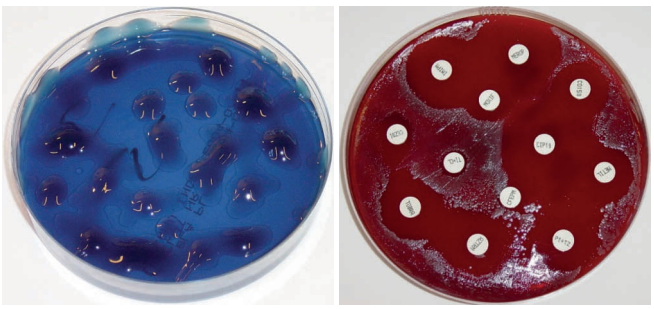

Fig. 9. (a) Growth of mucoid *P. aeruginosa* on a blue plate. (b) Antibiotic susceptibility testing of *P. aeruginosa*.

4.3. IgA and IgG antibodies against P. aeruginosa

In papers [1] and [2] we present and use a new method to diagnose antibodies against *P. aeruginosa* St-Ag and alginate: Twelve 6 mm in diameter paper discs (Fig. 10) with obtained serum or eluates of saliva or nasal secretions from each patient were examined for IgA and IgG antibodies against *P. aeruginosa* alginate and *P. aeruginosa* sonicate (St-Ag) (serogroups 1–17) using enzyme-linked immunosorbent assays (ELISA) as reported previously by our group [28,29]: Saliva and nasal secretion impregnated paper-discs were incubated on a shaker in dilution buffer to elute IgG and IgA antibodies. Phosphate-buffered saline +0.1% Tween-20 +NaCl 15 g/l was used for dilution, and the plates were washed three times with it.

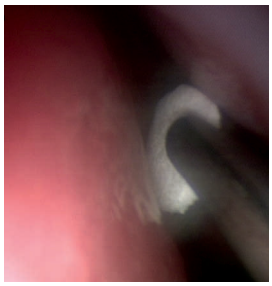

Fig. 10. A paper disc to collect secretions inserted in the nasal cavity.

4.3.1. Antibodies against P. aeruginosa alginate

Microtiter plates were coated with alginate purified from a mucoid CF *P. aeruginosa* strain as previously reported by our group [147]. The plates were coated and blocked in dilution buffer. Diluted serum, saliva and nasal secretions (see above) were added and allowed to react. After washing, horseradish peroxidase (HRP)-conjugated rabbit anti-human IgA (P0216) and anti-human IgG (P0214) were added and reacted.

4.3.2. Antibodies against P. aeruginosa St-Ag

A sonicated cell extract of *P. aeruginosa* serogroups 1–17 was used as standard St-Ag [28,29] and coated onto irrigated 96-well polystyrene plates. The plates were incubated and blocked with dilution buffer. Serum, saliva, and nasal secretion were diluted and allowed to react. After washing, horseradish peroxidase (HRP)-conjugated rabbit anti-human IgA (P0216) and anti-human IgG were added and left to react.

Fig. 11. ELISA procedures quantifying IgA and IgG in nasal and saliva secretions as well as serum.

4.3.3. ELISA

For all ELISAs, TMB-Plus media was added. The reactions were stopped after one hour at room temperature by adding 1 M H_2SO_4. The absorbance was measured at 450 nm on a plate reader. The results were expressed as optical density values (OD) (Fig. 11).

4.4. Additional methods

The following well-established methods were used in our studies: traditional immunohistochemistry in paper [2]; lung function test [148,149], body mass index standard deviation scores [150], specific anti-Pseudomonas IgG antibodies measured by ELISA [40] and precipitating antibodies measured by CIE [41] are all regularly used when evaluating the CF patients conditions, and were used in paper [4]. The CFQ-R (Cystic Fibrosis Questionnaire-Revised) has also recently been initiated in the CF centre to estimate the disease-specific health-related qualify of life [151], thus was logical to use in paper [4].
The sinonasal outcome test (SNOT-22, Table 4) used in paper [4] deals with sinonasal conditions [66], but also includes health-related questions that can be influenced by other CF-related conditions, e.g. cough; the SNOT-22 questionnaire is used worldwide when evaluating CRS.

4.5. Statistics

In all four papers [1–4], we tested whether data were continuous and if the comparisons fulfilled the criteria for normality and equal variance. The level of significance was set to $\leqslant 0.05$ (two-tailed). SAS 9.1.3 was used for calculations.
In paper [1], the non-parametric sign test was used to compare within patient samples of antibodies, while the data of the antibodies in paper [2] were unpaired, continuous and positively skewed distributed why Log_{10} transformations were made. The transformed data had an approximately normal distribution justifying an unpaired two-sample t-test for the means and a one way analysis of variance (ANOVA).
Receiver operating characteristic (ROC) curves was used to find the best cut-off values between the three lung infection groups if IgA was to be used as a diagnostic test [2]. A Spearman rank coefficient test was used to correlate nasal secretions and saliva in [2] as well.
A McNemar's test was used to compare the nominal data of postoperative frequencies of growth with the perioperative frequencies in paper [3] and to compare the change in lung infection status after FESS (paper [4]). In paper [4], a paired two-sample t-test for the means and an ANOVA was used for the rest of the comparisons.
The biggest statistical challenge was in paper [4]. When planning the study, we received statistical advice from Professor Torben Martinussen at the Department of Biostatistics in how to quantify the frequencies of positive cultures. The conclusion was that every lower-airway sample was registered and given the same weight regardless of the interval between the samples. Using a Spearman rank coefficient test, these results were then compared to the results of lower-airways samples where each

Table 4
SNOT-22 questionnaire [66]

1.	Need to blow nose	0	1	2	3	4	5
2.	Sneezing	0	1	2	3	4	5
3.	Runny nose	0	1	2	3	4	5
4.	Cough	0	1	2	3	4	5
5.	Postnasal discharge (dripping at the back of your throat)	0	1	2	3	4	5
6.	Thick nasal discharge (snot)	0	1	2	3	4	5
7.	Ear fullness	0	1	2	3	4	5
8.	Dizziness	0	1	2	3	4	5
9.	Ear pain	0	1	2	3	4	5
10.	Facial pain/pressure	0	1	2	3	4	5
11.	Nasal blokage	0	1	2	3	4	5
12.	Loss of taste and or smell	0	1	2	3	4	5
13.	Difficulty falling asleep	0	1	2	3	4	5
14.	Waking up at night	0	1	2	3	4	5
15.	Lack of a good night's sleep	0	1	2	3	4	5
16.	Waking up tired	0	1	2	3	4	5
17.	Fatigue	0	1	2	3	4	5
18.	Reduced productivity	0	1	2	3	4	5
19.	Reduced concentration	0	1	2	3	4	5
20.	Frustrated/restless/irritable	0	1	2	3	4	5
21.	Sad	0	1	2	3	4	5
22.	Embarrassed	0	1	2	3	4	5

0 = no problem; 1 = very mild problem; 2 = mild or slight problem; 3 = moderate problem; 4 = severe problem; 5 = problem as bad as it can be.
Please mark the most important items affecting your health (maximum of 5 items).

culture was given weight according to the period until the next culture.

4.6. Ethics

The study was approved by the local ethics committee (H-A-2008-141), and all patients gave informed consent. In patients < 18 years of age, consent was also obtained from their parents.
The inclusion for FESS was not a part of the study, solely the outcome. We also obtained consent for doing additional analyses on the bacteria/material obtained during FESS and BAL, the postoperative treatment and culturing, as well as for using questionnaires and data from the patient files. In paper [2], consent was used in order to obtain and analyze secretions and blood and for culturing; no change in treatment modality was made on behalf of these results.

5. Review of results

We found that the vast majority of CF patients have bacteria in their paranasal sinuses [1–4]. They are often colonised with CF-lung pathogens, especially *P. aeruginosa*, and there is a close correlation between the bacteriology of the sinuses and the lungs, including identical genotypes in the sinuses and lungs [1,2,4]). Importantly, the genotype remains unchanged over time. The chronically infected patients had the same *P. aeruginosa* genotype in their lungs for a median of 15 years as found in their sinuses, and up to 6 years in intermittently colonised patients, although the bacteria apparently had been eradicated from the lungs [1]).
Though the environment of the sinuses in many ways is similar to that of the lower airways, including anaerobic niches and biofilm formation, it differs by excessive presence of the non-phlogistic (does not induce inflammation) secretory-immunoglobulin A (s-IgA) [1,2]. Failure to eradicate CF-pathogens from the sinuses is probably a result of an inefficient local immune response: locally produced specific s-IgA binds Gram-negative bacteria on the mucosal surface, thereby reducing the inflammatory response by preventing antigen presentation inhibiting complement activation, inhibiting the recruitment of PMNs and thereby diminishing the oxidative burst [1]). This was visualized by immunohistochemistry showing excessive amounts of IgA-producing plasma cells in the sino-nasal tissue and IgA in the excretory ducts. It was also visualized by Gram-stained smears from the sinuses, where the bacterial biofilms were surrounded by very few and scattered PMNs in marked contrast to the pulmonary findings [1,2]).
With this background information, our new method to quantify IgA and IgG against *P. aeruginosa* antigen and against the

Table 5
Mean nasal and serum antibodies against *P. aeruginosa* [a]

	Nasal IgA	Serum IgA	Nasal IgG	Serum IgG	Nasal IgA / Serum IgA	Nasal IgG / Serum IgG	Nasal IgA / IgG	Serum IgA / IgG
Non-CF controls (n = 9)								
Alginate	0.17	0.05	0.09	0.12	3.29	0.92	2.19	0.57
St-Ag	0.63	0.51	0.28	0.96	1.74	0.24	2.93	0.56
Free from pathogenic bacteria (n = 32)								
Alginate	0.28* (0.20)	0.13 (0.08)	0.09* (0.05)	0.12* (0.09)	2.57* (1.83)	0.84* (0.40)	3.72 (2.71)	1.24* (0.75)
St-Ag	0.77* (0.28)	0.73* (0.57)	0.19* (0.13)	1.01* (0.45)	1.99 (2.28)	0.19* (0.13)	5.94* (4.85)	0.66 (0.34)
Intermittent PA (n = 24)								
Alginate	1.03 (0.68)	0.14 (0.14)	0.50 (0.50)	0.30 (0.26)	27.92 (83.42)	2.80 (3.64)	4.54 (5.24)	0.68 (0.67)
St-Ag	1.33 (0.35)	1.21 (0.87)	0.64 (0.59)	1.68 (0.64)	1.99 (1.87)	0.38 (0.27)	5.02 (6.58)	0.67 (0.34)
Chronic PA (n = 25)								
Alginate	1.70* (0.60)	0.45* (0.31)	0.63* (0.50)	0.85* (0.71)	4.84* (2.79)	1.16 (1.41)	4.35 (3.76)	0.82 (0.78)
St-Ag	1.46 (0.47)	1.65 (0.68)	0.96* (0.41)	2.52* (0.77)	1.06* (0.58)	0.38 (0.16)	1.81* (1.07)	0.66 (0.26)

[a] Standard deviations are shown in brackets. The ratios were first calculated for each individual and following the mean values were calculated.

P. aeruginosa-specific extracellular polysaccharide alginate, was used to compare nasal, saliva and serum concentrations with the patients' lung infection status (described in subsection 2.2.1) in a cross-sectional study [2]). A significant correlation ($p < 0.01$) was found between the *P. aeruginosa* lung infection status and the quantity of specific IgA in the nasal secretions and saliva; the intermittently colonised patients had the higher IgA concentrations than the non-infected patients (Table 5). This test may then be used as a supplementary tool for detecting CF patients with early lung colonisation. The theory background and our results indicate that the test actually reveals a *P. aeruginosa*-sinusitis, which again is a surrogate marker for lung infections due to the concordant bacteria in the upper and lower airways. In an upcoming prospective study we hope that the usefulness of the IgA test as a marker of *P. aeruginosa* sinusitis can be verified and that it will show a similar good sensitivity and negative predictive value as was the case when related to the lung infection status (Table 6).

Table 6
Combined nasal IgA St-Ag and alginate used to diagnose *P. aeruginosa* lung colonisation

	96% Sensitivity	81% Specificity
79% Positive predictive value	23 patients True positive	6 patients False positive
96% Negative predictive value	1 patient False negative	26 patients True negative

Sinus infections with CF-pathogens do not seem to be eradicated by the frequent oral and intravenous antibiotic therapies that CF patients receive. Conversely, in a prospective follow-up study [3] *P. aeruginosa, A. xylosoxidans* and *B. cepacia* complex could, in several cases, be eradicated from the sinuses or the quantity of colony-forming units were at least reduced, so the bacteria could not be re-detected by thorough sinus cultures for several months (Table 7). This was achieved by extensive sinus surgery and postoperative treatment (described in subsection 4.1.2). Achieving these results was a prerequisite for doing the research in our study reported in [4].

By the same procedure, probably as a consequence of the sinus bacteria being eradicated, a significant reduction in frequencies of lower-airway cultures with CF-pathogens was accomplished [4]. In particular, intermittently colonised CF patients with concordant CF-pathogens in the sinuses seem to benefit from the treatment strategy. As a consequence, the one-year prevalence of intermittent colonisation decreased by 38% after FESS and the one-year prevalence of non-colonised patients increased by 150% (Table 8). In addition, specific IgG for *P. aeruginosa* decreased and quality of life including sinonasal symptoms also improved. This was shown by a prospective, non-randomised, uncontrolled, intervention cohort study [4].

Table 7
Cultures from the left and right sides of the middle meatus and maxillary sinus perioperative and at follow-up. In conclusion, 21 patients had no re-growth at any time at any sinus during six months of follow-up.

Lung status at surgery	Perioperative	One month	Three months	Six months	Twelve months
LTX	24/24 (100%)	8/24 (33%)	9/20 (45%)	11/22 (50%)	9/20 (45%)
Chronically infected	25/26 (96%)	8/24 (33%)	12/24 (50%)	13/26 (50%)	8/18 (44%)
Intermittently colonised	55/66 (83%)	5/60 (8%)	11/60 (18%)	8/50 (16%)	12/48 (25%)

Table 8
Lung infection status (as described in subsection 2.2.1) in the CF patients at FESS and a year after FESS and adjuvant therapy

Main lung bacteria	Non-infected		Intermittently colonised		Chronically infected	
	Before FESS	After FESS	Before FESS	After FESS	Before FESS	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

6. Discussion

Bacterial sinusitis being a focus for lung colonisation and infection is supported by our finding of nearly all CF patients having bacteria in their sinuses and by frequent lower-airway cultures with CF-pathogens (*P. aeruginosa, A. xylosoxidans*, *B. cepacia* complex) correlating with a high frequency of concordant bacteriology in the sinuses. In our patients selected for FESS, 67% had concordant CF-pathogenic bacteria in the sinuses and lungs and additionally 5% had CF-pathogens in the sinuses that were not found in the lungs [4]. The high prevalence of bacterial sinusitis is despite massive intravenous and oral antibiotic treatment. Additionally, we often did not get any positive lower-airway cultures during BAL in intermittently lung-colonised patients even when we found CF-pathogens in the sinuses; this indicates that the sinuses are a more permanent focus than the lower airways. Our described prevalence of bacterial sinusitis is at the high end compared with other studies describing CF-sinus bacteriology (described in subsection 2.6.3), which is probably a result of our invasive and multiple-sample selection. Above all, we consider the risk of false positive results very small; the only way that samples can be cross-contaminated is via the anterior nasal cavity.

Few anaerobes and few fungus isolates were found in the sinuses. Anaerobes were found when doing molecular studies (unpublished material) but not even here were fungus frequently found. This is in contrast to our expectations, as CF-patients experience pulmonary problems with fungus and one study cited by EPOS found a high prevalence [46,74,152]. However, our findings are in accordance with the general low prevalence of fungus-sinusitis in Danish non-CF patients compared with the USA where the study was carried out.

In the early stage of lung colonisation, migration of CF-pathogens mainly occurs in a downward direction from the more permanent focus in the sinuses towards the lungs [24]. This migration occurs more frequently during the viral season where the nasal secretions are more liquefied [91]. The results in paper [4] further support this theory, as it may be concluded that if sinus surgery and adjuvant therapy reduce the frequencies of lower-airway bacteria, the sinuses are bound to influence the lower airways by downward migration of bacteria. Moreover, when evaluating the literature on this subject, including the previous papers from our group, I find it unquestionable that the sinuses play an important role in causing pulmonary colonisations and infections. It is more debateable what can be done to eliminate this risk of colonisation.

There is empirical evidence that the persistent sinus bacteria are facilitated by inflamed tissue obstructing the sinus ostia, lower antibiotic concentration than in the lungs due to lower blood perfusion in the sinus mucosa, that the infection is localised as an empyema in the sinuses and maybe also as intramucosal abscesses. Furthermore, our previous research [24] has shown that the bacteria develop resistant genotypes and phenotypes in the sinuses. In contrast to the nasal environment, where CF-polyps show various patterns of neutrophil-dominated acute and chronic inflammation [153], we found a reduced number of PMNs surrounding the biofilms on the sinus mucosa compared with the lungs. All these points taken together with our results that the upper airways are dominated by the non-phlogistic IgA [1,2], may explain the mechanism of why sinus bacteria are more persistent than in the lungs. In essence, what is important for the clearance of intermittent *P. aeruginosa* colonisation in the CF lungs is only partially functional in the sinuses, providing opportunities for the bacteria to adapt through evolution of resistance mechanisms.

Some non-infected CF patients were solely colonised with CF-pathogens in the sinuses [4]. It is likely that this represented their initial colonisation. Nevertheless, we cannot prove that these patients benefited from the treatment, and it is challenging to determine the prevalence of how often the colonisations initiated in the sinuses. We are confident that our prospective study on specific IgA in sputum and nasal secretions will prove useful in diagnosing *P. aeruginosa* sinusitis and thereby come closer to a conclusion. In fact, after we ended this study [2]), two of the four patients from the non-infected group with the highest IgA levels became intermittently lung-colonised with *P. aeruginosa*, suggesting that they were already sinus-colonised at the time of the study.

According to the Leeds criteria, CF-patients are cabable of having *P. aeruginosa* sinusitis but being categorised as being free from *P. aeruginosa* (non-infected) [17]. In my opinion, it would be clinically relevant to characterise CF infections both according to their sino-nasal bacteriology and according to the lower-airway colonisations/infections. This will require more focus on treating the upper airways, a general collaboration with ORLs, and that clinicians bear in mind that non-BAL lower-airway samples can be cross-contaminated from the upper airways. Furthermore, in order to characterise CF infections and select the right CF patients for FESS, it is essential to find a combination of tests that can diagnose CF-pathogenic sinusitis with high sensitivity. Nasal lavage, as described by Mainz [71] or in [2], is a very easy way to obtain samples with little patient

discomfort. However, these samples also contain bacteria from the upper pharynx and thereby do not solely represent sinus bacteria. It is also uncertain if saline from nasal irrigations represent material from all sinuses. In unpublished data, we have found a relatively low positive predictive value when doing middle meatus cultures in early intermittently colonised patients not previously having sinus surgery, which makes us conclude that this test cannot stand by itself. However, IgA can easily be obtained and quantified by ELISA, and if this is combined with regularly obtained cultures from the middle meatus, cultures from nasal irrigations and other paraclinical measures like serum antibodies and pulmonary function, we believe it has a high diagnostic value.

It may also be clinically relevant to subdivide intermittently colonised patients based on the colonisation pattern and bacteria genotypes as previously described [24]: (a) patients with single or multiple events of short colonisation periods (< 6 months) followed by eradication; (b) intermittently colonised patients with multiple recurrent colonisation events with the same genotype of bacteria and a low systemic immune response [1]; (c) patients with a rapid development of chronic lung infections with increasing precipitating antibodies. Thus, it is most likely that intermittently colonised patients from group b have an additional sinonasal infectious focus. This would help us to select patients for upper-airway treatment by FESS and/or conservative treatment.

Though we have shown that nearly all chronically infected CF patients have CF-pathogens in their sinuses, which in some cases could be eradicated [3,4]), we did not expect that they would have a significant decrease in positive lower-airway cultures [4]). In particular, four chronically infected patients had a pronounced effect of the treatment and we put forward the theory that such patients could be false-positive categorised if the lower-airway samples are cross-contaminated by the upper airways. Thus, the result of true chronically infected patients having an effect of the treatment is more uncertain. However, it is accepted that the lung damage in CF patients with chronic infections characteristically is focal [154] leading to a focal loss of alveoles and an annual decline of lung function of about 1–2% [78]. In that way, true chronically infected patients may benefit from having their sinus bacteria eradicated, as it may prevent further spread of the infection by aspiration from the sinuses to new areas of the lungs [155].

It can be argued that in paper [3] and [4] we gave no answers as to whether the same results could have been achieved by conservative treatment comprising nasal irrigations and endoscopical cleansing. Studies on otherwise healthy patients with CRS have shown that an ostial dimension should be > 4 mm to ensure that irrigations penetrate the maxillary sinus, and that the frontal sinuses are more difficult to irrigate [156,157]. By comparison, when using nebulizers the dimension requirements are thought to be smaller [139,158]. Literature addressing nasal irrigations in CF patients mainly focus on the maxillary sinuses, but one should remember that CF patients may have frontal sinuses, which contain CF-pathogens as often as the maxillary sinuses (paper [3] and unpublished data). To ensure permanent drainage from the sinuses adequate extensive surgery may be considered; this could comprise a modified endoscopic medial maxillectomy [128] or a Draf III [130], the latter ensuring drainage from the frontal sinuses, which are the most challenging sinuses to operate. We advocate that it is important to ensure permanent access to the sinuses, both to reduce symptoms of CRS but also to facilitate postoperative treatment preventing sinus infections and spread of bacteria to the lungs. We agree that more extensive surgery is needed in CF patients than in patients without CF, but have to await studies on FESS comparing surgical methods with postoperative clinical examinations, symptoms, adverse effects and cultures.

Fig. 12. Two methods of doing postoperatively nasal irrigations. (Nicely demonstrated by the author's 10-year-old son Bertram).

In paper [3] and in section 2.8, the possibilities of using different or additional ways to treat the upper airways are summarised but the most optimal combination is not yet defined. In addition, a synergistic effect has been suggested when using tobramycin and colistimethate sodium for inhalation, thus, this should also be considered when doing research on which drugs to use for nasal irrigations/nebulizations [159]. Especially when aiming at eradicating *A. xylosoxidans* and *B. cepacia* complex, one must be aware of their antibiotic susceptibility (described in section 2.4). While others have described a good effect of nebulizers such as the PARI sinus [139], the patients in our study have been able to choose between two devices for nasal irrigations (Fig. 12). We have no conflicts of interest and find the device in Fig. 12a creates a higher pressure than the one in Fig. 12b, thus the saline being more likely to penetrate the sinuses.

Finally, based on our findings, I want to stress the importance of focussing on upper airway bacteriology in CF patients, especially in the outpatient routine treatment and the importance of guidelines for upper-airway treatment being established. This requires collaboration between the CF physicians, microbiologists and ORLs.

6.1. Study strength and weaknesses

The major strength of our set-up is the establishment of a unique, effective, collaboration focusing on CF; the microbiologists and CF physicians have had a strong collaboration through many years. This project has allowed ORLs to be a part of this collaboration making it multidisciplinary. We have a very large group of CF patients, which are all seen on a monthly basis, which is very frequent compared with other CF centres. This results in an abundance of systematically collected data that can

be evaluated for the benefit of the CF patients. The patients seem very committed to the research, the adherence is high, and only two patients did not wish to be enrolled in the IgA study [2]) and only one patient turned down the offer of FESS [4]). The willingness to attend the postoperative controls and return the questionnaires was also high (80–99%).

A project is always strengthened by having one single committed coordinator; this improves adherence and reduces bias. To maintain and develop our high quality of treatment, I find it necessary that the ORLs are keep on seeing CF patients, evaluating their CRS symptoms, doing endonasal endoscopy and sino-nasal cultures. Furthermore, it is also important that the CF physicians on a standardized basis ask for CRS symptoms and focus on possible upper airway infections.

The main outcome of our four studies reported in [1–4] is based on culture results and antibody measurements. The Department of Clinical Microbiology has few, but very dedicated and experienced, laboratory technicians who are responsible for doing the bacterial and antibody CF analyses. Thus, the possibility of inter-observer errors is low.

In paper [2], a weakness is that the study was not prospective; that is why our hypothesis that high IgA actually reflects sinus colonisations cannot be finally proven. IgA against alginate can, in small concentrations, be present in non-infected individuals and IgA against St-Ag can cross-react with other Gram-negative bacteria and the test is therefore not totally specific towards *P. aeruginosa* [19,160]. Even if our theory is correct, one should bear in mind that it only should be used as a supplementary test creating awareness of possible colonisations.

In paper [3], a drawback might be that due to ethical considerations, only a minority of the postoperative samples were taken during general anaesthesia, which is why false negative culture results from the sinuses cannot be excluded. However, I was the only one who obtained the samples, the majority of patients had persistent opening to their sinuses, and the follow-up procedure was standardized. Except when doing FESS, the same point applies in all our studies where one can always discuss how representative the material is. There are advantages and disadvantages in concern of doing regular culture compared with molecular methods; these two methods will be compared in an upcoming paper from our group. In short, the true positive diagnostic value is high using both methods. Though the molecular methods in a few cases did detect CF-pathogens missed by conventional culture methods, a case was also seen where the abundance of different sinonasal bacteria resulted in a false-negative result of CF-pathogens by the molecular methods. Sinus samples could in both cases become cross-contaminated by bacteria from the anterior nasal cavity, but we find this fact clinically unimportant. What is important is that cross-contamination of lower-airway samples from the upper airways remains as a potential confounder. Hence, when the CF-pathogen bacteria were eradicated from the upper airways, there would be a smaller risk of false-positive lower-airway culture results.

Regardless of cross-contamination, CF-patients who do not show growth of CF pathogens for a longer period will get their antibiotic treatment reduced. Consequently, we could have analysed whether the use of antibiotics decreased as a consequence of the decreased positive lower-airway cultures, or if unchanged, if the higher rate of antibiotics compared with positive lower-airway cultures might have been a confounder. The reason that this analysis was not included in paper [4] was that we would have had to differentiate between types of administration (oral, inhalation and intravenous) and differentiate between prophylactic, eradication and maintenance therapy. We found that this would have taken focus from our main outcome.

A general weakness concerning papers [1,3] and [4] is that the FESS-project was step-wise initiated. As a consequence, at the beginning of the study period, we were more reluctant including patients for FESS, making FESS extensive, and encouraging patients to be thorough with the postoperative treatment. As the existing research on FESS in CF patients, including the extent and postoperative treatment, was very sparse, the FESS by itself has not been a part of the research but solely the outcome of an established treatment. As a consequence, some CF-patients with no symptoms of CRS have not been offered FESS as early as we would now recommend and some not at all. Furthermore, we have not done surgery so extensive and explored all sinuses if the symptoms were not present. On the other hand, these facts ought not to influence the results in a positive way. What may weaken the way our results can be interpreted is that we did not have a control group to the FESS group. Instead we have to use the knowledge of the natural history of CF. Generally, a confounder could be that patients' way of being treated changed during follow-up, but in our case the treatment strategy has mainly been unchanged throughout the study period [4].

We are aware that CF patients are a heterogeneous group with confounders such as different co-morbidities of CF, large age distribution, different lung infection patterns (including LTX), and a wide span in the use of medicine. Despite this, we found it most correct to include all patients; however, this should be kept in mind when interpreting our results, but we have partly dealt with the confounders by doing sub-analyses.

Finally, we now stand in a classical dilemma: the need for a randomized case-control study is in conflict with the positive results from paper [4] and the positive feed-back we have got from the vast majority of CF patients. This subject has been discussed with the Head of the Copenhagen Trial Unit, Centre for Clinical intervention Research. In conclusion, based on all our summarised results, our studies can be compared to a "Fase IV clinical trial" [161] and it would be unethical to randomise CF patients to FESS or no treatment. The next step is to randomise patients to either conservative treatment, minimal FESS or extensive FESS, and let the outcomes, especially postoperative IgAs and lower-airway cultures, be evaluated by someone blinded to the treatment.

7. Perspectives

The determination that the sinuses play a role in the initial colonisation and infection in CF patients opens a lot of unanswered questions and still requires an active role from ORLs.

First of all, we do not have the perfect tool to diagnose whether a CF patient has CF-lung-pathogenic bacteria in their sinuses without doing sinus surgery. A prospective study of nasal and saliva IgA against *P. aeruginosa* must be carried out, as well as prospective studies concerning cultures of nasal irrigations [71] compared with meatus media cultures [112] and perioperative findings. Research on whether biomarkers as BPI-ANCA [155] and other inflammatory markers can play a role in determining sinusitis is also advisable.

Secondly, our postoperative treatment have partly been empiric and based on knowledge from the CF-lungs. Studies involving animal experiments are recommended to determine the most suitable drug(s), dose and administration interval for nasal irrigations.

Thirdly, a prospective trial randomizing CF patients to either sinus surgery or conservative treatment with nasal antibiotic and saline irrigation is highly important.

Fourth, using molecular methods such as FISH [78], it will be interesting to determine the diversity of the bacteria in the sinuses, the bacteria–bacteria interaction, presence of biofilm, and how bacteria changes phenotype after sinus surgery. It will be of clinical interest if certain bacterial phenotypes and genotypes can be correlated with severity of the disease so aggressive treatment can be early initiated in these cases.

Finally, in the same way as CF patients, patients with primary ciliary dyskinesia (PCD) may have sinusitis initiating colonisation and infection though the mechanisms are different [162]. In general, there is a lack on research on PCD, which is why CF treatment often is used on this patient group [162]. I recommend that a study be done on extensive sinus surgery and adjuvant therapy in PCD patients.

8. Conclusion

With respect to the aims of this study (section 1.1), the following can be stated:

(a) There is a very high prevalence of CF pathogen sinusitis. The bacteria persist in the sinuses for years and can be a focus for initial lung colonisation and maintain the infection.
(b) In contrast to the lungs, the sinus inflammation is dominated by non-phlogistic specific IgAs; this facilitates persistence of bacteria.
(c) There is no single way of diagnosing CF-pathogens in the sinuses without being invasive. Nasal IgA may be a surrogate-marker for *P. aeruginosa* in the lungs and may be used as a supplementary diagnostic tool for *P. aeruginosa*-sinusitis.
(d) We have treated our patients with extensive FESS and standardized postoperative follow-up, i.v. antibiotics, prolonged nasal irrigation with saline and antibiotics in addition to nasal steroids. Further studies are needed to find the most effective treatment.
(e) By this treatment strategy (d), quality of life was improved, bacterial sinus foci could be eradicated and the frequency of pulmonary samples positive for CF pathogens could be reduced. This indicates a reduced CF morbidity.

Altogether, the CF-upper airways should not be neglected and ORLs can give a significant contribution to CF treatment.

Conflict of interest statement

The author has no conflict of interest to report.

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