Bacteriology and treatment of infections in the upper and lower airways in patients with primary ciliary dyskinesia: addressing the paranasal sinuses

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LIST OF PAPERS INCLUDED IN THIS THESIS

I) Simultaneous sinus and lung infections in patients with primary ciliary dyskinesia.

II) A longitudinal study of lung bacterial pathogens in patients with primary ciliary dyskinesia.

III) Bacterial evolution in PCD and CF patients follows the same mutational steps.

IV) Sinus surgery can improve quality of life, lung infections and lung function in patients with primary ciliary dyskinesia.

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OBJECTIVES

There has been a minimal focus on the role of the paranasal sinuses, their bacterial flora and the management of chronic rhinosinusitis (CRS) in patients with primary ciliary dyskinesia (PCD). Further, knowledge about the bacterial flora associated with acute and chronic pulmonary infections in PCD patients is limited.

Currently, PCD treatment and monitoring regimens are based on expert opinions and in addition extrapolated from clinical experience and research in related areas such as cystic fibrosis (CF) and non-CF bronchiectasis, despite the fact that PCD is a disease with a different pathophysiology that often requires specific treatment.

The treatment regimen for selected patients with CF comprising endoscopic sinus surgery (ESS) with adjuvant therapy can effectively treat sinus infections, reduce pulmonary infections and inflammation, and improve health-related quality of life (QoL) and self-reported sinus symptoms. The major objective of this thesis is to evaluate whether PCD patients can benefit from the experience we have gained in treating CF patients with a focus on the role of the sinuses as a possible infectious reservoir. In parallel, we focus on the PCD airway pathogens, especially Pseudomonas aeruginosa because this bacterium causes most of the morbidity and mortality in CF.

The present work benefits from a well-established collaboration among clinical microbiologists, PCD specialists, paediatric pulmonologists and rhinologists. The inclusion of the latter specialists to cover the upper airways has, in our opinion, narrowed the knowledge gap in CF and PCD sinus and lung research.

Based on the concept of the unified airways, the primary objectives of this thesis was to study

1. The putative role of the sinuses as bacterial reservoirs in PCD patients (paper I),
2. The bacterial flora associated with acute and chronic lung infections in PCD patients and changes in flo-
ra composition with increasing patient age or age at diagnosis (paper II).
3. The bacterial evolution and adaptation during chronic lung infections with *P. aeruginosa* in PCD compared to CF patients (paper III).
4. The impact of ESS with adjuvant therapy in treating sinus infections, CRS and the influence of sinus infections on the lower airways, including lung function (papers I and IV).

**BACKGROUND**

**Primary ciliary dyskinesia (PCD)**
PCD is an autosomal recessive genetic disease. To date, known mutations in more than 30 genes involved in ciliary structure and function have been characterized, and genetic testing can identify approximately 60% of phenotypically identified PCD patients. The prevalence of PCD is uncertain, but it is reported to be 1:10,000 to 1:20,000. Many less pronounced PCD phenotypes may remain undiagnosed, suggesting a higher prevalence. There are no reported differences in prevalence in terms of ethnicity or race, but it is higher in societies with high occurrence of consanguinity.

PCD diagnosis remains a challenge because there is no specific test for the disease. The European Consensus guidelines recommend a combination of tests. Nasal nitric oxide measurements is useful as a preliminary screening test, although the diagnosis is based on the presentation of the characteristic clinical phenotype in combination with ciliary ultrastructural defects verified by electron microscopy (EM), abnormal ciliary movements visualized on high-speed video recordings or a genetic mutation recognized to cause PCD.

The respiratory tract is mainly lined with ciliated pseudostratified columnar epithelium. Cilia are motile hair-like organelles that transport respiratory mucus. Normal ciliary ultrastructure and examples of normal and abnormal EM recordings are shown in figure 1.

**Figure 1: Ultrastructure and electron microscopy of cilia**

A. Simplistic drawing of transverse section of a respiratory motile cilium. Normal motile cilia contain 9 outer doublets of microtubules and a central pair, called 9 + 2 axonemal appearance. Dynein arms are attached and contain enzymes for ATP hydrolysis to produce power. Nexin links connect the doublets and stabilize the structure, while radial spokes join the outer doublets with the central pair. When activated, the dynein arms slide one microtubule-duplet relative to another and because these are coupled by nexin links, the entire axoneme bends.

B. Electron microscopy of normal motile cilia. Electron microscopy showing lack of outer dynein arms as one example leading to primary ciliary dyskinesia.

Genetic defects lead to non-functional cilia in anatomical areas comprising motile cilia, including the respiratory tract, Eustachian tubes, ventricles of the brain and Fallopian tubes. PCD is predominantly an airway disease that clinically presents with neonatal respiratory distress, coughing, conductive hearing loss due to otitis media with effusion (OME), rhinitis, recurrent acute sinusitis, CRS, recurrent or chronic lung infections with bronchiectasis, progressively declining lung function and, in severely affected patients, respiratory failure and death. The median age at death has been reported to be 65 years. However, given the rarity of the disease and because PCD does not have a unique ICD-10 code, this statement remains unproven. Life expectancy of the general population in Denmark in 2015 was 78.5 for males and 82.7 years for females (statistics Denmark, www.dst.dk). Roughly, 50% of PCD patients have situs inversus totalis or other organ laterality defects. Congenital heart defects are observed in 5% of the patients. Males are practically infertile owing to dysmotility of the spermatozoa since the sperm tail shares ultrastructure with motile cilia.

**History**

In 1904, Siewert published a case report of a 21-year-old male with situs inversus, bronchiectasis and signs of sino-nasal disease. In 1933, Kartagener reported four patient cases with the triad of situs inversus, bronchiectasis and chronic mucosal nasal disease with nasal polyps, and this disease entity was subsequently named Kartagener’s syndrome. In 1976, first Afzelius and shortly after Pedersen and Mygind discovered using EM that in patients with this triad, cilia were immotile due to ultrastructural defects, and the disease was subsequently termed immotile cilia syndrome. However, later studies revealed that often, the cilia are not completely immotile but beat stiffly or asynchronously, thus justifying its present name of primary ciliary dyskinesia. In this name, primary distinguishes the condition from secondary reasons for defective ciliary movement such as airway infection, inflammation and muco-stasis. Defective mucociliary clearance is also observed in CF patients; however, cilia are normal in both structure and function whereas mucus is changed as explained beneath.

**Cystic fibrosis (CF)**

CF is the most common life-threatening inherited disease among the Caucasians, with an incidence of 1:2,500 in Europe. However, there are regional differences, and the incidence in Denmark is 1:4,700. CF is caused by mutations in the cystic fibrosis transmembrane regulator protein. Defects in this gene cause abnormal ion transport across epithelial borders accompanied by depletion of the periciliary liquid layer and failure of mucus detachment from the submucosal glands, resulting in the formation of thick, dehydrated mucus that impairs mucociliary clearance (MCC).

**Mucociliary clearance (MCC) in PCD and CF**

Inhaled pathogens are trapped in the airway mucus and then phagocytosed or removed by MCC in healthy individuals, in which constantly beating cilia propel the mucus with entrapped pathogens to the oropharynx, where it is cleared by swallowing, coughing and expectoration. Impaired MCC leads to the stagnation of mucus in the respiratory tract, which predisposes individuals to bacterial infections.

Infection and subsequent inflammation cause structural lung damage, contributing to bronchiectasis and declining lung function, which are hallmarks of both CF and PCD. In PCD, the composition of mucus is presumably normal. However, during prolonged or chronic infection and inflammation, the
DNA and actin released from polymorphonuclear leukocytes may increase viscosity of the mucus 34,35.

In CF, MCC is affected by dehydrated sticky mucus that impedes normal ciliary movement. Cough clearance is weakened in CF owing to depletion of the airway surface liquid, but in contrast is preserved in PCD 36.

Airway inflammation in both PCD and CF are dominated by neutrophil infiltration 37. In 2006, Bush et al. 35 reported no difference in the rheology or transport properties of sputum in PCD and CF, but even more pronounced inflammation in PCD compared to that in CF, as measured by interleukin-8 levels, suggesting similar inflammation and action of the host immune systems in PCD and CF.

Despite these similarities, PCD patients probably have a more favourable prognosis. Moreover, given the different basic biological mechanisms behind PCD and CF, it may be unreasonable to simply transfer CF research and treatment protocols to PCD patients.1,38 This is exemplified in the response to human deoxyribonuclease (rhDNase) inhalation. In the case of CF, rhDNase can improve lung function and reduce the number of pulmonary exacerbations 39, but in patients with non-CF-bronchiectasis, including patients with PCD, a short-term double blind placebo controlled study found no improvement in clinical parameters 40.

In fact, such treatment may even be harmful in patients with idiopathic bronchiectasis 41. Accordingly, rhDNase is not recommended in PCD 42. This may also be the case with other inhaled or intravenous antibiotics, which may not be used in same dosing regimens due to toxic effects in PCD not seen in CF. Yet, adopting CF treatment protocols in PCD remain clinical practice.

THE UPPER AIRWAYS

Paranasal sinus anatomy and development

The paranasal sinuses are mucosa-lined pneumatized cavities in the cranial bone that drain mucus into the nasal cavity and are ventilated via the nasal cavity. Most adults have four pairs of sinuses: the maxillary sinuses located infra-orbitally, ethmoids situated between the eyes in the ethmoid bone, sphenoid sinuses posterior to the ethmoids and frontal sinuses located in the forehead above the eyes.

The maxillary and ethmoid sinuses are air-filled at birth. The sphenoids are pneumatized from two years of age and mature in size at puberty. The frontal sinuses are the last to develop, starting at or shortly after two years of age, and they reach full size after puberty. Hypoplasia or aplasia of the frontal or sphenoid sinuses is found in >50% of adult patients with PCD 44, figure 2. Abnormal sinus anatomy is almost universal in CF 45. By contrast, frontal sinus hypoplasia is found in nearly 15% of the general population and sphenoid sinus hypoplasia is extremely rare 43.

Computed tomography (CT) is the optimal modality for visualizing the bony structures of the sinuses, and it is mandatory before ESS for diagnostics and surgical planning. Owing to abnormal sinus anatomy, image-guided surgery is preferable for treating PCD. Based on a systematic review and meta-analysis an Australian expert panel suggested that image-guided surgery is optional but helpful when performing ESS in CF patients 46. This recommendation may be transferable to PCD and we find it obligatory.

Figure 2. Computed tomography images of the paranasal sinuses.

Management of CRS in PCD

Currently, CRS management in PCD focuses on relieving symptoms. Therapeutic strategies are inspired from CRS treatment in
the general population, and they include sinonasal irrigation with saline, topical steroids and long-term antibiotics. However, to the best of our knowledge, no prospective studies have evaluated the medical or surgical treatment of CRS in PCD, and many questions about the impact of CRS in PCD remain unanswered.

Undoubtedly, sinonasal irrigation with saline is a beneficial and safe method for assisting upper airway clearance in PCD, as it is in the general population. Nasal irrigation can remove stagnant mucus, which may contain entrapped pathogens. It is inexpensive, fast and practical even for children.

Topical nasal steroids have a well-documented positive effect on CRS (level Ia evidence, Grade A strength of recommendation), and it may be reasonable to believe that administration can reduce mucosal inflammation in PCD, as it does in general CRS patients. Nevertheless, inhaled steroids are not recommended for the management of lower airway inflammation in PCD with the exception of PCD patients having overlapping atopic disease, and they have no clear effect on CF patients with CRSwNP presumably because airway inflammation, including nasal polyps, is dominated by neutrophils. Similarly, airway inflammation in PCD has neutrophilic predominance, and future studies should evaluate any potential effect of anti-inflammatory drugs, perhaps non-steroidal, on the upper airways in PCD.

Systemic corticosteroids are advisable for treating some CRSwNP patients. They can control the inflammatory response, reduce polyp size and alleviate CRS symptoms, but there is a risk of severe side effects, including growth suppression in children and osteoporosis in adulthood. Long-term medical therapy with macrolides has proven effective in treating CRSsNP in the general population and may thus be beneficial in treating PCD patients with CRS as well. However, no studies have evaluated the effect of antibiotics on CRS in PCD, although a randomized controlled trial is currently addressing this issue (www.bestcilia.eu).

ESS can ventilate and drain the sinuses and is a well-established CRS treatment, improving QoL in non-PCD CRS patients where medical therapy has failed. Early intervention seems to improve CRS-related outcomes. Essentially, ESS with adjuvant therapy can eradicate pathogenic sinus bacteria, postpone chronic lung infection (with timely intervention before chronic lung infection status), improve self-reported sinus symptoms and QoL, and stabilize lung function in selected patients with CF. In PCD, Frija-Masson et al. found a relatively short time period between the first isolation of *P. aeruginosa* and chronic lung infection and speculated about the importance of a more aggressive treatment regimen when this bacterium is isolated for the first time. Given that the sinuses may be the initial site of colonization, early intervention addressing the upper airways may be of paramount importance.

Only a few small case studies or solely retrospective case series with correspondingly low levels of evidence, have evaluated the potential benefits of CRS treatment with ESS in PCD. However, these studies, listed in table 2, consistently showed subjective benefit, but none of them addressed the timing of intervention.

Table 2. ESS studies in PCD (including case reports and case series).

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**Bacterial flora in the upper airways**

The paranasal sinuses in CRS patients are often colonized with bacteria. In non-PCD patients with CRS, the sinuses are often colonized with coagulase-negative staphylococci, *Staphylococcus aureus*, *Streptococcus viridans*, *Corynebacterium* species and anaerobes, and a few of these pathogens may contribute to the inflammatory component of CRS. However, the exact role of bacteria in CRS pathogenesis is unknown.

Although the pattern of bacterial flora in the lower airways has been studied extensively in CF and to a certain degree in PCD, the upper airways have attracted attention only recently in CF and almost no attention in PCD.

In CF, *P. aeruginosa* and *S. aureus* are the most frequently isolated pathogens from the sinuses; other common bacteria include *Haemophilus influenzae* and coagulase-negative staphylococci. Supplementary and important CF GNB lung pathogens can also colonize the sinuses, such as *Achromobacter xylosoxidans* and *Burkholderia* spp. In CF, sinus bacteria can migrate between the upper and lower airways, thus connecting or uniting the two compartments.

The bacterial flora of PCD sinuses has not been investigated previously. However, in 1982, in a study of 15 Danish PCD patients, Pedersen and Mygind found that *H. influenzae* was the most frequent pathogen in the nasal cavity, followed by *Streptococcus pneumoniae*, *S. aureus* and *P. aeruginosa*.
THE UNIFIED AIRWAYS

The unified airways theory is well-accepted, especially for allergic airway disease (hay fever and asthma). Further, many patients with non-specific inflammatory disease in the upper airways simultaneously have an associated lower airway inflammation, as demonstrated by the high prevalence of CRS and nasal polyps in patients with non-allergic asthma. In most areas, the upper and lower airways comprise identical epithelium and they have many overlapping functions such as conduction, heating and moisturizing of air, and filtration of particles.

We and other researchers have documented that CF patients can house a bacterial reservoir in their sinuses. Further, re-colonization from the sinuses is frequently observed in lung-transplanted CF patients. The transfer of P. aeruginosa may be promoted when patients have a viral infection owing to liquefaction of upper airway secretions, which will ease aspiration to the lungs. Bacterial migration is presumably both ways.

In CF, P. aeruginosa or other GNB can colonize the sinuses before the patient’s lungs are infected, and it is hypothesized that sinus bacteria can initiate and maintain deleterious lung infections. Eradication of sinus bacteria with ESS and adjuvant therapy can reduce lung colonizations and infection, and stabilize lung function in selected CF patients with intermittent lung colonization. Thus, ESS with adjuvant therapy should in our opinion be an important part of CF management.

Prior to this thesis it was unknown whether P. aeruginosa colonizes the sinuses in PCD patients and if the sinuses in PCD similarly function as bacterial reservoirs.

THE LOWER AIRWAYS

PCD patients are susceptible to bacterial lung colonization and infection due to defective MCC and stagnant respiratory mucus. The resulting lung inflammation can lead to irreversible lung damage, including bronchiectasis and a decline in lung function. Bronchiectasis is present from infancy, affects more than 50% of children and is universal in adult PCD patients, signifying disease progression. The progression of lung disease in PCD is individual and inconstant. However, mean annual declines in FEV1% of 0.5, 0.6 and 0.7 have been reported, and 20% or more of patients may progress to respiratory failure.

P. aeruginosa can colonize and infect the lungs of patients with PCD and may cause lung inflammation and destruction, as in CF. However, the bacterial flora in the lower airways of PCD patients have only been described in a few studies, and several pathogens can contribute to pulmonary disease in PCD, including S. aureus, H. influenzae, non-tuberculous mycobacteria, Moraxella catarrhalis and S. pneumoniae, which resembles the bacteria most commonly isolated from BAL in patients with non-CF bronchiectasis.

We wanted to examine whether the bacterial flora associated with acute and chronic lung infections in PCD are age-dependent or correlated to age at diagnosis. Further, neither the definition nor the importance of chronic lung infections or sinus infections has been considered in PCD previously.

Therapy

The classical treatment regimen of pulmonary disease in PCD consists of two primary factors: antimicrobial therapy and airway clearance. In Denmark, all patients are followed at the Danish PCD Centre (see Methods section). However, a paper from the European Task Force on PCD in children concluded that management varied widely across European centres, making a direct comparison of studies difficult. The European and American consensus statements both recommend full or shared care in PCD- or CF-specialized centres, with two to four annual clinical evaluations. However, regarding PCD, only the European recommends regular involvement of ear-nose and throat (ENT) specialists and, apparently, the focus is OME and not the sinuses. The extent to which these guidelines are followed is uncertain.

PSEUDOMONAS AERUGINOSA AND PATHOADAPTATION

The present thesis focuses on P. aeruginosa - an opportunistic Gram-negative bacterium that can be isolated from many environments in water and soil. It rarely infects healthy humans, but can cause lethal infections in immune-compromised patients and in patients with severe COPD. The prevalence of P. aeruginosa lung colonization in PCD is approximately 10% in cross-sectional studies. Davis et al. reported that this species had been isolated in 25% of PCD children in their cohort.

Two patterns of infection exist: 1) short-term lung colonization followed by clearance of the bacteria (intermittent colonization) and 2) long-term infection (chronic infection).

Adaptation of a bacterium to changing environmental conditions is facilitated by phenotypic and genetic adaptations. The adapted bacteria have improved sustainability in a given environment. Chronic lung infections with P. aeruginosa in CF patients are associated with genetic and phenotypic changes in the infecting bacterial isolates.

Consequently, the phenotypic characteristics of P. aeruginosa isolates from chronically infected patients are markedly different from the characteristics presented by the initial isolate in the patient, which often resembles wild-type environmental species characterized by rapid growth, being non-mucoid and susceptible to antibiotics.

In contrast, the phenotypic characteristics of P. aeruginosa isolates from chronically infected CF patients show slow growth, antibiotic resistance, lack of motility, protease overproduction, changed cell envelope and overproduction of alginate (mucoidity).

The majority of genetic changes include mutations in genes known to improve the fitness or resistance of the bacteria. Mutated forms of these genes are popularly called ‘pathoadaptive genes’. Presumably, the evolutionary process from the early wild-type geno- and phenotypic characteristics to the features of chronically adapted isolates is driven by the frequent presence of antibiotics, the host immune system and inflammatory response, nutrient availability, oxygen levels and the structure and composition of respiratory mucus. However, their relative contribution and importance are unknown.

MATERIAL AND METHODS

Patients

Approximately 120 patients are diagnosed with PCD in Denmark. Denmark has a population of 5.7 million. Thus, there should be around 300 PCD patients in Denmark, signifying diagnostic challenges. Patients follow a routine protocol and they are intentionally...
appointed with four clinical examinations annually, including lung function testing with spirometry, measurement of body mass index (BMI) and obtaining of lower airway samples for culture as recommended by the European Respiratory Society Task Force. At least one annual blood sample is analysed for precipitating antibodies against relevant lung pathogens. Multiple measurements are reserved for patients with increasing antibody levels or decreasing lung function.

**Endoscopic sinus surgery (ESS) and bronchoalveolar lavage (BAL)**

ESS combined with per-operative BAL under the same anaesthesia was performed in two studies (I, IV). The indications of ESS were:

1. search for a sino-nasal infectious focus in patients’ colonized in their lungs with *P. aeruginosa*,
2. search for a sino-nasal or unidentified pulmonary infectious focus in patients with an increase (≥2) in serum precipitating antibodies (see below) against *P. aeruginosa* or
3. severe CRS symptoms according to the European position paper on rhinosinusitis and nasal polyps (EPOS) with unsatisfactory effect of medical treatment comprising nasal irrigation with saline and topical nasal steroids.

Before ESS, all patients completed the SNOT-22 questionnaire (see below) and were clinically examined for symptoms and clinical signs of CRS, including an endoscopic fibre optic examination of the nose, and a CT scan of the paranasal sinuses with slice thickness ≤ 1 mm used for surgical planning and for image guidance during surgery.

The aim of the surgical procedure was to obtain representative mucous and mucosal samples for microbial culture, to ventilate and drain all sinuses and make them accessible for postoperative nasal irrigation. The surgical procedure was standardized in paper IV: a navigation system was used based on the preoperative CT scan for pre-surgical planning and image guidance during surgery. The surgical procedure was performed under general anaesthesia, and it included uncinectomy, anterior ethmoidectomy and, when indicated, frontal- and sphenoid sinus anthropotomies, figure 3. At the end of the surgical procedure, all accessible sinuses were irrigated with colistimethate sodium (CMS) (3 million international units dissolved in 30 cc isotonic saline). All operative details and complications were recorded.

**Image guidance**

When performing extensive sinus surgery you may get in close proximity to critical and vital structures such as the olfactory fossa, the skull base, the ethmoid and carotid arteries and the orbit. Further, abnormal sinus anatomy, which is frequent in PCD, may increase the risk of surgery. Image guidance during ESS can reduce the rate of surgical complications. Consequently, it was used in all PCD patients. The system used (The Fusion™ ENT Navigation System, Medtronic) is electromagnetic-based and consist of a computer, a tracking system and specially designed navigation instruments. The patient’s CT scan is uploaded into the system and when the image guidance is registered the system allows for tracking of navigation instruments in real time in three planes (axial, coronal and sagittal), as shown on the monitor in figure 3 A.

**Adjuvant therapy**

Postoperative adjuvant therapy was standardized in (IV) and included two weeks of systemic antibiotic therapy according to susceptibility testing of the bacteria cultured from the BAL and/or sinuses and twice daily nasal irrigation with saline and topical nasal steroids (spray mometasonfuroate 100 milligrams) for at least three months. Clinical follow-up was performed after 14 days, three, six and 12 months, where crusts in the nose or sinus cavities and secretions were cleansed. CMS was intentionally administered in the sinuses twice daily for six months if *P. aeruginosa* was cultured in the sinuses samples obtained during surgery. Nasal administration of CMS has proven safe and well tolerated in CF.

**Sino-nasal outcome test 22 (SNOT-22)**

A translated and validated Danish version of the SNOT-22 questionnaire was used to evaluate the effect of CRS on health-related QoL in PCD patients. It contains 22 questions, each with a score ranging from 0 to 5. Thus, the maximum score is 110. It has been validated and is used worldwide to evaluate outcomes after ESS in CRS patients. It has also been used to evaluate effects of ESS on patients with CF. In most cases, the patients were old enough and capable to complete the questionnaire. However, in a few cases, parental assistance or support from the treating physician (MA) was needed.

**Antibiotic treatment of lung pathogens in PCD**

PCD patients in whom pathogenic bacteria have been isolated from the lower airways are treated with systemic antibiotics even if clinical symptoms are absent, which is in accordance with international guidelines. Oral antibiotic treatment with amoxicillin with or without clavulanic acid is administered for 14 days when *H. influenzae*, *M. catarrhalis* or *S. pneumoniae* are isolated from lower airway secretions. Growth of *P. aeruginosa* is treated with inhaled CMS and orally administered ciprofloxacin for 30 days. Elective intravenous antibiotic treatment according to susceptibility testing is administered for 14 days every three months to PCD patients chronically infected with *P. aeruginosa*. Prophylactic antibiotic treatment is not recommended, but it is often used, and currently being investigated in a randomized controlled trial (www.bestcilia.eu).
**Lung infection status**

No international consensus on categorizing pulmonary infections in patients with PCD exists. In this thesis, we extrapolated criteria and definitions developed for CF to the lung infection status of patients with PCD. We used the modified CF Leeds criteria, and accordingly patients can be categorized into three groups with regard to pulmonary infections:

1. Chronically infected, when >50% of the preceding 12 months’ cultures were positive for a specific pathogen;
2. Intermittently colonized, when <50% or less of the preceding 12 months’ cultures were positive for a specific pathogen;
3. Free of infection, when no growth occurred in the lungs in the previous 12 months.

Patients should have at least four evaluable samples annually to be classified. However, we made an exception relative to the criteria and introduced the use of serum antibodies (precipitins) in PCD. Specific antibodies against *P. aeruginosa* are measured annually using crossed immunoelectrophoresis. Normal values are 0–1. In CF, precipitins ≥ 2 are correlated with the extent of lung inflammation, immune complex-mediated tissue damage and related to chronic lung infection and poor prognosis. We classified a PCD patient as *chronically lung infected* when a lower airway culture was positive in combination with 2 or more precipitins against the concordant bacterium, which resembles the Copenhagen criteria used in CF.

### REVIEW OF THE RESULTS

**Paper I**

Simultaneous sinus and lung infections in patients with primary ciliary dyskinesia.

In this study, bacterial sinusitis was reported for the first time in PCD. All included patients (n = 7) had bacterial sinusitis, and five patients (71%) had simultaneous sinus and lung infections with the same pathogen, which was *P. aeruginosa* in four out of five cases (80%) and *H. influenzae* in one case (20%). Based on a combination of these early findings and the similarity of MCC dysfunction between PCD and CF, it seems likely that the sinuses in PCD can function as a bacterial reservoir, especially for *P. aeruginosa*, causing repeated lung colonization and infection, as in CF. Another important finding in this study was that two patients with intermittent lung colonization by *P. aeruginosa* before ESS remained free from lung colonization by *P. aeruginosa* for at least one year after ESS, and in one of these patients, sinusitis caused by *P. aeruginosa* was detected. Further, three out of four patients showed decreasing levels of serum antibodies against *P. aeruginosa* after this treatment regimen. This work was hypothesis-generating, and the potential benefits of combined ESS and adjuvant therapy on *P. aeruginosa* sinusitis and lung colonization was verified in a larger prospective studies (paper IV). Paper I will be reviewed further below, together with paper IV.

**Paper II**

A longitudinal study of lung bacterial pathogens in patients with primary ciliary dyskinesia.

Concurrently we examined the bacterial flora associated with acute and chronic pulmonary infections in PCD. In accordance with previous studies, we found that PCD patients are infected with a unique set of pathogens acquired in age-dependent sequences. *H. influenzae* was the most frequent microorganism, and other common pathogens in children and young adults were *S. pneumoniae, M. catarrhalis*, and *S. aureus*, whereas *P. aeruginosa* dominated in adults. This bacterial flora resembles the flora in patients with non-CF bronchiectasis.

Studies have reported a cross-sectional prevalence of lung colonization with *P. aeruginosa* of 10% in PCD, but aspects such as chronic infections, definition of chronic infection and whether the prevalence of *P. aeruginosa* is age-dependent or correlated with the age at diagnosis required further attention.

In CF we learned that a high prevalence of chronic infection should encourage a search for new treatment regimens, including ESS with adjuvant therapy. Our study revealed that *P. aeruginosa* frequently colonizes the lower respiratory tract in PCD patients, with a median PePR of 32%, corresponding to 11–44 intermittently lung-colonized Danish PCD patients annually; most patients (69%) had been lung-colonized with *P. aeruginosa* at some point. This in accordance with a recent study by Shah et al., in which *P. aeruginosa* was isolated in 68 of 151 (44%) adult PCD patients.

In CF, the incidence of *P. aeruginosa* increases with age, and these bacteria causes most of the morbidity and mortality associated with the condition. Correspondingly, we found significantly increased prevalence with increasing age in PCD and observed that early diagnosis was correlated with a significantly lower prevalence of *P. aeruginosa*. Similarly, Shah et al. found that late diagnosis was associated with increased *P. aeruginosa* colonization and reduced lung function. This highlights the importance of timely PCD diagnosis, which is supported by Ellerman and Bisdag, who showed stabilization of lung function decline after initiation of adequate antibiotic treatment and physiotherapy. However, this was among selected patients with few observations and later Martin et al. in a comprehensive longitudinal study concluded that early diagnosis did not protect against lung function decline. In CF, early diagnosis and adequate airway therapy is of paramount importance from the viewpoint of reducing morbidity and mortality.

In Danish CF patients, the prevalence of chronic bacterial lung infection with *P. aeruginosa* is seven percent in children and 47% in adults. In our PCD cohort, the incidence of chronic infection was strikingly higher than reported hitherto. Based on the definitions and the criteria developed for CF patients, 42 out of 107 patients (39%) met the criteria for chronic infection at least once. In a recent study, Frija-Masson and colleagues observed chronic *P. aeruginosa* infection in 27% of adult PCD patients, and Maglio and Boon et al. reported chronic *P. aeruginosa* infection in 5% of patients by using different criteria. However, the criteria we used for chronic infection seem valid because 10 out of 12 patients (83%) maintained the same clone type for years, as determined by PFGE, thus substantiating factual chronic airway infection and not re-colonization with environmental isolates. Nevertheless, the pattern of chronic infection with *P. aeruginosa* showed intermediate periods where patients did not conform to the definitions of chronic infection. However, 83% of the patients maintained a unique clone type for years when their lungs were re-colonized. This distinctive infection pattern may support the hypothesis of a sustainable non-pulmonary bacterial reservoir in PCD for repeated pulmonary infection, which may be the sinuses, as in the case of CF. It should be noticed though, that patients may also acquire the bacterium from a persistent source.
in the environment or by transmission from other PCD or CF patients. Sharing of *P. aeruginosa* clone types between PCD and CF was shown by WGS in paper (III).

Thus, the progression of lung disease in PCD may result from recurrent lung infection/pulmonary exacerbations, chronic infection and a change in the dominant lower airway pathogens. The importance of acute infection was substantiated in a study by Sunther et al. 110, who recently discovered that 25% of PCD patients fail to recover lung function after an acute pulmonary exacerbation. However, most previous studies have not found any correlation between lung bacteriology and lung function in PCD 13,21,85. Nevertheless, both Boon et al. 51 and Frija-Masson et al. found that PCD patients colonized and infected with *P. aeruginosa* had significantly poorer respiratory function than other patients. Similarly, Rogers et al. 14 documented correlations of *P. aeruginosa* abundance with age and declining lung function.

Another novel finding in this study is that no single clone type of *P. aeruginosa* is responsible for chronic lung infection in PCD because many clones can establish persistent infections, similar to CF 94. Thus, it seems probable that many wild-type *P. aeruginosa* isolates can initiate pulmonary colonization in PCD patients and then adapt to the stressful environment via genotypic and phenotypic changes, and establish chronic infection. Consequently, we examined phenotypic evolution and changes in genetic content during chronic infection with *P. aeruginosa* in PCD patients (paper III).

**Paper III**

Bacterial evolution in PCD and CF patients follows the same mutational steps.

PFGE is useful for typing of bacteria. However, the method does not provide an in depth understanding of the bacterial evolution over time. By WGS of 35 *P. aeruginosa* isolates sampled from 12 PCD patients we have studied the bacterial evolution in vivo for the first time in PCD. The analyses verified that different clone types of *P. aeruginosa* can establish persistent infections in PCD patients. Presumably, the initial infection is caused by environmental isolates, and when chronic infection is successful, the isolate adapts to the airways by gradually accumulating pathoadaptive mutations and phenotypic characteristics.

We sought to identify the genes targeted by beneficial mutations that optimize bacterial fitness and found genetic evolution in eight genes among the *P. aeruginosa* isolates. Remarkably, mutations in six of these genes are known to be important for adaptation to the CF airway 94. Among these were Mex genes that encode efflux pumps leading to antibiotic resistance, and Muc and AlgU which are important for transition to the mucoid phenotype. Mucoidity is an important risk factor for chronic infection and is correlated with poor prognosis in CF since the mucoid phenotype grows as biofilms in CF airways 111.

Unpredictably, we did not witness a clear phenotypic adaptation in the PCD isolates, which is in contrast to the genotypic alteration, and most of the PCD isolates continued to grow rapidly, were susceptible to antibiotics, non-mucoid and without increased biofilm formation.

Possibly, the phenotypic traits may evolve at a slower pace, and the interval between the first and last isolates in our cohort was short, with an average timespan of 2.4 years.

Nevertheless, the parallel genotypic changes indicate similar selective pressures on the bacterial isolates in PCD and CF constituting the frequent presence of antibiotics and the host’s inflammatory response rather than the immediate environment comprising the structure and composition of the mucus. This similarity in evolution may support a similar therapeutic strategy in PCD as that in CF, including the choice of antibiotic therapy and ESS. This hypothesis may be supported by the study of Ratjen et al. 37, who found equal response to antibiotic therapy for pulmonary exacerbation with regard to lung function tests between CF and PCD.

Though studies of bacterial micro-evolution may seem far from daily clinical work, a better understanding of evolutionary processes may help identifying new treatment options. WGS of bacterial isolates including *P. aeruginosa* has proven clinically useful and cost effective with regard to surveillance of multi drug resistance and in monitoring of transmission between patients 112. Further, such micro-evolutionary traits may prove useful for the development of new drugs, diagnostic markers or predictions of persistent infections. Identification of new target could be a susceptible phenotypic characteristic or a specific treatment could be used to affect the with-in host evolution and thus direct this in a desired direction 94. For example administration of one antibiotic may enhance susceptibility for another, a principle termed collateral sensitivity 113.

**Paper IV**

Sinus surgery can improve quality of life, lung infections and lung function in patients with primary ciliary dyskinesia.

Paper IV is the first prospective study of ESS in PCD patients. It showed that bacterial sinusitis and bacterial co-colonization are frequent despite regular systemic antibiotic therapy. This indicates that the sinuses constitute a bacterial focus, where systemic antibiotics have only a small impact, similar to CF 114. Table 3 lists the bacteria cultured from the sinus samples obtained during ESS, and mentions whether we consider these bacteria pathogenic.

Table 3: List of sinus bacteria isolated from samples obtained during 31 sinus surgeries

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Number of patients harbouring the bacteria</th>
<th>Pathogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>12</td>
<td>Yes</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>5</td>
<td>Yes</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>1</td>
<td>Yes</td>
</tr>
<tr>
<td><em>S. maltophilia</em></td>
<td>2</td>
<td>Unknown</td>
</tr>
<tr>
<td>Coagulase negative staphylococcus</td>
<td>11</td>
<td>No</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>3</td>
<td>Yes</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>1</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Propionibacterium acnes</em></td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td><em>Propionibacterium species</em></td>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>1</td>
<td>No</td>
</tr>
</tbody>
</table>

The data are cumulative due to co-infection. Sinus bacteria from one patient (Patient #2 in paper IV and patient #8 in paper I) overlap.

Notably, the most frequently cultured bacterium from the sinuses was *P. aeruginosa*, which was isolated in 12 out of 31 cases (39%).
Figure 4 shows an intraepithelial abscess in the left maxillary sinus. The abscess was not cultured, but the sinus cultures grew *P. aeruginosa*.

Figure 4. View of the left maxillary sinus of a PCD patient using a 30° endoscope.

We found simultaneous sinus and lung infections with identical pathogens in the majority of the patients in (I, IV): in five out of seven patients in paper I and in 13 out of 21 patients with bacterial sinusitis in paper IV, 64% altogether. In ten patients (36%), the identified specific pathogen was *P. aeruginosa*. This supports the unified airways theory.

Other common sinus bacteria were *H. influenzae* and *S. pneumoniae*, which are also among the most common lung pathogens in PCD, as demonstrated in paper II and others. The role of sinus co-infection is unidentified but some bacteria may suppress others, and it is noteworthy that co-infections were more common in the sinuses compared to the lungs.

In a published and comparable study on CF patients with sinus samples obtained during ESS, *P. aeruginosa* was isolated from the sinuses in 27 out of 55 cases (49%), demonstrating that *P. aeruginosa* is a common and important sinus pathogen in both PCD and CF.

The optimal sinus bacterial eradication regimen is debatable. Doth et al. found that the impact of intravenous antibiotic treatment on inflammatory markers is considerably lower in the sinuses compared to the lungs of CF patients. This may be the case in PCD as well. Thus, active local treatment of sinus infections comprising surgery and/or local antibiotic treatment may be superior. However, it should be noted that sinus penetration of topically administered drugs increases with ostial size, and the universal CRS in PCD with inflamed mucosa may limit access, especially in non-operated patients. Further, it may be challenging to eradicate an infectious focus such as the one shown in figure 4 without surgical intervention.

Sinus hypoplasia or aplasia was identified in 58% of the patients, which is in accordance with the findings of Pifferi et al. Osteitis, neo-osteo genesis and new bone formation have been used synonymously in the literature and can be defined as inflammatory bony processes associated with *P. aeruginosa* sinusitis and previous surgery. Thus, persistent mucosal disease or bacterial rhinosinusitis may lead to osteitis, recalcitrant CRS and sinus hypoplasia in PCD patients. In comparison, otitis media can affect bone development and decrease pneumatization of the middle ear. However, this remains unproven in the case of PCD.

Nasal polyps were identified in 42% of the patients in paper IV, including in two out of six children (<17 years) (33%), figure 5. This is higher than the numbers reported previously and an accidental finding of nasal polyposis in children should lead the clinician to suspect PCD or CF.

Figure 5. Nasal polyps in a child with PCD.

Nasal polyps in a 13-year-old girl with PCD.

Important goals when treating patients with chronic lung disease are maintaining lung function and improving QoL. The introduction of our multidisciplinary team’s new treatment regimen comprising ESS with adjuvant therapy to PCD patients had a positive impact on the lower airways and significantly ameliorated CRS symptoms. Postoperatively, patients tended to have fewer positive lower airway cultures and improved lung function. In addition, 4 out of the 16 patients in paper IV and 2 out of the 7 patients in paper I (i.e. 6 out of 23 patients = 26%), where infection was the indication for surgery, remained free of lung infection with *P. aeruginosa* during follow-up for at least six months.

It is tempting to speculate that ESS with adjuvant therapy can eradicate sinus bacteria and, thus, reduce lung re-colonization from the sinuses, but further evidence is needed to support this hypothesis, preferably from a multicentre randomized controlled trial.

Previous case series have suggested subjective improvement in QoL after ESS in PCD (Table 2). We have for the first time used a specific sino-nasal questionnaire and confirmed that improvement in sino-nasal-related QoL can be expected shortly after ESS and last for at least 12 months, which is in accordance with the clinical experience related to CF patients. Finally, ESS using image guidance and devoted surgeons is a safe procedure in PCD, and no severe or persistent complications were noted.

Our research has led to the following conclusions which may be relevant for clinicians who are treating PCD patients. By ESS

- there may be a 25% chance of eradicating *P. aeruginosa*. The best result is probably achieved for patients with early infections (before chronic lung infection).
- lung function may improve.
- improvement in sino-nasal related QoL for at least 12 months is likely achieved. This may require high patient compliance including regular follow-up in the ENT outpatient clinic, daily nasal irrigation with saline and topical nasal steroids.
- there is a potential need for revision surgery, which based on clinical experience of treating CF patients and their sinuses, is about 33% within three years.
- thus far, we have observed no peri-operative severe complications, and ESS has proven to be safe in patients with CF. However, in general, ESS puts subjects at risk for haemorrhage, pain, infection and damage to neighbouring organs, including the eyes and the brain. However, these risks are reduced using image-guidance.
• it is uncertain whether the patients may achieve the same positive results with adjuvant therapy alone and no surgery, which remains to be studied.

STUDY STRENGTHS AND WEAKNESSES

The strengths of my studies are the establishment of a well-functioning PCD multidisciplinary team of clinical microbiologists, pediatric pulmonologists and rhinologists. In Copenhagen, the inclusion of the latter specialists was introduced in CF in 2007 and with this thesis transferred to PCD. In addition, the Danish PCD Centre handles a large number of patients with regular clinical visits. Thus, abundant clinical and para-clinical data, including sputum samples were available. Danish PCD patients are committed to research and are willing to adhere to protocols. This was exemplified in paper IV, where we had 100% follow-up after three months, and there were no drop-outs at the time of writing this thesis. The involvement of one committed health care person in all steps from pre-surgical planning through surgery to the final postoperative follow-up, and the fact that patients had easy access to ENT medical expertise if required are other advantages.

In the most comprehensive study of lung bacterial pathogens to date in PCD (II) we found that *P. aeruginosa* is a common and important lung pathogen with a lethal potential. We genotyped bacteria using PFGE (II) and WGS (III) to verify factual chronic infection rather than recolonization with another isolate, which is original. Finally, we performed the largest prospective study with the longest follow-up to date of ESS in PCD (IV). These strengths substantiate our conclusions and for the first time in PCD it was possible to propose evidence based guidelines and recommendations for ESS including indications, benefits and risks.

Further, our studies of sinus and lung pathogens (I, II, III, IV) have made us competent to suggest the first criteria for categorizing lung infection status in PCD (page 41). So far, varying criteria for chronic lung infection have been applied in the literature. Sunther et al. 110 defined persistent infection as at least two positive cultures of the same microorganism at least three months apart during 12 months. Other researchers defined chronic *P. aeruginosa* infection as the presence of this bacterium for at least six months, with at least three positive cultures 11,20,85. Widespread use of the suggested infection status tool could be advantageous when choosing treatment strategies, for hygiene precautions and for designing or comparing clinical studies.

Our cohort may represent a selected group of PCD patients with the most severe phenotypes, which is a limitation. In addition, in (I, IV) we favored inclusion of patients’ lung infected with *P. aeruginosa*. Consequently, there is a risk of selection bias that may reduce the generality. However, most patients including patients free of infection experienced a long-lasting (≥1 year) improved QoL after ESS with adjuvant therapy.

A few patients were solely sinus colonized with *P. aeruginosa* and categorized as free of infection when criteria adopted from CF were used. The sinuses may represent the initial colonization site or a persistent infectious focus, and categorization as free of infection is obviously misleading unless the ‘free’ anatomical area is described. Even though it is not possible to evaluate, we probably have postponed or even spared pulmonary colonization and infection in a few and potentially all of these patients according to our experience from CF 2. Thus, we advocate that a sinus status should be included in the infection criteria (page 41).

It can be argued that conservative medical therapy comprising nasal irrigation and systemic antibiotics could have achieved the same positive results as demonstrated after ESS with adjuvant therapy in (I, IV). Since we did not include a control group this cannot be ruled out. Nevertheless, nasally administered saline and antibiotics may only irritate the nose and not reach the sinuses in non-operated patients. Still, our results must be verified prospectively in different cohorts at other centers, and clearly, a randomized controlled trial is necessary.

Further, neither the surgery performed nor the postoperative treatment in paper I was standardized, which may have led to underestimation of the possible benefits of this treatment regimen.

In paper IV, we could have evaluated whether ESS can prevent or reduce pulmonary exacerbations because this may be a valuable end-point in clinical trials involving PCD patients 110. Other potential clinically relevant end-points are the use of antibiotics, hospitalization, absenteeism and the new PCD-Qol 122. The latter was not validated and published when we initiated the studies, but it will be used in future studies at our institution.

Further, the extent of surgery can be debatable. We applied extensive ESS to ensure representative samples from all involved sinuses because bacteria can be isolated from the maxillary, ethmoid, frontal and sphenoid sinuses. In addition, extensive surgery facilitates postoperative cleansing, thus creating the possibility to examine the sinuses endoscopically with a sufficient overview during follow-up. In this way, recurrent disease may be noticed earlier.

Only low-level evidence supports the chosen local adjuvant therapy. Inhalation therapy with CMS is often used in PCD in Denmark when *P. aeruginosa* is cultured from the lungs. Therefore, we used this drug locally in the sinuses. However, optimal dosage and administration form is uncertain, but the dose was chosen based on the fact, that the pharmacokinetics and pharmacodynamics of CMS against *P. aeruginosa* is dose-dependent 123. Comparably, the effect of topical nasal steroids in PCD is tentative 60.

Culture results are a major outcome throughout this thesis. Cultures were performed and analysed by skilled laboratory technicians at the Department of Microbiology, Rigshospitalet, who have gained extensive experience from CF over many decades, thus minimizing the risk of errors. Nevertheless, using classical culture methods and not molecular methods may lead to an underestimation of bacterial diversity and ignored difficult-to-grow bacteria 14,114. However, the experience from CF patients in our centre does not indicate that this is a major problem 125.

SUGGESTED CRITERIA FOR GRADING AIRWAY INFECTIONS IN PCD

Optimized infection status criteria for PCD and CF could include a potential sinus focus and serum antibodies in a TNM-like system, called PUS, table 5.

Table 5. Suggested criteria for staging of GNB colonization/chronic infection in PCD: Pulmonary, Upper airways, Serum antibodies (PUS).
To answer the primary aims of this thesis:

1. In PCD, there is high prevalence of bacterial sinusitis and simultaneous sinus and lung colonization with identical pathogens, indicating that the sinuses may constitute a potential bacterial reservoir for recurrent bacterial lung infections. This is in accordance with the unified airways theory.

2. ESS can ameliorate CRS symptoms for at least 12 months and improve lung infection status in selected PCD patients, thus postponing chronic lung infection. Lung function can be stabilized in selected PCD patients after ESS with adjuvant therapy.

3. PCD patients are infected by a unique set of pathogens acquired in an age-dependent order. Early infections are dominated by *H. influenzae, M. catarrhalis* and *S. aureus*, while the prevalence of *P. aeruginosa* increases with age. *P. aeruginosa* is a major pathogen in both the upper and the lower airways in PCD. Early PCD diagnosis and adequate PCD therapy may protect the lungs from colonization and infection with *P. aeruginosa*.

4. Many clone types of *P. aeruginosa* can establish persistent infections in both CF and PCD patients, where *P. aeruginosa* gradually adapts to the airways through mucociliary clearance, causing increasing knowledge about the diversification and adaptive ability of *P. aeruginosa* may facilitate new and better treatment regimens. It may be relevant to study the microevolutionary events in PCD patients who acquire their first *P. aeruginosa* isolate as this may allow a better understanding of the initial colonization phase from wild type environmental species to a possible persistent clone type.

OVERALL CONCLUSIONS

To answer the primary aims of this thesis:

1. MCC is decreased in patients with COPD, and *P. aeruginosa* chronically colonizes the lungs of >10% of patients with severe disease accompanied by bronchiectasis. These patients may have bacterial sinusitis as well and a study with extensive ESS in patients with severe COPD and lung infection by *P. aeruginosa* is suggested.

2. We observed a tendency to a reduction in pulmonary infections and an improvement in lung function tests after ESS with adjuvant therapy in PCD. Clearly a randomized controlled trial is needed, preferably a multicentre randomized controlled trial to increase power and further elucidate the role of ESS on the lower airways.

3. The genotypic and phenotypic adaptations of *P. aeruginosa* may begin in the sinuses, as in CF. We are planning to establish a biobank for sinus and lung bacterial isolates for future studies on PCD because increasing knowledge about the diversification and adaptive ability of *P. aeruginosa* may facilitate new and better treatment regimens. It may be relevant to study the microevolutionary events in PCD patients who acquire their first *P. aeruginosa* isolate as this may allow a better understanding of the initial colonization phase from wild type environmental species to a possible persistent clone type.

PERSPECTIVES

Several related project are currently ongoing and require further research:

1) It is essential to identify suitable PCD candidates for ESS and to intervene in a timely manner, as our experience from CF shows that patients with early infections benefit the most from this regimen. This may include identification of patients with *P. aeruginosa* sinusitis. Swaps from the middle meatus in the nose or nasal lavage may help identify these patients, but in our experience (unpublished), there may be a risk of false negative results.

Another possibility is the measurement of immunoglobulin A (IgA) because IgA values in saliva or nasal secretions increase in CF patients with GNB lung colonization. In a prospective setting, data evaluating the diagnostic value of secretory IgA in the saliva and nasal secretions of CF patients are under way. These results may be transferable to PCD.

2) The dose, drug(s) of choice and modality for adjuvant therapy need further investigations. Hopefully our group’s ongoing study of nasal flushing will elucidate how or when nasal irrigation and topical antibiotics enter the sinuses and for how long these fluids remain there.

3) Many PCD patients (33%) display normal ciliary ultrastructure on EM, suggesting that other factors can affect ciliary function. Ciliary movements may be controlled by a series of signalling events and stimulation of extraoral taste receptors, can increase ciliary beat frequency. Our research group are currently investigating aberrant receptor signalling in PCD.

Besides the ongoing projects and related publications other ideas have emerged from working with this thesis. Three future projects are suggested below:

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SUMMARY

The respiratory tract is lined with motile cilia that transport respiratory mucus. Primary ciliary dyskinesia (PCD) is a chronic genetic disease caused by mutations in genes responsible for ciliary structure and function. Non-functional airway cilia impair the mucociliary clearance (MCC), causing muco-stasis, lung infections.
and destruction, chronic rhinosinusitis (CRS) and hearing impairment. It is of paramount importance to postpone chronic lung infection mainly with Gram-negative bacteria (GNB) in patients with an impaired MCC. When successful, lung function can be stabilized and quality of life (QoL) improved.

In this thesis, we evaluated whether PCD patients can benefit from the experience we have gained from our operative approach towards the paranasal sinuses in cystic fibrosis (CF) patients. In CF, it has been established that bacterial sinusitis can be a focus for initial lung colonization and chronic lung infection. Combined endoscopic sinus surgery (ESS) and adjuvant therapy can eradicate sinus bacteria, reduce pulmonary infections and improve quality of life (QoL).

Currently, approximately 120 patients are diagnosed with PCD in Denmark and all are affiliated with the Danish PCD Centre. Patients included in this thesis were recruited from this cohort. In papers (I, IV), we found that the most frequent sinus pathogen was P. aeruginosa, which was isolated in 12 out of 31 (39%) patients who underwent ESS. In searching for a non-pulmonary infectious focus, we observed simultaneous sinus and lung infections with identical pathogens in two out of three patients. This supports our hypothesis of a bacterial reservoir in the sinuses. Next (II), we examined the bacterial flora associated with acute and chronic pulmonary infections in PCD. A high prevalence of chronic infections encouraged a search for new treatment regimens, including ESS with adjuvant therapy, to impact the course of infection. We revealed that P. aeruginosa frequently colonizes the airways in PCD and during the 11-year study period a total of 42 out of 107 (39%) patients fulfilled the definition of chronic lung infection at some point. Importantly, 10 out of 12 patients (83%) with chronic lung infection had the same clone type of P. aeruginosa for years, as determined by pulsed field gel-electrophoresis (PFGE), thus substantiating factual chronic airway infection. Further, we found an increase in the prevalence of P. aeruginosa with age and observed a negative association between early PCD diagnosis and prevalence of P. aeruginosa. This indicates a positive effect of early diagnosis and initiation of therapy.

In paper (III), we performed whole genome sequencing (WGS) of P. aeruginosa isolated from the same 12 patients who were included in the PFGE analysis in paper II. By sequencing and phenotypically characterizing multiple isolates from the same patients we were able to study the within-host bacterial evolution for the first time in PCD. The analyses provided detailed insight into how P. aeruginosa evolves in PCD when they are stressed by the host immune system and antibiotics. We verified the persistence of clonal lineages and confirmed that different clone types of P. aeruginosa can establish persistent infections in PCD patients. Further, we showed that P. aeruginosa acclimatizes gradually to the PCD airway by accumulating pathoadaptive mutations and phenotypic characteristics similar to those of CF. Such information may provide valuable clinical information, and as an example we identified mutations in genes responsible for the development of antibiotic resistance.

Based on our research we conclude that P. aeruginosa is a major pathogen in PCD and that future research should focus on preventing or eradicating these bacteria. Implementing ESS with adjuvant therapy to PCD patients (I, IV) significantly ameliorated CRS symptoms. Further, postoperatively patients tended to have fewer positive lower airway cultures and better lung function; approximately one out of four operated patients, in search of an infectious focus, remained free of lung colonization with P. aeruginosa during follow-up for at least six months. Based on these results, it is tempting to speculate that ESS with adjuvant therapy can eradicate sinus bacteria and thereby reduce lung re-colonization from the sinuses. However, further evidence is needed to support this hypothesis, preferably from a multicentre randomized controlled trial.

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