Sinonasal pathology in Cystic Fibrosis

Maaike Berkhout

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Promotiereeks HagaZiekenhuis

Het HagaZiekenhuis van Den Haag is trots op medewerkers die fundamentele bijdragen leveren aan de wetenschap en stimuleert hen daartoe. Om die reden biedt het HagaZiekenhuis promovendi de mogelijkheid hun dissertatie te publiceren in een speciale Haga uitgave, die onderdeel is van de promotiereeks van het HagaZiekenhuis. Daarnaast kunnen promovendi in het wetenschaps-magazine HagaScoop van het ziekenhuis aan het woord komen over hun promotieonderzoek.

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Sinonasal pathology in Cystic Fibrosis

Maaike Berkhout

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Chapter 1

General introduction and outline of this thesis

1 General introduction and outline of this thesis

This thesis comprises the pathology of the mucosa of the nose and the paranasal sinuses in patients with Cystic Fibrosis (CF). The prevalence, the impact on patients and the possible treatment options of this pathology will be described. In this introduction the disease CF is discussed, followed by pathology of the mucosa of the nose and the paranasal sinuses in detail. Finally, the specific aims of the thesis are displayed.

CYSTIC FIBROSIS

History

CF is a relatively common genetic disorder in the Netherlands, with currently approximately 1530 patients with CF and with one in 5250 babies born with this disease (Dutch CF Registry Annual Data Report 2014). It is a life-shortening disease with high morbidity and mortality.

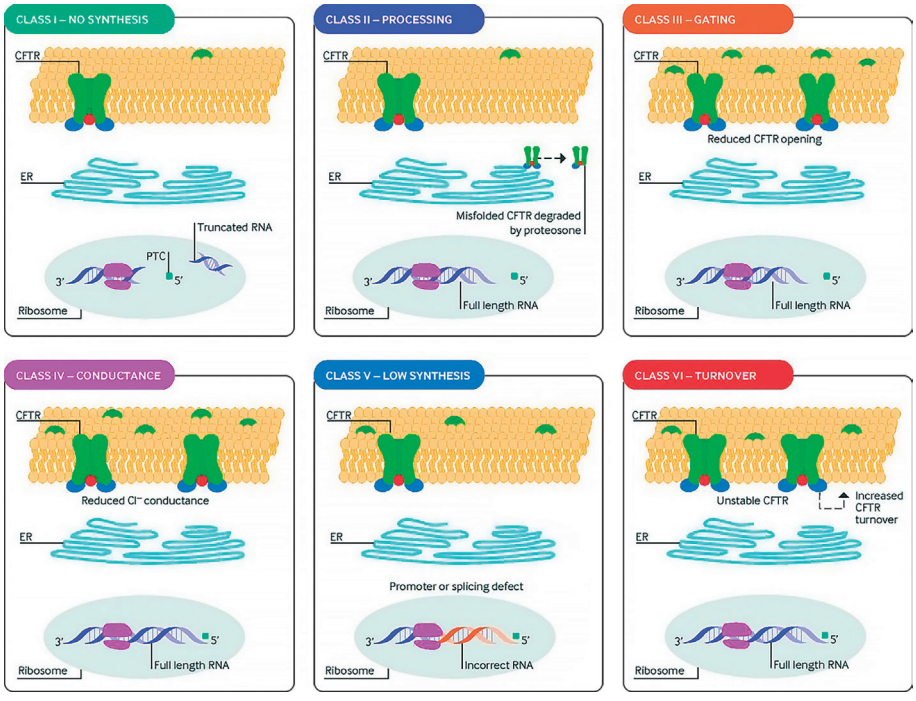
In 1938 CF was first reported as a separate disease following autopsies on malnourished children in the United States. These children showed malabsorption of fat and protein, which lead to growth failure, steatorrhea and lung infection which were often fatal[1]. The autopsies showed thick mucus plugs in predominantly the pancreas, but also other mucus producing glands. This 'new' disease appeared to run in families and showed an inheritance pattern of an autosomal recessive disease[1].

During a heat wave in 1948 in New York, children with CF suffered a high morbidity and mortality. Pediatrician di Sant' Agnese discovered that the sweat of these children contained extremely high levels of sodium and chloride[2]. This finding could not be directly related to the mucus plugs in the pancreas. The excess of sodium and chloride, however, did lead to a useful diagnostic test for CF, in which the concentration of electrolytes were measured in sweat[3]. This test showed elevated levels in patients without pancreas insufficiency, creating an understanding of a milder form of CF. Years of research followed, trying to find the basic defect of this complicated disease. In 1983, studies on sweat glands by Quinton, showed that an abnormal chloride transport was the basic defect in CF[4]. This work was followed by other researchers that showed increased sodium reabsorption as a consequence of this disturbed chloride transport[5,6]. This completed the picture that the abnormal electrolyte transport lead to fluid reabsorption and eventually to inspissated mucus[7].

The genetic foundation of CF was discovered in 1985 on the long arm of chromosome 7 and in 1989 the definitive CF gene was found[8-10]. The protein product of the CF gene was called the CF Transmembrane conductance Regulator (CFTR), a chloride channel present in many epithelial cells across the body. The CFTR is present in the epithelial membranes of sweat glands, pancreas, airway, liver, intestines, biliary tree and vas deferens. Dysfunction of CFTR results in elevated sweat chloride levels, pancreatic insufficiency, airway disease characterized by infection and bronchiectasis, intestinal obstruction, biliary cirrhosis and absence of the vas deferens, often in combination. Since 1989 approximately 2000 different CF mutations have been identified (genet.sickkids.on.ca). These mutations can be classified into six groups based on their effect on the CFTR protein.

Class I mutations represent defects in the production of CFTR, class II mutations result in a defect of processing, class III mutations lead to defective CFTR channel regulation, class IV mutations cause a decreased CFTR channel function, class V mutations result in a reduced amount of normal CFTR at the surface of the membrane and class VI mutations result in a reduced amount of normal CFTR because of a decreased stability of the protein at the surface[11,12].

Figure 1. CFTR mutation classes[12]



Research showed class I-III mutations are associated with a severe phenotype and class IV-VI mutations with a milder CF phenotype[13]. Correlations between genotype and phenotype were found for pancreatic function, lung function, age at diagnosis, nutritional status and *Pseudomonas aeruginosa* colonization[14-16].

Clinical features of CF

With the CFTR protein present in several organs, CF is a multi-organ disease. The most important clinical features of this disease and their general therapies are described below.

Pulmonary pathology account for the highest morbidity and mortality in patients with CF[17,18]. Research has shown that the lungs are normal at birth, but soon acquire viral but predominantly bacterial infections[19,20]. The most common bacterial pathogens in patients with CF are *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Burkholderia* species[17]. After the first encounter with a pathogen, eradication with an intensive regime of antibiotics is possible[21]. However, despite these efforts, many adult patients become chronically infected with one of these pathogens. P aeruginosa is particularly renowned for the rapid decline in lung function it causes[22,23]. Chronic infections cause irreversible damage to the lungs and eventually a decline in lung function[24,25]. Since the respiratory tract constitutes both the lower airways (LAW) and the upper airways (UAW), the paranasal sinuses and the nasal cavity are affected by the underlying CFTR defect as well. Infections in the UAW can have several implications, which will be discussed in detail in the next paragraph.

The first manifestation of CF was found in the pancreas[26]. Pancreatic insufficiency is present from birth, but can aggravate during life. This leads to nutritional problems and often to gradually diminishing islets of Langerhans which results in CF-related diabetes mellitus[27].

Dysfunction of the CFTR protein in the liver results in obstructive biliary cirrhosis, portal hypertension and often gallstones[28]. The intestines in patients with CF also contain thick contents, which can result in intestinal obstruction[29]. The last major complication of CF is pathology in the reproductive system. Men often have a congenital bilateral absence of the vas deferens and women often suffer of an impaired fertility due to irregular hormonal cycles and thick cervical mucus[30].

Therapy

Treatment of the pulmonary pathology is based on two key factors; clearance of the inspissated mucus and antimicrobial therapy. Clearance of the mucus can be achieved mechanically by coughing techniques or with special equipment.

Moreover, drugs to decrease the viscosity of the mucus such as human desoxyribonuclease and hypertonic saline, are frequently used to facilitate clearance[31]. Systemic and locally administered antibiotics are the cornerstones of antimicrobial therapy. Antibiotics in aerosols are very effective since high concentrations can be achieved without systemic side effects[32]. The final recourse, if lung function becomes critical, is a lung transplantation. Life can be extended with a new pair of lungs, however because of the shortage of donor lungs, many patients die on the waiting list.
Pancreatic insufficiency can be treated by supplementation of pancreatic enzymes, often in combination with proton pump inhibitors to enhance their efficacy. The malnutrition and deficit of fat soluble vitamins A, D, E and K is counteracted by supplementation of these vitamins and intake of extra calories. CF-related diabetes mellitus is treated with antidiabetic oral medication and/or insulin. Ursocholic acid is used for treating the pathology of the liver and in extensive liver cirrhosis sometimes transplantation of the liver is performed[33].
The treatment of intestinal obstruction consists of laxatives and in extreme cases surgical removal is the final recourse.
Male infertility can be treated with in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI). Pregnancy in women with CF often requires intensive monitoring and treatment, but can be achieved without permanent decline in pulmonary condition[34].

Increased life expectancy

A consequence of all these therapies is that the life expectancy of a CF patient has increased significantly during the last four decades. In the 1970's the mean survival of patients with CF in industrialized countries was 14 years[17]. Median survival is currently approximately 40 years (Annual Data Report USA 2014). Improved life expectancy has allowed the focus of treatment to expand to include quality of life.
Rhinosinusitis is one of the conditions associated with CF and is known for having a negative influence on a patient's quality of life[35]. The following chapter of this introduction will describe the disease of the nose and the paranasal sinuses in patients with CF in detail.

Sinonasal manifestations of CF

The main manifestations of CF in the head and neck region is chronic rhinosinusitis with (CRSwNP) or without (CRSsNP) nasal polyps.
The most common complaint of a CF patient with CRSwNP is nasal obstruction, patients with CRSsNP often report facial pain and headache[36].

However, as with non-CF patients, CF patients can display a broad range of symptoms that accompany rhinosinusitis and nasal polyps. Interestingly, research has shown CF patients to tend to underreport their symptoms of CRSsNP or CRSwNP[37,38]. This could be due to the chronic character of the disease or due to other symptoms that surpass the sinonasal disease, such as pulmonary or gastrointestinal disease.
Physical examination characteristics of sinonasal disease in CF are often nasal polyps and thick nasal discharge. Most patients with CF show opacification of the paranasal sinuses at the CT scan. Moreover, anatomical abnormalities such as hypoplasia and aplasia predominantly of the frontal and sphenoidal sinuses, medial bulging of the lateral nasal wall, demineralization and medial displacement of the uncinate process are described[39,40]. In addition, osteitis/neoosteogenesis of the sinus walls and mucoceles have been reported as radiographic abnormalities in CF[40].

The prevalence of CRSwNP or CRSsNP varies widely across reported studies, probably due to different definitions and a varying mean age of the study populations. Babinski and colleagues reported the prevalence of rhinosinusitis as 92.9%[41]. This is considerably higher than the prevalence of CRS in the general population (10.9%)[42]. However, Babinski and colleagues defined rhinosinusitis by the presence of clinical symptoms and cytologic examinations of nasal mucosa, which is different from the regular definition of rhinosinusitis[43].
Nowadays rhinosinusitis is defined as an inflammation of the nose and paranasal sinuses characterized by two or more of the following symptoms: nasal blockage/congestion/obstruction, nasal discharge (anterior or posterior), facial pain/pressure and/or a reduction or loss of smell. One of them should be either nasal blockage/congestion/obstruction or nasal discharge. Along with these symptoms either endoscopic signs of nasal polyps, mucopurulent discharge primarily from the middle meatus, edema/mucosal obstruction primarily in the middle meatus and/or mucosal changes within the ostiomeatal complex and/or sinuses on a CT-scan have to be present[43]. The prevalence of nasal polyps is more accurately documented across the CF literature. Reported prevalences of CRSwNP range from 32% to 56% in children and adults with CF respectively[44-46].
Symptoms of CRSsNP or CRSwNP impact the quality of life of a patient. In non-CF patients CRS have been shown to negatively influence several aspects of quality of life with an even greater impact on quality of life than chronic heart failure, back pain or angina[35,47].

Besides influencing quality of life, substantial evidence has recently been gathered that microorganisms from the UAW show similarities with microorganisms from the LAW[48-50]. In 1997 Walter and colleagues were the first to address a possible bacterial reservoir function of the UAW patients with CF[48]. They showed that patients with CF after lung transplantation harboured identical strains of *Pseudomonas aeruginosa* in their lung allografts. These data support the hypothesis that a reservoir of *P. aeruginosa* remains, most likely in the UAW or trachea. From this part of the respiratory tract this pathogen can recolonize the lung allograft, causing serious infections. Another longitudinal study in children with CF showed cultures from the middle meatus positive for *P. aeruginosa* preceded LAW cultures positive for this pathogen[50]. Recently evidence has been gathered that the paranasal sinuses can be a protected harbour for, which can subsequently spread to the lungs causing lung infections[51]. The paranasal sinuses can be the ideal environment for *P. aeruginosa* to adapt to the host and create survival strategies. Inhibition of polymorphonuclear cells comprise the local and systemic inflammatory reaction[52] and low oxygen tension in sinus mucosa stimulates bacterial growth[53]. In children with CF the sinuses harboured *P. aeruginosa* lineages that showed biofilm formation, antibiotic resistance, downregulation of motility and virulence factors and alginate overproduction. These characteristics increase the potential of the pathogen to establish chronic infection in the upper and the lower part of the airways in CF[51].

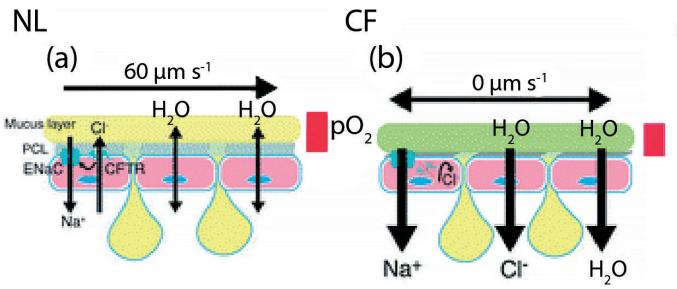
PATHOPHYSIOLOGY

The pathophysiology of sinonasal disease in CF is not fully understood. Numerous studies were performed to elucidate the association of rhinosinusitis with or without nasal polyps and CF. Factors associated with rhinosinusitis in CF are presented in this introduction.

Increased viscosity of mucus

The most evident factor in the pathophysiology of CF is the increased viscosity of the airway surface liquid. Respiratory epithelium lines the LAW (lungs and trachea) and the UAW (paranasal sinuses, nasal cavity, tuba auditiva, middle ear, mastoid antrum, oral cavity, pharynx and larynx). The epithelium consists of ciliated cells and goblet cells and is covered with a layer of periciliary fluid (sol layer) and a layer of mucus (gel layer). The viscosity of both layers is increased in CF due to the defective CFTR protein. Consequently, the mucociliary function is reduced (Figure 2)[54-56].

Figure 2.[56]



Mucus clearance of airway surfaces in normal (NL) and CF patients. PCL; periciliary layer, ENaC; epithelial sodium channel, Cl-; chloride, Na+; sodium, H_2O; water.

(a) Normal airways with active mucus clearance of approximately 60µm s-1. (b) In CF, chloride is not or less transported via the apical membrane which leads to an increased absorption of sodium followed by water.

The combination of decreased mucociliary clearance and tenacious mucus cause obstruction of the sinus ostia, which leads to hypoxia in the sinus cavity. Hypoxia further decreases mucociliary function and ultimately this can lead to mucus stasis and bacterial overgrowth in the sinuses. Furthermore, hypoxia can also comprise CFTR transcription, which in turn leads to increased viscosity of the gel and sol layers[53].

Genetic factors

Mutations in the CFTR gene result in the clinical manifestations of CF. However, the translation from genotype to phenotype is currently unclear. Since CRS is highly prevalent in CF patients, CFTR mutation could be directly responsible for this disease. Studies investigating the prevalence of CFTR mutations amongst non-CF patients with CRS, showed an increase in mutations in the CFTR gene in these patients. These results suggest that mutations in the CFTR gene might be associated with the development of rhinosinusitis and nasal polyps in the general population[57-60].

Pinto's linkage analysis, aimed at identifying genetic loci influencing susceptibility for CRS, demonstrated that the strongest linkage signal was identified on chromosome 7q31.1-7q32.1, an area which includes the CFTR locus[61].

Genetic factors could influence the susceptibility of rhinosinusitis through decreasing CFTR protein function, but also through regulation of the inflammatory cascade[62,63].

CFTR function is decreased in carriers of a CFTR mutation[64]. However, the residual CFTR function is enough to be free of the most serious symptoms of CF. Nevertheless, the prevalence of CRS in carriers of a CFTR mutation is much higher compared to the general population[57,58].

Finally, one study concluded that CFTR mutations which result in significant reduction of CFTR protein function are correlated with more severe expression of sinonasal disease[45].

Immunity

Many studies have been performed to compare rhinosinusitis in CF patients and rhinosinusitis in non-CF patients[65-69]. Although both groups present with similar disease characteristics, the pathogenesis is not comparable.

CF patients differ in both innate and adaptive immunity from patients with CRS with or without nasal polyps. Furthermore, research showed histopathological differences between these two patient groups[70-72].

The innate immune system of patients with CF and CRSsNP or CRSwNP is characterized by an upregulation of surfactant protein A1, A2 and D, human β defensin 2 and Toll-like receptor 2 and 9[65,66]. This upregulation is likely the result of the substantial bacterial infections that accompany CRS in CF[65].

Differences in the arachidonic acid metabolism, such as an increased COX-1 and COX-2, could imply an increased inflammatory reaction in patients with CF compared to non-CF CRS patients[67,68].

The difference in inflammatory mediators between CF patients with CRS and non-CF CRS patients is best summarized by stating that CF patients

predominantly suffer from neutrophilic inflammation and that non-CF patients suffer from inflammation that is more dominated by eosinophils[65,68,72]. Inflammatory cytokines such as IL-8, interferon γ, IL-6, IL-17, IL-1β, lipoxin A4 and MPO are more prominent in CF patients with CRS compared to non-CF CRS patients. This suggests an increased Th1 mediated inflammatory reaction. On the contrary Th2 cytokines such as IL-4, IL-5, IL-10, eotaxin, ECP, IgE are increased in non-CF CRS patients[66,69].
Histopathological studies show a predominance of mucous glands in sinonasal epithelium of patients with CF, in contrast to the predominance of serous glands in non-CF patients. Furthermore, the glandular ducts in CF patients are dilated compared to those of non-CF patients[70]. Also, goblet cell hyperplasia is seen in sinus specimens of CF patients[71].

OUTLINE OF THIS THESIS

Sinonasal disease and its impact on the general health of patients with CF is a subject which is under-researched. This thesis focuses on the prevalence of various aspects of sinonasal disease, its impact on quality of life and possible treatment of this disease in patients with CF.
Chapter 2 to 4 describe the results of an observational cross-sectional study in a group of adult patients. In chapter 2 the variety of microorganisms found in the UAW of patients with CF and its influence on general health are discussed. Chapter 3 addresses the prevalence of rhinosinusitis and nasal polyps in adult patients and the correlation between genotype and phenotype of sinonasal disease. Chapter 4 highlights an unexpected finding on the condition of temporal bones of CF patients.
To gain more knowledge on the prevalence and the onset of sinonasal disease an observational cross-sectional study in children with CF was conducted.
Chapter 5 summarizes the results from this study.
In chapter 6 the safety of nasal irrigations with colistin and tobramycin, as a potential new treatment option for patients with CF, is investigated.
During these studies, new drugs targeting the molecular defect in CF rapidly developed. In chapter 7 a promising and new finding regarding sinonasal disease in a young CF patient is highlighted.
This thesis is completed in chapter 8 with a summary, a general discussion and recommendations for future research on sinonasal disease in CF.

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Chapter 2

Importance of bacteriology in upper airways of patients with Cystic Fibrosis

M.C. Berkhout, E. Rijntjes, L.H. el Bouazzaoui, W.J. Fokkens, R.W. Brimicombe, H.G.M. Heijerman

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2 Importance of bacteriology in upper airways of patients with Cystic Fibrosis

ABSTRACT

Background

Recently the influence of the upper airways (UAW) on the general health of a patient with Cystic Fibrosis (CF) has been acknowledged. Surprisingly the microbiology of the upper compartment of the airways receives barely any attention in the treatment of CF. The aim of the present study was to investigate the microbiology of the upper airways in adult patients with CF, to correlate these findings with cultures from the lower airways (LAW) and with clinical characteristics.

Methods

In this cross-sectional study bacteriological and clinical data were gathered from 104 adult patients with CF. UAW samples for culture were collected by nasal lavage and middle meatal swabs; LAW cultures were performed on expectorated sputum or cough swabs. Each patient performed the Rhinosinusitis Outcome Measure (RSOM-31).

Results

In 72 patients (69.2%) UAW cultures yielded microorganisms other than normal nasal flora and in 50 patients (48.1%) *Pseudomonas aeruginosa* grew from the UAW cultures. Similarity between UAW and LAW cultures was determined in 50.0% of these 72 patients. In 3 patients *P. aeruginosa* was cultured from the UAW after successful eradication of *P. aeruginosa* from the LAW. *P. aeruginosa* in the UAW did not influence symptoms of sinonasal disease compared to other microorganisms.

Conclusions

Comparison of UAW and LAW cultures in adult patients with CF showed one or more concordant microorganism in 50.0% of the patients. *P. aeruginosa* was most frequently cultured from the UAW. *P. aeruginosa* can be cultured from the UAW after eradication therapy which may suggest persistence of *P. aeruginosa* in the UAW. We feel this is may be a motive to include the UAW in eradication therapy in Cystic Fibrosis.

INTRODUCTION

Patients with Cystic Fibrosis (CF) are prone to develop rhinosinusitis due to the defect in the Cystic Fibrosis Transmembrane Regulator (CFTR) protein. The membrane lining the paranasal sinuses and the nose is identical to the membrane lining the lungs. As in the lower airways (LAW) the defect CFTR protein results in viscous mucus. Consequently mucociliary function is reduced, which facilitates bacterial colonization and eventually infection leading to rhinosinusitis[1,2]. In the past decades infection of the lower airways was the most prominent focus in treatment protocols for CF. Over the years infection of the upper airways (UAW) gradually gained more attention in Cystic Fibrosis.

Previous research in the microbiology of the UAW in CF displayed that *Haemophilus influenzae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were most frequently cultured from the UAW[3,4]. Since several studies showed concordance between microorganisms in the UAW and the LAW in CF, the hypothesis evolved that the UAW might influence the pulmonary status of the patient[5].

Mainz et al. demonstrated identical genotypes of *P. aeruginosa* and *S. aureus* isolates of UAW and LAW in Cystic Fibrosis[6]. They suggested a reservoir function of the UAW for these pathogens. This hypothesis was strengthened by the recent study of Mainz et al.[7]. In their case report they showed persistence of identical *P. aeruginosa* genotypes in CF UAW prior to and after lung transplantation. Moreover Hansen et al. displayed that the paranasal sinuses can be a protected niche of adapted clones of *P. aeruginosa*, which can intermittently spread this pathogen to the lungs and facilitate subsequent chronic lung infections[8]. Research has demonstrated that middle meatal cultures and maxillary sinus aspirates showed the same pathogens in 80% of the cases[9]. Based on these findings nasal cultures might give insight in the microbiology of the paranasal sinuses. Surprisingly, nasal cultures are only occasionally implemented in the regular care of CF patients.

In Cystic Fibrosis pulmonary infection with *P. aeruginosa* rapidly deteriorates the course of lung disease[10,11]. Since the mucosa of the UAW is similar to the mucosa of the LAW, a similar effect of *P. aeruginosa* on the UAW might be expected. Therefore it is interesting to investigate if this pathogen influences sinonasal disease differently from other pathogens. However, the influence of *P. aeruginosa* in the sinonasal area on sinonasal disease is scarcely investigated.

Early infections with *P. aeruginosa* can be treated with tobramycin inhalation solution (TOBI) in order to eradicate this pathogen from the LAW[12]. However the sinonasal area is not included in this eradication regimen. Based on the above mentioned arguments, this may be an omission.

The present study was part of a larger study in rhinosinusitis and nasal polyps in adult patients with CF. The aim of the present study was to investigate the microbiology of the UAW in adult patients with CF and to correlate these findings with cultures from the LAW and clinical characteristics of the patients (symptoms of sinonasal disease).

STUDY DESIGN

Patients

Adult patients from the CF centre of the Haga Teaching Hospital, with a diagnosis of Cystic Fibrosis, based on a positive sweat test and/or genotype were considered eligible for the present study. The intention was to include 100 patients in this study. The study was conducted from April 2011 to February 2012 and was approved by the local medical ethics committee.

Microbiology

Upper airway cultures were collected by nasal lavage and middle meatal cultures. Under endoscopic control cultures from the area around the left and right sinus ostia were taken with a swab. For the nasal lavage the patient was asked to breathe in and hold their breath with the head reclined. Next 5 ml of sterile isotonic saline was inserted into each nostril with a 10 ml sterile syringe. After 10 s the patient expectorated the solution from the nose into a sterile plastic container. The two techniques of culturing the UAW were compared by the number of different microorganisms they yielded. Lower airway cultures were collected from expectorated sputum. In case no sputum was available a cough swab was taken. Preferably the UAW and LAW cultures were collected on the same day. When the UAW culture grew a bacterial or fungal species different from the LAW culture or when the UAW culture grew a microorganism but the LAW culture did not, LAW culture results from the preceding two years were extracted from the medical record. All specimens were cultured in the same laboratory. The samples were streaked on a chocolate agar, blood agar with aztreonam/colistine, *Burkholderia cepacia* agar, Cystine-Lactose-Electrolyte Deficient agar (CLED agar) and Sabouraud agar. All samples were incubated aerobically at 37 °C and were

analysed for growth of bacteria, fungi and yeast after 8–16 h and every 24 h during 7 consecutive days. Each microorganism cultured from the UAW was registered and allocated to three groups: 1. "No growth" of bacteria or fungi after culture except for nasal flora, 2. "Pathogenic microorganisms" containing microorganisms that are regarded as pathogenic for CF patients: e.g. *P. aeruginosa*, *Methicillin-sensitive S. aureus*, *Methicillin-resistant S. aureus*, *Stenotrophomonas maltophilia*, *Aspergillus fumigatus, H. influenzae*, *Burkholderia cepacia* complex, *Klebsiella pneumoniae* and *Klebsiella oxytoca*. *Escherichia coli* and *Proteus mirabilis* were considered pathogenic if the patient's LAW were infected with these bacteria 3. "Non-pathogenic microorganisms" comprise microorganisms that are generally considered as non-pathogenic for CF patients such as *Alternaria alternata* and *Penicillium* species.

Symptoms of sinonasal disease

In this study symptom burden was measured with the validated quality of life questionnaire 'Rhinosinusitis Outcome Measure' (RSOM-31)[13]. Since previous experience showed that patients had difficulties to distinguish between the 'Magnitude Scale' and the 'Importance Scale', in the present study only the 'Magnitude Scale' was measured (J Piccirillo personal communication). Symptom burden was analysed from the total score on the RSOM-31 divided by the number of completed items, resulting in a 'mean per item' (range 0–5, with higher scores representing a worse quality of life). Because the RSOM-31 contains questions about cough and dyspnea, the disease Cystic Fibrosis can influence the outcome of this questionnaire. Therefore the nasal domain of the RSOM-31 was analysed separately. The mean per item of the total RSOM and of the nasal domain were compared for patients with no growth after culture, non-pathogenic microorganisms and pathogenic microorganisms in the UAW. Also the RSOM scores for subjects with *P. aeruginosa* and without *P. aeruginosa* were compared.

Data and statistics

Data in this study were analysed with SPSS for Windows, version 17.0. The primary outcome parameter was the outcome of the UAW cultures. Every microorganism was registered. Descriptive statistics were used for describing the frequency of the microorganisms. Due to the complexity of the analysis, the similarity of microorganisms in UAW and LAW, was performed manually by comparing all microorganisms cultured from the UAW and the LAW. The influence of microorganisms in the UAW on the symptoms of sinonasal disease was investigated using a one-way ANOVA. The influence of *P. aeruginosa* specifically was tested with an independent t-test. A p-value of < 0.05 was considered statistically significant.

RESULTS

Patients

Of the 123 patients that were invited to participate in the study, 104 patients gave informed consent and were included in the study. The descriptive characteristics of the 104 subjects are displayed in Table 1. The mean age of the subjects was 34.8 years (range 19–63) and the percentage of male subjects was 52.9%. In total 59 subjects (56.7%) reported one or more sinus surgeries in the past.

Table 1.

Descriptive statistics of 104 adult patients with Cystic Fibrosis

Parameter	Total (n = 104)
Mean age, years (range)	34.8 (19–63)
Male (%)	55 (52.9)
Previous sinus surgery (%)[a]	59 (56.7)
P. aeruginosa in UAW cultures (%)	50 (48.1)
P. aeruginosa in LAW cultures concurrent with UAW cultures (%)[b]	38 (36.5)
P. aeruginosa in LAW cultures of preceding 2 years (%)[c]	74 (71.2)
Lung function:	
Mean FEV1% predicted (range)[d]	63.4 (25–114)
Mean FVC % predicted (range)[d]	84.3 (31–137)

[a] By patient report.

[b] Obtained at the same time as the UAW culture.

[c] Based on LAW cultures of the preceding 2 years, including cultures concurrent with UAW cultures.

[d] Percentage of predicted value based on reference values specific for sex, weight and height.

Microbiology

In 32 patients (30.8%), UAW cultures grew only normal nasal flora. In 72 patients (69.2%), UAW cultures yielded microorganisms other than normal nasal flora (Table 2). The most prevalent microorganism cultured from the UAW was *P. aeruginosa* (48.1%).

Table 2.

Distribution of microorganisms in upper airway cultures of 104 adult patients with Cystic Fibrosis

Microorganism[a]	Number of patients (% of total population; n = 104)[b]
Nasal flora	98 (94.2)
Pseudomonas aeruginosa	50 (48.1)
Staphylococcus aureus	10 (9.6)
Penicillium species	6 (5.8)
Escherichia coli	5 (4.5)
Stenotrophomonas maltophilia	5 (4.8)
Aspergillus fumigatus	4 (3.8)
Proteus mirabilis	2 (1.9)
Haemophilus influenzae	1 (1.0)
Moraxella catarrhalis	1 (1.0)
Serratia marcescens	1 (1.0)
Acinetobacter species	1 (1.0)
Burkholderia cepacia complex	1 (1.0)
Klebsiella pneumoniae	1 (1.0)
Klebsiella oxytoca	1 (1.0)
Methicillin-resistant Staphylococcus aureus	1 (1.0)
Alternaria alternata	1 (1.0)

[a] Results based on cultures from nasal lavage or nasal swabs.

[b] Samples from individual patients could yield more than 1 microorganism, so the total number of microorganisms is greater than the total number of patients.

LAW cultures were collected on the same day as the UAW cultures in 92 (88.5%) patients and within a week in the remaining patients. LAW samples were expectorated sputum in 98 patients and cough swabs in 6. In 36 patients, the microorganism cultured from the LAW was also cultured from the UAW. Among patients in whom the UAW culture yielded a microorganism not identified in the concurrent LAW culture (n = 36), LAW cultures from the preceding 2 years were extracted from the medical records. Among these patients, 21 had historical LAW cultures positive for at least one microorganism identified in the UAW culture.

Microorganisms from the UAW were divided into three groups based on the pathogenicity of these microorganisms in CF patients. In 67 subjects (64.4%) pathogenic microorganisms were cultured from the UAW, in 5 patients (4.8%) non-pathogenic microorganisms and in 32 patients (30.8%) the UAW cultures showed no growth after culture except nasal flora.

In total 3 patients were not able to complete the nasal lavage. In 24 (23.8%) of the 101 patients the results of the swabs and lavage were completely identical. In 27 patients (26.7%) cultures from both swabs yielded more different bacterial or fungal species than cultures from nasal lavage. In 17 patients (16.8%) more different species were cultured from the lavage compared to both swabs. In two patients (2.0%) both techniques produced different species, but the amount of different species was similar.

P. aeruginosa in UAW after eradication of *P. aeruginosa* from the LAW

Surprisingly, UAW cultures of 3 patients showed *P. aeruginosa* after eradication therapy of this pathogen from the LAW. In all three patients *P. aeruginosa* was successfully eradicated from the LAW, defined as three successive negative LAW cultures in 6 months[14]. In all three patients eradication therapy consisted of an oral quinolone (ciprofloxacin) for 3 weeks and nebulised tobramycin for 6 weeks.

Symptoms of sinonasal disease

The total RSOM score and the nasal domain subscore did not differ significantly between patients from whom normal nasal flora, pathogens or non-pathogenic microorganism were isolated from the UAW (Table 3). Similarly, total RSOM and nasal domain scores did not differ significantly between those patients from whom *P. aeruginosa* was or was not isolated from the UAW (data not shown).

Table 3.

Influence of microorganisms on symptoms of sinonasal disease in adult patients with CF

	No growth after culture n = 32	Non-pathogenic microorganisms n = 5	Pathogenic microorganisms n = 67	*p*-value
Total score RSOM-31[a] (Magnitude Scale only, mean per item (SD))	1.10 (0.74)	1.12 (0.56)	1.20 (0.69)	0.806
Nasal domain of RSOM-31[a] (Magnitude Scale only, mean per item (SD))	1.58 (1.02)	1.17 (0.78)	1.47 (0.83)	0.596

[a] Range of mean per item: 0–5, with a higher score indicating a worse quality of life.

DISCUSSION

In this study we systematically investigated the upper airways of a large cohort of adult patients with Cystic Fibrosis. Since most of the research in microbiology of the UAW is performed in children[5,15] or in small numbers of adults[16,17], knowledge on the microbiology of the UAW in adults is scarce. Data from this study may represent the current situation for adult patients with CF and may be a guide for treatment and monitoring protocols.

In 72 patients (69.2%) of the study population the UAW cultures produced microorganisms other than nasal flora. Noteworthy is the total amount of microorganisms of 91, due to the fact that the sinonasal area of one patient can host more than one microorganism. Results from the UAW cultures show that *P. aeruginosa* is the most prevalent microorganism in the UAW of adults with CF, followed by *S. aureus*. These results conform to previous research[16,17]. However studies in children showed a more prominent role for *S. aureus* and *H. influenzae*[15]. In the present study the latter was only cultured from the UAW of one patient. An explanation for this finding could be that *P. aeruginosa* has the capacity to overgrow other bacteria including the H.influenzae and that adult patients are more likely to be colonized with *P. aeruginosa* than children. Previous research supports that the most common bacteria in the UAW of CF patients vary with age[2].

One limitation of our study is that only the phenotypes of the microorganisms were analysed. Microorganisms with a similar phenotype could, however, be different in genotype. Nevertheless Muhlebach et al. showed 83% of the microorganisms that were similar in phenotype, were also similar in genotype[18].

Currently no consensus on the best technique of culturing microorganisms from the UAW is defined. Mainz et al. suggested in a recent study[6] that nasal lavage was the method of choice for sampling the UAW. In contrast, our results show that the endoscopic samples targeting for the sinus ostia produced slightly more different bacterial or fungal species than nasal lavage. Moreover Araujo et al. demonstrate that middle meatus and maxillary sinus cultures presented the same pathogens in 80% of the cases[9]. However, it is important to realise that in the present study no sinus aspirates were collected and therefore no golden standard was available. We feel that without adding nasal lavage as a culturing technique, some microorganisms can be missed. Altogether we believe that both techniques combined obtain the most representative cultures from the

UAW in CF patients. However, this means that for routine cultures of the UAW an otorhinolaryngologist is necessary, which might be complicated to implement in regular care. Moreover one has to note that more invasive culture techniques, such as sinus aspirates during sinus surgery resulted in a much higher prevalence of bacteria[17]. In our study the UAW cultures of 30.8% of the patients showed no growth except nasal flora, whereas Rasmussen et al. showed this percentage was only 1.8% from sinus aspirates[17]. Finally it is important to remember that nasal lavage may represent bacteria from the pharynx and therefore give an overestimate of bacterial growth in the UAW.

An interesting result from this study is that in three patients who successfully completed an eradication therapy of the LAW after early infection with *P. aeruginosa*, the UAW cultures showed *P. aeruginosa*. All of these patients fulfilled the criteria for eradication, that is three successive negative LAW cultures in 6 months[14]. These findings can be explained in two different ways: *P. aeruginosa* in the UAW can be a new acquisition of the pathogen in the UAW or *P. aeruginosa* in the UAW was never eradicated from this compartment of the airways. The latter hypothesis is endorsed by several studies[8,19,20,21], suggesting a reservoir for *P. aeruginosa* in the UAW. Persistence of *P.aeruginosa* in the UAW after eradication therapy might lead to cross-infection to the LAW[6,8]. Therefore we believe this could be an explanation for failure of eradication therapy or early recurrence of *P. aeruginosa* after eradication therapy. A limitation of the present study, however, is that it lacks a longitudinal evaluation of both UAW and LAW cultures. For this reason, we can only speculate about a possible cross-infection, instead of collect evidence for this statement. Despite this limitation we feel these results may justify frequent cultures of the UAW especially in patients who completed eradication therapy or patients in which *P. aeruginosa* was never cultured from the LAW. Moreover these findings challenge the current definition of eradication, where cultures from the UAW might be added in the definition. Unfortunately, to date, no eradication therapy is available for *P. aeruginosa* in the UAW. Therefore further research in the treatment of *P.aeruginosa* in UAW, for example by topical antibiotics, is necessary.

In line with the above mentioned results, in four patients the UAW cultures grew *P.aeruginosa* whilst their LAW cultures did not grew this pathogen for one year. According to the standard definition[22] they were therefore classified as free of infection. We postulate that the *P. aeruginosa* from the UAW can incidentally disperse to the LAW, causing intermittent infection of the lungs. Possible mechanisms for the dispersion could be post nasal drip and pharyngeal aspiration

during sleep[23]. Altogether we believe these results emphasize the role of the UAW in initiating or maintaining lung infection. Therefore we suggest that addition of effective treatment of the UAW could improve the pulmonary status of the CF patient and could result in a longer *P. aeruginosa*-free period.

P. aeruginosa is known to deteriorate lung disease in Cystic Fibrosis and may therefore decline the quality of life[11]. The influence of this pathogen on the UAW is, however, not clarified. Since the mucosa of the paranasal sinuses is similar to the mucosa lining the lungs, a comparable influence of *P. aeruginosa* might be expected. However, the results of this study showed no correlation between the presence of *P. aeruginosa* in the UAW and symptoms of sinonasal disease.

In conclusion, upper airway cultures from 104 adult patients with CF, showed in 72 patients (69.2%) microorganisms other than normal nasal flora. Sixty seven of them (93.1%) were considered pathogenic microorganisms for CF patients with *P. aeruginosa* being the most prevalent pathogen in the UAW. In 36 of the 72 patients (50.0%) the UAW cultures and LAW cultures showed at least one microorganism that was similar in phenotype. Interestingly, in 3 patients who successfully completed eradication therapy of *P. aeruginosa* from the LAW, UAW cultures displayed *P. aeruginosa*. No influence of pathogenic microorganisms and more specific *P. aeruginosa* on symptoms of sinonasal disease was demonstrated. We feel that the results of *P. aeruginosa* after eradication therapy may suggest persistence of *P. aeruginosa* in the UAW. Therefore we believe the UAW should occupy an important role in the treatment of Cystic Fibrosis, especially in eradication therapy.

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Chapter 3

Sinonasal manifestations of Cystic Fibrosis: A correlation between genotype and phenotype?

M.C. Berkhout, C.J. van Rooden, E. Rijntjes, W.J. Fokkens, L.H. el Bouazzaoui, H.G.M. Heijerman

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3 Sinonasal manifestations of Cystic Fibrosis: A correlation between genotype and phenotype?

ABSTRACT

Background
Patients with Cystic Fibrosis are prone to develop sinonasal disease. Studies in genotype–phenotype correlations for sinonasal disease are scarce and inconclusive.

Methods
In this observational study several aspects of sinonasal disease were investigated in 104 adult patients with CF. In each patient a disease specific quality of life questionnaire (RSOM-31), nasal endoscopy and a CT scan of the paranasal sinuses were performed. Patients were divided into two groups, class I–III mutations and class IV–V mutations, based on their CFTR mutations.

Results
The prevalence of rhinosinusitis in adult patients with CF was 63% and the prevalence of nasal polyps 25%. Patients with class I–III mutations had significantly smaller frontal sinuses, sphenoid sinuses, more opacification in the sinonasal area and more often osteitis/neoosteogenesis of the maxillary sinus wall compared to patients with class IV and V mutations.

Conclusion
These data suggest more severe sinonasal disease in patients with class I–III mutations compared to patients with class IV–V mutations.

INTRODUCTION

Cystic Fibrosis (CF) predisposes a patient to pathology of the nose and the paranasal sinuses. The mucosa lining the nose and paranasal sinuses is similar to the mucosa of the lower airways. Therefore pathology in both compartments of the respiratory system is expected to be quite identical. In the sinonasal system the CF distinctive viscous mucous results in impaired ciliary function, mucous stasis and consequently bacterial superinfection. Moreover, research showed other factors are associated with the tendency of CF patients to develop sinonasal disease. Evidence suggests that heterozygotes for the Cystic Fibrosis

Transmembrane Regulator (CFTR) mutation are predisposed to develop chronic rhinosinusitis compared to the normal wild-type CFTR population[1,2]. This might indicate a direct influence of the CFTR mutation in the aetiology of CF sinonasal disease. Another factor that might play a role in the development of sinonasal disease in CF is the environment of the sinuses. Research showed that the immune response in the sinuses is weak, bioavailability of antibiotics is low and the environmental conditions increases antibiotic-resistance of pathogens in the sinuses[3]. Altogether this may result in chronic and recalcitrant rhinosinusitis in patients with CF.

Rhinosinusitis has been proven to negatively influence a patient's quality of life[1,4]. Moreover pathogens from the sinuses can migrate to the lungs and cross-infect the lungs[3,5]. Often the upper airways barely receive attention in the routine care of the CF patient. Especially in adult patients the upper respiratory tract is not regularly examined. Therefore the actual impact of sinonasal disease in adult patients cannot be estimated properly.

Since the identification of the CF mutation in 1989 approximately 1939 different mutations have been described[6]. These mutations can be classified according to their impact on the function of the Cystic Fibrosis Conductance Transmembrane Regulator (CFTR) protein. Class I–III mutations are associated with a severe phenotype of CF. Mutations in class IV–V are associated with a milder CF phenotype[7].

Considerable amount of studies have been performed to find a correlation between the genotype and the phenotype of CF[7,8]. Correlations between genotype and phenotype were found for pancreatic function, lung function, age at diagnosis, nutritional status and *Pseudomonas aeruginosa* colonisation[8,9].

Research in a genotype–phenotype correlation for sinonasal disease is scarce and the results are inconclusive. Jorissen and co-workers showed ΔF508 homozygosity was a risk factor for paranasal sinus disease in CF[10]. Other investigators showed nasal polyps were more frequent in patients with the genotype consisting of both 'strong' mutations compared to patients with a genotype consisting of unknown or 'mild' mutations[11]. However, no association between sinonasal disease and genotype was found in the study of Cimmino et al.[12]. Moreover, with their research Sakano et al. displayed no association with the pathology seen on a sinus CT scan and the severity of CF[13]. In summary, the current data of a possible genotype–phenotype correlation for sinonasal disease

remain inconsistent. Investigating genotype–phenotype correlations can lead to more knowledge on the pathogenesis of sinonasal disease in Cystic Fibrosis. To date this pathophysiology is poorly understood. Besides, a possible correlation can have clinical implications.

In the present prospective study several aspects of sinonasal disease were examined and correlated with genotype in a large group of adult patients with CF.

STUDY DESIGN

Patients

Adult patients who regularly attend the Cystic Fibrosis centre of the Haga Teaching Hospital were studied. All subjects with a diagnosis of CF, based on a positive sweat test and/or genotype were considered eligible for this study. The intention was to include 100 patients. The present study was performed from April 2011 to February 2012 and was approved by the local medical ethics committee.

Genotype

Patients were divided in two groups; subjects with class I–III mutations and patients with class IV–V mutations. Subjects who were homozygous or compound heterozygous for class I–III mutations were allocated to the class I–III mutation group and patients who carried at least one class IV–V mutation were addressed to the class IV–V mutation group. In patients where one or two CFTR mutations were unknown the sweat test was used to confirm the diagnosis of CF. In those patients pancreatic function and age at diagnosis were used to allocate patients to one of the two groups. Pancreatic function is known to correlate well with genotype[8]. Moreover age at diagnosis correlates with genotype as well[9,14]. Considering the age at diagnosis a cut-off value of 10 years was used in this study. A subject that was pancreatic sufficient and the age at diagnosis was > 10 years was considered as carrying a class IV–V mutation. Pancreatic insufficiency and age at diagnosis < 10 years was considered among the class I–III mutation group.

Rhinosinusitis and nasal polyps

In this study rhinosinusitis is defined as an inflammation of the nose and paranasal sinuses characterised by two or more of the following symptoms: nasal blockage/congestion/obstruction, nasal discharge (anterior or posterior), facial pain/pressure and/or a reduction or loss of smell. One of them should be either

nasal blockage/congestion/obstruction or nasal discharge. Along with these symptoms either endoscopic signs of nasal polyps, mucopurulent discharge primarily from the middle meatus, oedema/mucosal obstruction primarily in the middle meatus and/or mucosal changes within the ostiomeatal complex and/or sinuses on a CT-scan had to be present[1]. In the present study symptoms are measured with the Rhinosinusitis Outcome Measure (RSOM-31). Since this RSOM is a quality of life questionnaire each symptom was scored on a 6-point Likert scale. A symptom was included in the definition of rhinosinusitis when subjects rated the symptom with a 2 ('mild or slight problem') or more.

The presence of nasal polyps was defined as an endoscopically visualised grape-like lesions in at least one nasal cavity following a decongestant (xylometazoline 0.1%).

Quality of life

Disease specific quality of life (QoL) was measured with the 'Rhinosinusitis Outcome Measure' (RSOM-31)[15]. This validated questionnaire consists of 31 items on rhinosinusitis and each item is scored on a 'Magnitude Scale' and an 'Importance Scale'. Since previous experience showed that patients had difficulties in distinguishing between the 'Magnitude Scale' and the 'Importance Scale', in the present study only the 'Magnitude Scale' was measured (J Piccirillo personal communication). The RSOM-31 contains seven domains: nasal, eye, sleep, ear, general, practical and emotional. Disease specific QoL was analysed from the total score on the RSOM-31 divided by the number of completed items, resulting in a 'mean per item' (range 0–5, with higher scores representing a worse quality of life). Since the RSOM-31 contains pulmonary symptoms such as cough and dyspnea, the disease Cystic Fibrosis can influence the outcome of this questionnaire. Therefore the nasal domain of the RSOM-31 was analysed separately.

Computed tomography

Computed tomography of the paranasal sinuses was performed (Toshiba Aquilion 16). Patients were situated with their head tilted backwards until the palatum durum was positioned perpendicular to the bench. Axial computed tomography was performed with a slice thickness of 0.5 mm and images were reconstructed at 1.0 mm. The scan ranged from the upper border of the frontal sinuses to the lower part of the teeth in the maxilla. No intravenous contrast was used.

Paranasal sinus opacification was assessed using the Lund–Mackay score (L–M score). This validated staging system grades every sinus as 0: normal, 1: partial opacification or 2: total opacification. These points are applied to the maxillary, anterior ethmoid, posterior ethmoid, sphenoid and frontal sinus on each side. The ostiomeatal complex is graded as 0: patent or 1: occluded. The Lund–Mackay score ranges from 0–24. Since CF patients often display aplasia of one or more sinuses the Lund–Mackay score had to be adjusted for this study. The absence of one or more sinuses distort the L–M scores and makes comparison between patients incorrect. Therefore the total L–M score was divided by the amount of developed sinuses or components of the sinonasal system, resulting in a L–M score per component of the sinonasal system. This adjusted L–M score ranged from 0–2. An experienced radiologist (C.J.v.R) analysed all CT-scans and was blinded for genotype and previous study results of the patients.

The volume of the sinuses were analysed with an Aquarius Intuition software (TeraRecon®). For each sinus the circumference on each slice was drawn manually and the total volume of the sinus was calculated subsequently. Volumes of the left and right sides were added up. Only the frontal, sphenoid and maxillary sinuses were measured. Aplastic sinuses were calculated as 0 cm^3.

Moreover increased bone density and irregular thickening, in this study called 'osteitis/neoosteogenesis', of the maxillary sinus wall was evaluated and graded as 0: absent, if both maxillary sinuses did not show osteitis/neoosteogenesis and as 1: present, if one of the maxillary sinuses showed signs of osteitis/neoosteogenesis.

Finally the anatomy of the ostiomeatal complex (OMC) was evaluated. Both left and right OMC were assessed. The anatomy of the OMC was scored as 0: normal on both sides, 1: abnormal anatomy on one or both sides due to sinus surgery in the past or 2: abnormal anatomy on one or both sides without sinus surgery in the past. To ascertain if patients had sinus surgery in the past, surgery reports were requested. In case no surgery reports were available, one experienced otorhinolaryngologist (E.R.), whom was ignorant to previous data of the patients, evaluated the scan and assessed if the patients had surgery on the OMC.

Data and statistics

Data in this study were analysed with SPSS for Windows, version 17.0. The aspects of sinonasal disease were reported by descriptive characteristics such as mean, standard deviation (SD) and percentage. To determine whether the proportion in

the study group takes a particular value (from literature) a z test for a proportion was used. For every aspect of sinonasal disease a comparison between patients with class I–III mutations and patients with class IV–V mutations was made. A chi-squared analysis was applied to rhinosinusitis, nasal polyps and osteitis of the maxillary sinus wall. The RSOM-scores, PSV of the frontal and sphenoid sinuses were analysed using a non-parametric test. The L–M scores and PSV of the maxillary sinuses were analysed using parametric tests. A *p*-value of < 0.05 was considered statistically significant. Since nine hypothesis tests were performed on one population the data had to be corrected for multiple testing. In this study a Holm–Bonferroni correction was applied.

RESULTS

Patients

One hundred and twenty three patients were invited to participate in this study, of which 104 gave informed consent and were included. The mean age of all 104 subjects was 34.8 years (SD: 11.1) and the percentage of male subjects was 52.9%. Fifty-nine patients (56.7%) reported a history with one or more surgeries in the sinonasal area. In total 10 patients were on oral steroids and 41 patients on nasal steroids during the study. The descriptive statistics of the subjects separated for the different mutation classes are displayed in Table 1. This table shows the mean age of patients with class IV–V mutations was significantly higher than the mean age of patients with class I–III mutations (p–value < 0.001). All other descriptive parameters did not differ significantly between the two groups.

Table 1.

Descriptive statistics of 104 adult patients with Cystic Fibrosis

Parameter	Mutation class IV-V n = 31	Mutation class I-III n = 73	*p*-value
Mean age, years (SD)	40.5 (10.7)	32.4 (10.4)	0.000*
Male, n (%)	14 (45.2)	41 (56.2)	0.304
Previous sinus surgery, n (%)	19 (61.3)	40 (54.8)	0.541
Lung function:			
Mean FEV1 % predicted (SD)	66.3 (25.4)	62.1 (25.5)	0.389
Mean FVC % predicted (SD)	87.5 (18.1)	83.0 (19.0)	0.265
Oral steroids (%)	1 (3.2)	9 (12.3)	0.150
Nasal steroids (%)	9 (29.0)	32 (43.8)	0.158

* *p*-value <0.05

Genotype

In 3 patients one CFTR mutation was unknown and in one patient both of the CFTR mutations were unknown. Table 2 shows the distribution of the genotypes among the 104 study patients. The last column states to which group the patients were assigned. The characteristics of the 3 patients with one unknown mutation are displayed in Table 2. One of the three patients was assigned to the class I–III mutation group and 2 were allocated to the IV–V mutation group. The subject with both unknown mutations was diagnosed at 42 years, but was pancreatic insufficient secondary to a pancreatitis. Since this patient had typical characteristics of 'mild disease' he was assigned to the class IV–V mutation group. In total 73 subjects were allocated to the class I–III mutation group and 31 to the class IV–V mutation group.

Table 2.

Distribution of genotypes and their mutation classes

Genotype	Frequency; n (%)	Class of mutation
F508del/F508del	61 (58.7)	I-III
F508del/3849+10kbC	2 (1.9)	IV-V
F508del/N1303K	2 (1.9)	I-III
F508del/R1162X	2 (1.9)	I-III
F508del/A455E	12 (11.5)	IV-V
F508del/3272-26A>G	5 (4.8)	IV-V
F508del/E528X	1 (1.0)	I-III
F508del/S1251N	3 (2.9)	IV-V
F508del/R75Q	1 (1.0)	IV-V
F508del/G542X	2 (1.9)	I-III
F508del/1717-1G>A	1 (1.0)	I-III
F508del/Ser489X	1 (1.0)	I-III
F508del/4382delA	1 (1.0)	-[a]
F508del/L1077	1 (1.0)	I-III
F508del/1813insC	1 (1.0)	-[b]
A455E/S1251N	1 (1.0)	IV-V
A455E/E60X	1 (1.0)	IV-V
3272-26A>G/G970R	1 (1.0)	IV-V
3272-26A>G/R1162X	1 (1.0)	IV-V
F508del/UNK	2 (1.9)	-[c]
R117H-7T/UNK	1 (1.0)	-[d]
UNK/UNK	1 (1.0)	-[e]
Total	**104 (100.4)**	

[a] Patient with pancreatic sufficiency and diagnosed at 19 years of age (class IV-V)

[b] Patient with pancreatic insufficiency and diagnosed at 9 years of age (class I-III)

[c] One patient with pancreatic insufficiency and diagnosed at 4 years of age (class I-III)

One patient with pancreatic sufficiency and diagnosed at 46 years of age (class IV-V)

[d] Patient with pancreatic sufficiency and diagnosed at 39 years of age (class IV-V)

[e] Patient with pancreatic insufficiency secondary to chronic pancreatitis and diagnosed at 42 years of age (class IV-V

Rhinosinusitis and nasal polyps

Sixty-five patients (62.5%) met the EPOS criteria of the definition of rhinosinusitis. This is significantly higher (p-value < 0.0001) than the 10.9% of chronic rhinosinusitis observed in the general population[16]. Table 3 shows the percentage of rhinosinusitis in patients with class I–III mutations was slightly higher than

in patients with class IV–V mutations, but the difference was not statistically significant (*p*-value = 0.14).

In 26 patients (25.0%) nasal endoscopy showed nasal polyps on one or both sides. This observed prevalence is significantly higher (*p*-value < 0.0001) than the 2.4% observed in the general population[17]. Of the 26 CF patients with nasal polyps 21 carried class I–III mutations and 5 class IV–V mutations (*p*-value = 0.17).

Table 3.

Aspects of sinonasal disease divided for severity of CF

Aspect of sinonasal disease	Mutation class IV-V n = 31	Mutation class I-III n = 73	*p*-value
Rhinosinusitis; n (%)	16 (51.6)	49 (67.1)	0.135
Nasal polyps; n (%)	5 (16.1)	21 (28.8)	0.173
RSOM-31 total score; mean (SD)	1.21 (0.77)	1.15 (0.66)	0.672
RSOM-31 nasal domain; mean (SD)	1.44 (0.97)	1.51 (0.85)	0.536
L-M score per component[a]; mean (SD)	0.62 (0.41)	0.85 (0.32)	0.007*
PSV frontal sinuses[b] (cm^3); mean (SD)	5.09 (5.00)	2.34 (2.96)	0.002*
PSV sphenoid sinuses[b] (cm^3); mean (SD)	3.79 (4.58)	1.74 (2.18)	0.000*
PSV maxillary sinuses[b] (cm^3); mean (SD)	18.83 (8.42)	17.17 (7.70)	0.331
Osteitis maxillary sinuses; n (%)	21 (67.7)	67 (91.8)	0.002*

[a] Lund-Mackay score per component of sinonasal system

[b] PSV = Paranasal Sinus Volume

* Statistically significant after Holm-Bonferroni correction

Quality of life (RSOM-31)

The mean total score on the RSOM-31 of the total study population was 1.17 (SD: 0.70). The mean score on the nasal domain was 1.49 (SD: 0.88). Table 3 displays the RSOM scores for the two groups based on mutation classes. Statistical analyses showed no significant difference between these two groups for the total score and the nasal domain (*p*-value = 0.67, respectively *p*-value = 0.54).

Computed tomography

Of the study population 15 patients showed aplasia of both frontal sinuses (20.0% class IV–V, 80.0% class I–III). Eight patients had one aplastic frontal sinus (12.5% class IV–V, 87.5% class I–III). Moreover two patients displayed aplasia of one sphenoid sinus (both with class I–III mutations). In one patient very extensive sinus surgery leads to indistinct boundaries of the sinuses and therefore this

patient was not included in the analysis of the L–M scores. This patient was carrying two class I–III mutations.

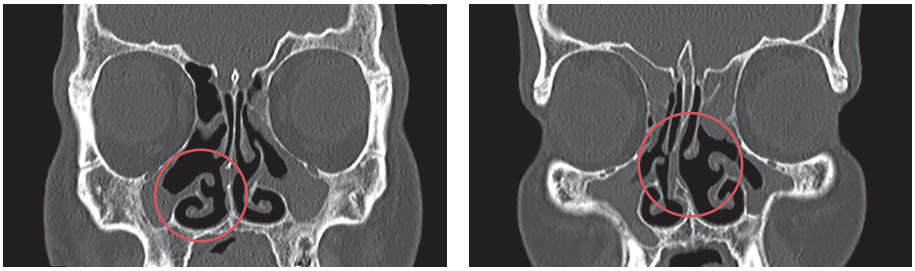
The mean L–M score per component of the sinonasal system for 103 patients was 0.78 (SD: 0.37). Table 3 shows the L–M scores in patients with class I–III mutations were significantly higher compared to patients with class IV–V mutations (*p*-value = 0.007).

Paranasal sinus volume was analysed in all 104 patients. The mean volume of the left and right frontal sinuses together was 3.16 cm^3 (SD: 3.87). For the sphenoid sinuses the mean volume was 2.35 cm^3 (SD: 3.21). The mean volume of both maxillary sinuses together was 17.66 cm^3 (SD: 7.92). Table 3 presents the volumes of these sinuses for patients with class IV–V mutations and class I–III mutations. The frontal sinuses and the sphenoid sinuses of patients with class I–III mutations were significantly smaller compared to patients with class IV–V mutations (*p*-value = 0.002, respectively *p*-value = 0.000).

Among the study population 88 patients (84.6%) had signs of osteitis/ neoosteogenesis in one or two maxillary sinuses. This percentage was significantly higher in the group of patients with class I–III mutations compared to patients with class IV–V mutations (*p*-value = 0.002, Table 2).

Analysis of the ostiomeateal complex showed a normal anatomy on both sides in 43 patients (41.3%), an abnormal anatomy on one or both sides with sinus surgery in the past in 45 patients (43.3%). In 16 patients (15.4%) the CT-sinus showed an abnormal anatomy of the ostiomeatal complex without any reported sinus surgery in the past or without signs of previous sinus surgery on the CT-scan. This abnormal anatomy often involved the uncinate process, where this process was projected medially instead of laterally (Fig. 1a and b).

Figure 1.



a. Abnormal anatomy of uncinate process on right side, normal anatomy on left side

b. Bilateral abnormal anatomy of uncinate process

DISCUSSION

The sinonasal system is gradually receiving more attention in the treatment of the multi-organ disease Cystic Fibrosis. Pathology of the nose and the paranasal sinuses in CF patients have been observed previously. Only recently more substantial research in this area was initiated. Where previous studies include a rather heterogeneous study population with children and adults with CF, the present study focuses on a large group of only adult patients.

Table 1 showed the characteristics of the two groups were almost comparable. However, the mean age differed significantly, with a higher mean age in the patients with class IV–V mutations. One could speculate this is the result of the fact that class IV–V mutations often leads to a higher life expectancy. For this reason the group of patients with class IV–V mutations contained relatively older patients compared to the group of patients carrying class I–III mutations.

Since CF predisposes a patient to rhinosinusitis, the high prevalence of this complication was expected. In total 65 patients fulfilled the criteria of the EPOS for rhinosinusitis. This prevalence among CF patients is considerably higher compared to the prevalence in the general population. However, the prevalence in the general population from the GA2LEN study was the prevalence of chronic rhinosinusitis. One limitation of this study is that the time aspect of the rhinosinusitis in the study population is unknown. Where in the GA2LEN study was asked for symptoms > 12 weeks in the previous 12 months, we only asked for symptoms in the last 2 weeks. Therefore one might suggest that these two prevalences cannot be compared to each other, although considering the

CT-scans of these patients, a chronic course of this rhinosinusitis is suspected. The investigators feel the high prevalence of rhinosinusitis and nasal polyps among adult patients with CF should indicate a closer follow-up of this pathology by the pulmonary physician as well as the otorhinolaryngologist.

In the present study we chose to use a 'mean per item' outcome of the RSOM-31 to ensure we could include the incomplete questionnaires in the analyses. However, this method is not previously used in other studies. To compare data of CF patients with data of patients suffering from chronic rhinosinusitis (CRS), we could calculate 'mean per item' scores in other studies. For the study of Dietz de Loos and colleagues[18] this calculated mean total RSOM score in patients with CRS and nasal polyps was 2.13 and for patients with CRS without nasal polyps this was 2.16. Compared to our results of 1.21 and 1.15 for the class IV–V mutation group and the class I–III mutation group, respectively, the reported symptoms of CF patients were lower. Results of Hissaria et al. confirm this observation with a mean total RSOM score of 2.2 in patients with sinonasal polyposis[19]. This could suggest that CF patients tend to underestimate the rhinosinusitis symptoms. One could speculate this is explained by a more chronic or perhaps congenital course of rhinosinusitis in CF patients or this could be due to other pathology, for example pulmonary and gastrointestinal disease, that outweigh sinonasal disease.

In this study we used a modified Lund–Mackay score. Since many CF patients display aplasia of the frontal sinuses, the original L–M scoring system cannot be used accurately in these patients. Therefore the investigators chose to use an adjusted L–M score, the L–M score per component of the sinonasal system. For this reason comparison with literature required adaptation of the data. Dietz de Loos et al. showed a mean L–M score of 18 in 137 patients with CRS and nasal polyps and a mean score of 5 in 97 patients with CRS but no nasal polyps[18]. Corrected for the 4% of the general population with two aplastic frontal sinuses[20], the L–M score per component of the sinonasal system would be 1.51 for patients with CRS and nasal polyps and for patients with CRS without nasal polyps this would be 0.41. Our CF population had a mean L–M score intermediate of those two CRS subpopulations of 0.78.

In our study patients with class I–III mutations had significantly smaller frontal and sphenoid sinuses compared to patients with class IV–V mutations. For the maxillary sinuses this difference did not reach statistical significance. These findings were supported by several studies[20,21,22,23]. To date the exact pathogenesis of abnormal paranasal sinus development is unknown. A commonly held

hypothesis is that chronic sinusitis during development decreases sinus pneumatization, leading to smaller sinuses[20]. Considering this hypothesis, one could speculate patients with class I–III mutations develop chronic sinusitis earlier in life compared to patients carrying class IV–V mutations. Consequently, pneumatization is reduced or not even initiated, resulting in hypoplastic or aplastic sinuses respectively. The fact that the maxillary sinus is relatively well developed and no difference between the two study groups was detected, could be explained by the timing of pneumatization. The maxillary sinuses pneumatize prenatally, while the frontal and sphenoid sinuses develop after birth. Since sinusitis can only develop postnatal, it can interfere with the pneumatization of the frontal and sphenoid sinuses more compared to pneumatization of the maxillary sinus. Interestingly, Chang et al. contradict the previous hypothesis and state that sinus hypoplasia precedes sinus infection in a porcine model of CF. They suggest a direct influence of the CFTR protein on sinus development[24]. The residual CFTR function in patients with class IV–V mutations as opposed to no CFTR function in the class I–III mutation group could explain our observation of smaller sinuses in severe CF. The volume of the ethmoid sinus was not analysed in this study, since this was technically difficult. Where the other sinuses had distinct boundaries to draw a circumference, the ethmoid sinus has less distinct boundaries. For this reason only opacification of the ethmoid sinuses was evaluated. Like the maxillary sinus the ethmoid sinus pneumatizes prenatally. None of the patients showed aplastic ethmoid sinuses, however the investigators did see signs of hypoplasia, in example fewer ethmoid cells. This observation was in concordance with data from Eggesbo and colleagues[20].

Osteitis/neoosteogenesis in patients with rhinosinusitis is associated with increased severity of inflammation[25]. The extent of osteitis has been correlated with the Lund–Mackay score, duration of symptoms and previous surgery[26]. Our findings that patients with class I–III mutations showed significantly more often osteitis of the maxillary sinus compared to patients with class IV–V mutations, could indicate the patients with class I–III mutations experience increased severity of inflammation in the paranasal sinuses compared to the class IV–V mutation group.

Although the exact pathogenesis of sinonasal disease is not entirely clarified, one could speculate on a rationale for the sinonasal phenotype to be affected by CFTR genotype. One explanation could be that class I–III mutations result in higher viscosity of the sinonasal mucus and therefore rhinosinusitis is more easily developed compared to class IV–V mutations. Furthermore, the sinonasal system could be directly related to the function of the CFTR protein at the apical

membrane of the sinonasal mucosa. Wang and co-workers[2] showed that carriers of a CFTR mutation are predisposed to develop chronic rhinosinusitis compared to the normal wild-type CFTR population. Previous research showed that heterozygote carriers of CFTR mutations have approximately 50% of the normal CFTR function compared to wild-type CFTR, which is sufficient to remain free of disease[27]. Patients with class IV–V mutations have a decreased CFTR function, but they often have residual CFTR function. This residual function could result in sinonasal disease, but not as severe as in patients with no CFTR function, such as patients with class I–III mutations. Thus, CFTR function could be directly related to the development of rhinosinusitis.

To our knowledge, this is the first study to describe the specific abnormal anatomy of the uncinate process in patients with CF. Previously, medial bulging of the lateral nasal wall, aplasia/hypoplasia of the uncinate process and demineralization of the process have been observed in CF patients[23,28-31]. According to the investigators this bulging and demineralization were associated with a maxillary sinus mucocele. Absence or hypoplasia of the uncinate process may follow maxillary sinus hypoplasia[32]. However, in our study population we did not find any signs for a maxillary mucocele. Moreover the anatomy we observed did not resemble hypoplasia. The uncinate process was projected medially towards the nasal septum, instead of projected in an upwards direction. Remarkably this anatomy results in a wide and open infundibulum, which in theory facilitates good drainage from the sinus to the nasal cavity.

To date, the pathogenesis of sinonasal disease in CF is not fully elucidated. Research in children with CF is needed to investigate the beginning of sinonasal disease in CF. The results of the present study emphasise that rhinosinusitis in CF patients is chronic, but unfortunately the onset of this extensive pathology is unknown.

In summary this observational study showed the prevalence of rhinosinusitis in adult patients with CF was 63% and the prevalence of nasal polyps was 25%. Patients with class I–III mutations had significantly smaller frontal sinuses, sphenoid sinuses, more opacification in the sinonasal area and more often osteitis/neoosteogenesis of the maxillary sinus wall compared to patients with class IV–V mutations. Despite this considerable sinonasal pathology, patients do not estimate these problems as very troublesome. For this reason the investigators recommend a regular examination of the sinonasal area in all CF-patients.

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Chapter 4

Temporal bone pneumatization in Cystic Fibrosis: A correlation with genotype?

M.C. Berkhout, C.J. van Rooden, R.C. Aalbers, L.H. el Bouazzaoui, W.J. Fokkens, E. Rijntjes, H.G.M. Heijerman

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4 Temporal bone pneumatization in Cystic Fibrosis: A correlation with genotype?

ABSTRACT

Objectives/Hypothesis
Paranasal sinus pneumatization in patients with Cystic Fibrosis (CF) is less extensive compared to the general population and seems to be correlated to CF genotype. Interestingly, in CF patients temporal bone pneumatization (TBP) is more extensive compared to the general population, and middle ear pathology is generally uncommon in CF. It is debated whether TBP is influenced environmentally or genetically. The aim of the present study was to investigate pneumatization of the temporal bone in patients with CF and to correlate this with genotype and paranasal sinus volume.

Study Design
Prospective collection of data.

Methods
In 104 adult CF patients, computed tomography of the temporal bone and the paranasal sinuses was performed. TBP was graded using a validated scoring system. Patients were divided into two groups, mild and severe CF, based on their mutations in the CF transmembrane conductance regulator gene.

Results
Of the 31 patients with mild CF, 71% had extensive TBP, and of the 73 patients with severe CF, 82% had extensive pneumatization of the temporal bone. TBP did not differ significantly for CF genotype, and TBP was not correlated to paranasal sinus volume.

Conclusions
Whereas paranasal sinus pneumatization in CF patients seems to be related to CF genotype among other influencing factors, this study showed no correlation between TBP and CF genotype. TBP was not correlated to paranasal sinus volume. Hypothetically, in CF, pneumatization of the temporal bone is under a different influence than paranasal sinus pneumatization.

INTRODUCTION

Paranasal sinus pneumatization in patients with Cystic Fibrosis (CF) is decreased in comparison to the general population[1,2]. This results in smaller maxillary sinuses and smaller or even absent frontal and sphenoid sinuses in CF patients. To date the pneumatization process in the human skull is not entirely understood. It is still debated whether the postnatal pneumatization is influenced environmentally or genetically[3]. A commonly held hypothesis is that an early onset of chronic rhinosinusitis (CRS) results in an impaired pneumatization of the paranasal sinuses[1,4]. Because many CF patients are expected to have this early onset CRS, the decreased pneumatization could be explained. However, studies comparing CF CRS patients with non-CF CRS patients showed a difference in pneumatization of the paranasal sinuses between these groups. Pneumatization patterns in non-CF CRS patients were not comparable to the pneumatization patterns in CF CRS patients[2,5]. These results could suggest that the decreased pneumatization is not secondary to the sinonasal disease, but perhaps more genetically driven. Recently Chang and colleagues showed that in CF piglets the underdevelopment of the sinuses was seen prior to the onset of sinus disease[6]. According to the authors, these results suggest that sinus hypoplasia is a direct consequence of the CF mutation as opposed to a consequence of sinonasal disease[6].

Within the CF population, decreased pneumatized paranasal sinuses have been correlated to specific mutations in the CF transmembrane conductance regulator (CFTR) gene[4,7,8]. Woodworth et al. demonstrated that ΔF508 homozygosity is related to smaller paranasal sinuses compared with other mutations in the CFTR gene[7].

Given that the temporal bone is also subjected to pneumatization, one might expect that in CF patients this pneumatization is different from the general population and might differ for CF genotypes. Temporal bone pneumatization (TBP) begins prenatally, but primarily occurs during postnatal growth. The pneumatization process starts at around 2 years of age and is completed between 10 and 15 years of age[9]. Chronic middle ear infections can impede pneumatization of the temporal bone[10]. Because the middle ear and the auditory tube are also lined with respiratory epithelium, viscous mucus and infection are expected to cause middle ear pathology in patients with CF. Moreover, one could hypothesize that sinonasal disease predisposes a CF patient to otitis media and consequently a decreased TBP.

Interestingly, research in the pneumatization of the temporal bone showed a greater pneumatization in patients with CF compared to healthy controls[8,11]. Based on the abovementioned genetic influence of CF genotype on paranasal sinus pneumatization, one could hypothesize that the development of the temporal bone is also influenced by CF genotype. One study found that a greater TBP was related to delta F508 homozygosity when compared to other mutations in the CFTR gene[8].

In the present study, TBP in adult patients with CF was investigated and correlated with CF genotype and paranasal sinus volume (PSV) as a surrogate parameter of paranasal sinus pneumatization.

MATERIALS AND METHODS

Patients

Adult patients treated in the Haga Teaching Hospital CF center, with a diagnosis of CF based on a positive sweat test and/or genotype, were considered eligible for this study. The study was conducted from April 2011 to February 2012 and was approved by the medical ethics committee of South-West Holland.

Genotype

Participating patients were divided into subjects with mild CF and subjects with severe CF. Class I–III mutations were considered as severe CF, and class IV–V mutations were classified as mild CF[12]. Patients with two different mutations in the CFTR gene were allocated to one of these groups according to their mildest mutation. In patients with one or two unknown CFTR mutations, pancreatic status was used to allocate these patients to the mild or severe CF group. Research showed a strong relationship between CF genotype and pancreatic status, in which class I–III mutations were associated with pancreatic insufficiency and class IV–V mutations with pancreatic sufficiency[13]. Therefore, in this study subjects with a pancreatic insufficiency were considered to be patients with severe CF, and pancreatic-sufficient patients were assigned to the group with mild CF.

Computed Tomography

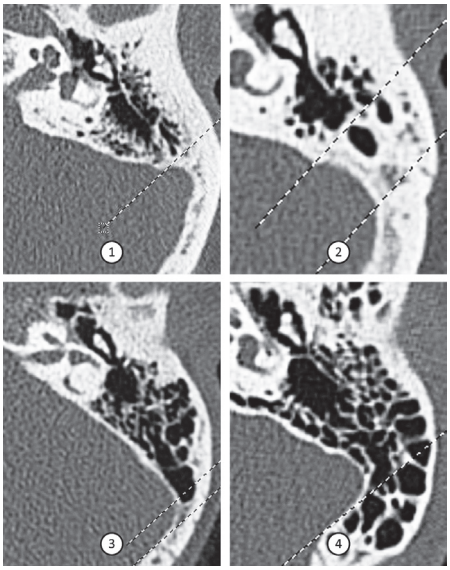
In every patient, computed tomography of the temporal bones and the paranasal sinuses was performed (Toshiba Aquilion 16; Toshiba Europe, London, UK). Patients were situated with their head tilted backward until the palatum durum was positioned perpendicular to the bench. Axial computed tomography was

performed with a slice thickness of 0.5 mm, and images were reconstructed at 1.0 mm. The scan ranged from the upper border of the frontal sinuses to the lower part of the teeth in the maxilla. No intravenous contrast was used.

TBP

In this study, TBP was measured using the validated grading scale described by Han and coworkers[14]. This scale used the sigmoid sinus as a reference structure. With this method, the section where the malleoincudal complex appeared as an ice cream cone shape was used. Three parallel lines angled at 45° in the anterolateral direction were applied at positions in which each line crossed the most anterior point of the sigmoid sinus, the most lateral aspect of the sigmoid sinus, and the most posterior point of the sigmoid sinus. TBP was classified in four categories: 1) pneumatization remained anterolateral to the line that was drawn at the most anterior point; 2) pneumatization extended beyond the most anterior line, but not beyond the line crossing the most lateral aspect of the sigmoid sinus; 3) pneumatization extended to the space between the lines drawn at the most lateral aspect and at the most posterior point; and 4) pneumatization extended beyond the line drawn at the most posterior point (Fig. 1). Left and right temporal bones were analyzed separately.

Figure 1.



At the slide where the malleoincudal complex appeared as an ice cream cone shape, three parallel lines were drawn. (1) Pneumatization remained anterolateral to the line that was drawn at the most anterior point; (2) pneumatization extended beyond the most anterior line, but not beyond the line crossing the most lateral aspect of the sigmoid sinus; (3) pneumatization extended to the space between the lines drawn at the most lateral aspect and at the most posterior point; and (4) pneumatization extended beyond the line drawn at the most posterior point.

Assessors

Two assessors (M.C.B., R.C.A.) independently measured TBP. In case of disagreement between the TBP scores of the two assessors, an experienced radiologist (C.J.v.R) gave a decisive opinion, which was considered as the final score for TBP. All assessors were blinded for the genotype of the patients and for previous outcome parameters, such as PSV.

PSV

To analyze whether the pneumatization process of the temporal bone was correlated to the pneumatization process of the paranasal sinuses, the correlation between TBP and paranasal sinus pneumatization was investigated. PSV was used as a surrogate parameter for paranasal sinus pneumatization.

The volume of the sinuses was analyzed with Aquarius Intuition software (TeraRecon, Foster City, CA). For each sinus, the circumference on each slice was drawn manually, whereupon the software program calculated the surface. All

these surfaces were added up to generate the total volume of the sinus. Only the frontal, sphenoid, and maxillary sinuses were measured. Together these volumes were used as total PSV. Aplastic sinuses were regarded as 0 cm3.

Data and Statistics

Average TBP was calculated using the left and right TBP together. To ensure the patients' TBP of the left and right side did not differ considerably, a Cohen κ was calculated. In case of substantial difference between the two sides, both sides were analyzed separately.

The interobserver correlation was assessed by Cohen κ. Consensus on the value of κ is that agreement is poor if $\kappa \leq 0.20$, fair if $0.21 \leq \kappa \leq 0.40$, moderate if $0.41 \leq \kappa \leq 0.60$, substantial if $0.61 \leq \kappa \leq 0.80$, and good if $\kappa > 0.80$.

An insufficient number of patients would hinder proper statistical analyses; therefore, the four categories of TBP were divided into two groups: less extensive TBP = score 1 to 2 and extensive TBP = score 2.5 to 4. The Cohen κ for interobserver correlation was applied to the original four categories of TBP.

To evaluate a possible correlation between TBP and genotype, a χ^2 test was used. The correlation between TBP and PSV was analyzed using a Mann-Whitney U test.

RESULTS

Patients

One hundred and four patients gave informed consent and were included in the study. The mean age of all subjects was 34.8 years (standard deviation = 11.1), and 52.9% was male. Table 1 shows the descriptive statistics of the 104 patients separated for the two severity groups. The mean age of the patients with mild CF was significantly higher compared to the mean age of the patients with severe CF.

Table 1.

Descriptive statistics of 104 adult patients with Cystic Fibrosis

Parameter	Mild CF; n = 31 (mutation class IV-V)	Severe CF; N = 73 (mutation class I-III)	*p*-value
Mean age, years (SD)	40.5 (10.7)	32.4 (10.4)	0.000*
Male, n (%)	14 (45.2)	41 (56.2)	0.304
Lung function:			
Mean FEV1 % predicted (SD)	66.3 (25.4)	62.1 (25.5)	0.389
Mean FVC % predicted (SD)	87.5 (18.1)	83.0 (19.0)	0.265

**p*-value <0.05

CF = Cystic Fibrosis; FEV1 = forces expiratory volume at 1 second; FVC = forced vital capacity; SD = standard deviation.

Genotype

In three patients one CFTR mutation was unknown and in one patient both of the CFTR mutations were unknown. One of the three patients was pancreatic insufficient and therefore assigned to the class I–III mutation group, and two patients were pancreatic sufficient and were allocated to the IV–V mutation group. The subject with both unknown mutations was diagnosed at 42 years, but was pancreatic insufficient secondary to pancreatitis. Because this patient had typical characteristics of mild disease, he was assigned to the class IV–V mutation group. In total, 31 patients were classified with mild CF and 73 patients with severe CF.

Assessors

In 17 of the 208 analyzed temporal bones (8.2%), the two observers disagreed and a deciding opinion was necessary. The Cohen κ for interobserver correlation was 0.85 ($p = .000$).

TBP and Genotype

The average TBP separated for genotype is presented in Table 2. Although the percentage of extensive TBP in patients with severe CF was higher compared to patients with mild CF, this difference did not reach statistical significance ($p = .20$).

Table 2.

Average TBP separated for genotype

Average temporal bone pneumatization*	Mild CF; n = 31 (mutation class IV-V)	Severe CF; n = 73 (mutation class I-III)	*p*-value
Less extensive TBP (category 1-2), n (%)	9 (29.0)	13 (17.8)	
Extensive TBP (category 2.5-4), n (%)	22 (71.0)	60 (82.2)	
Total	31 (100)	73 (100)	0.20

*Average TBP was calculated using the left and right TBP together.

CF = Cystic Fibrosis; TBP = temporal bone pneumatization.

Comparison between the left and right TBP within one patient showed a Cohen κ of 0.48, which means a moderate agreement between both sides. For this reason, TBP was analyzed separately for the left and right side of the subject. Table 3 presents the TBP for patients with mild CF and patients with severe CF. For both sides, TBP was not significantly different between patients with mild CF and patients with severe CF (left: $p = .20$; right: $p = .96$).

Table 3.

Temporal bone pneumatization for left and right side separately

	*LEFT**		*RIGHT**	
	Mild CF; n (%)	Severe CF; n (%)	Mild CF; n (%)	Severe CF; n (%)
Less extensive TBP (category 1-2)	9 (29.0)	13 (17.8)	13 (41.9)	31 (42.5)
Extensive TBP (category 2.5-4)	22 (71.0)	60 (82.2)	18 (58.1)	42 (57.5)
Total	31 (100)	73 (100)	31 (100)	73 (100)
p-value	0.20		0.96	

*Because the left and right TBP in one patient often were not identical, the left and right TBP were analysed separately. This results in two TBP scores per patient and therefore in a total of 208 temporal bones analysed.

CF = Cystic Fibrosis; TBP = temporal bone pneumatization.

The correlation between TBP and PSV was analyzed for average TBP as well as for the left and right side separately. For the latter analysis, PSV was also separated for the left and right side of the patient.

Results are displayed in Table 4. Statistical analyses showed no correlation between PSV and TBP.

Table 4.

Paranasal sinus volume and temporal bone pneumatization

Temporal bone pneumatization	Paranasal sinus volume (cm^3); mean (SD)		
	AVERAGE TBP	*LEFT*	*RIGHT*
Less extensive TBP (category 1-2)	23.69 (12.92)	12.54 (7.47)	10.94 (5.04)
Extensive TBP (category 2.5-4)	23.04 (11.92)	11.63 (5.93)	11.66 (6.88)
p-value	0.79	0.89	0.99

SD = standard deviation; TBP = temporal bone pneumatization.

DISCUSSION

To our knowledge, this is the first study to compare TBP in a large group of adult patients with CF. The only study in which TBP was compared between CF patients with different genotypes is the study of Seifert and coworkers[8]. They found that ΔF508 homozygosity correlated with greater TBP. In contrast with their results, we did not find a significant difference in TBP between patients with mild CF and patients with severe CF. However, the percentage of extensive TBP in patients with severe CF was higher compared to the patients with mild CF, thereby showing the same trend as in the study of Seifert et al. The difference in statistical results could be due to the groups used in both studies not being similar. Classification in severe and mild CF is based on the CFTR mutation classes, as mentioned in the study design. Patients with ΔF508 homozygosity belong to the group of severe CF patients. However, many other mutations result in severe CF. Therefore, the study of Seifert et al. is in theory not comparing patients with severe CF and patients with mild CF. Moreover, we had to subdivide the TBP into two categories, because statistical analysis was not possible using the original four categories. Seifert et al., however, did use the original four categories. For these reasons we have to be careful when comparing both studies.

Whereas we did not find a correlation between genotype and TBP, in the same study population we did find a correlation between paranasal sinus pneumatization and genotype[15]. Because the mastoid air cells and the sinuses are in close contact with each other and are lined with similar mucosa, one would expect the pneumatization processes to be correlated. However, the present

study did not show a correlation between PSV and TBP. This is confirmed by other studies showing no correlation between the volume of the paranasal sinuses and the mastoid air cells[16,17].

To date the pneumatization process in the human skull is not entirely understood. It is debated whether the postnatal pneumatization is influenced genetically or environmentally[3]. Seifert et al. hypothesize that the defective CFTR protein directly influences the function of the mastoid air cell mucosa, with changes in gas exchange and pressure and consequently an influence on TBP[8]. Todd and Martin studied TBP in patients with CF and in the general population and found an increased TBP in patients with CF[11]. They hypothesize that the mucosa of the auditory tube and the middle ear in CF patients is different in, for example, composition of goblet cells compared to non-CF patients. Twelve years later this was confirmed by the results of Yildirim and coworkers[10]. They performed histologic examination of the temporal bone of patients with CF and found lower densities of goblet cells in the mucosa of the auditory tube and middle ear of patients with CF compared to the middle ear mucosa and auditory tube of a non-CF population. This could explain the low prevalence of ear pathology in CF patients. Moreover, they suggest a role for expression of mucin genes. Differences between mucin gene expression in the epithelium of the sinuses and in the epithelium of the middle ear might explain the discrepancy between sinonasal pathology and middle ear pathology in CF patients.

The degree of pneumatization of the temporal bone does not have direct clinical implications. Indirectly, however, the results of this study can be meaningful for clinicians treating patients with CF. A decreased TBP is an objective measure for a history of chronic ear infection[10,11]. No difference in TBP between patients with mild CF and severe CF could indicate that these patients do not differ in middle ear pathology. Moreover, the overall well-preserved TBP in CF patients[11] might indicate that CF patients do not suffer from middle ear pathology often. Furthermore, these findings challenge the belief that poor mucociliary transport induces middle ear disease.

A limitation of this study was the scale that is used for grading TBP. The grading scale is based on pneumatization that is in the posterior direction. However, during analysis in some patients, the investigators noticed pneumatization in a more anterior direction. Sometimes this pneumatization was more extensive in the anterior direction than in the posterior direction. Therefore, these patients would be graded as having less extensive TBP, although they had an extensively pneumatized temporal bone.

Another limitation was that information on the otologic history of the patients was not collected. Therefore, we could not correlate these retrospective data to the pneumatization of the temporal bone. However, we did investigate the middle ear on the same day the CT scan was performed. Only two of the 104 patients had signs of unilateral otitis media with effusion. From an article of Hadfield and coworkers, we learned that otitis media with effusion is scarce among patients with CF[18].

CONCLUSION

Data from 104 adult patients with CF showed no difference in TBP between patients with mild CF and patients with severe CF. Moreover, no correlation between PSV and TBP was found. Hypothetically, the TBP is under a different influence than the paranasal sinus pneumatization. The exact mechanism has not yet been elucidated.

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Chapter 5

CT-abnormalities, bacteriology and symptoms of sinonasal disease in children with Cystic Fibrosis

M.C. Berkhout, F. Klerx-Melis, W.J. Fokkens, M. Nuijsink, W.M.C. van Aalderen, H.G.M. Heijerman

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5 CT-abnormalities, bacteriology and symptoms of sinonasal disease in children with Cystic Fibrosis

ABSTRACT

Background

Sinonasal pathology in adults with Cystic Fibrosis (CF) is common but the extent of CT-abnormalities and symptoms of sinonasal disease in children with CF and the age of onset are less frequently studied.

Methods

In this observational, cross-sectional study 58 children with CF from two CF centres were included. All subjects completed a questionnaire regarding sinonasal symptoms, underwent a CT scan of the paranasal sinuses, and in each subject a culture of the upper airways was performed. Subjects were divided in 6 age cohorts (0–2, 3–5, 6–8, 9–11, 12–14 and 15–17 years) and were divided into severe and mild CF based on their CFTR mutation. Opacification of the sinonasal system of the subjects was compared with opacification on MRI-scans of an age-matched control group without CF.

Results

Most frequently reported symptoms were nasal obstruction and posterior/anterior nasal discharge. Opacification was abundant in every age cohort of the study group and was significantly more compared to the control group. In patients with severe CF the opacification was higher than subjects with mild CF. Upper airway cultures showed predominantly *Staphylococcus aureus*, *Haemophilus influenzae* and *Pseudomonas aeruginosa*.

Conclusion

CT-abnormalities indicating sinonasal disease and symptoms are present from shortly after birth which may argue for a thorough examination of the upper airways in children with CF.

INTRODUCTION

The prevalence of sinonasal pathology in patients with Cystic Fibrosis (CF) is high. Due to the defect Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein, stasis of viscous mucous and impaired mucociliary transport are believed to contribute to the development of chronic rhinosinusitis (CRS). However, not all predisposing factors are identified yet[1]. Acknowledgement of sinonasal disease in CF patients is important because symptoms of rhinosinusitis can decrease a patient's quality of life. Furthermore the paranasal sinuses can constitute a niche for pathogens from which cross infection to the lungs can occur and lung infections can be facilitated[2].

Recently more aggressive treatment strategies of sinonasal disease have been applied to improve pulmonary outcomes. Early detection and treatment of pathogens in the upper airways can prevent or postpone cross infection of the lungs[3,4]. For early detection however, it is necessary to gain data on the clinical characteristics of sinonasal disease from children with CF.

In adult patients with CF the prevalence of rhinosinusitis is approximately 63% and the prevalence of nasal polyps around 25%[5]. The exact prevalence in children with CF, however, is unknown. In the literature the prevalence of nasal polyps ranges from 32 to 45%[6]. For ethical concerns studies performed in children with CF often did not include children in the 0 to 4 age group. Moreover investigations to ascertain the diagnosis of rhinosinusitis, such as a CT scan and nasendoscopy are often omitted in children. For these reasons a proper statement on the prevalence among children in different ages was difficult to obtain.

Clinical manifestations of sinonasal disease in CF which can be found on additional examination include nasal polyps, abnormalities on computed tomography (CT) of the sinuses and cultures from the upper airway positive for CF pathogenic microorganisms. In adults with CF *Pseudomonas aeruginosa* is most frequently cultured from the upper airways, followed by *Staphylococcus aureus*.

CT scans of the paranasal sinuses often show opacified sinuses, which indicates inflammation of the epithelium lining the sinuses[7]. Furthermore, hypoplasia and aplasia of the frontal and sphenoid sinus have been described[7,8]. Another radiographic finding in patients with CF is osteitis/neoosteogenesis predominantly of the maxillary sinus walls[7]. This has been hypothesized to be associated with increased severity of inflammation[9]. These developmental and

inflammatory changes in adult patients indicate a chronic course of rhinosinusitis. However, to date the age of onset of the sinonasal pathology is unknown.

Sinonasal manifestations of CF seem to be associated with genotype, with more severe sinonasal disease in patients with class I–III mutations in the CFTR gene[5,10]. However, research in this possible correlation is scarce.

In the present study several aspects of sinonasal disease in children with CF were investigated. This is one of the scarce studies that includes CF children from the age of 0 years[11,12]. The aim of this study was to gain more knowledge on CT-abnormalities, microbiology and symptoms of sinonasal disease in children with CF.

STUDY DESIGN

Patients

This multicentre, observational study was performed in two CF-centres in the Netherlands (Academic Medical Centre (AMC) in Amsterdam and Haga Teaching Hospital in The Hague). From September 2013 to September 2014 children with a diagnosis of Cystic Fibrosis, based on a positive sweat test and/or a confirmed CF genotype, from 0 to 17 years of age were enrolled in this study. Subjects were divided into 6 cohorts of age; 0–2, 3–5, 6–8, 9–11, 12–14 and 15–17 years. The intended number of patients was ten in each cohort. The subjects were divided into two groups according to the mutations in their Cystic Fibrosis transmembrane regulator (CFTR) gene. Patients who were homozygous or compound heterozygous for class I–III mutations were considered as having severe CF. Subjects who carried at least one class IV–VI mutation were assessed as having mild CF. If one or both of the mutations were unknown, pancreatic function and age at diagnosis were used to allocate subjects to one of the two groups. Since a strong correlation between pancreatic function and severity of CF is reported[13] and age at diagnosis is known to correlate well with genotype[14] in this study patients with pancreatic insufficiency or an age at diagnosis < 10 years were considered as having severe CF. History of sinonasal surgery and current drug use were recorded for each subject. The study was performed in accordance with the Declaration of Helsinki and was approved by a local medical ethics committee. Written informed consent was obtained from both parents/caretakers and assent was obtained from a participant of 12–17 years old.

Computed tomography

Computed tomography (CT) of the paranasal sinuses was performed in all subjects. In the Haga Teaching Hospital the SOMATOM Definition Flash CT scan was used and in the AMC the Philips Brilliance CT scan. In both hospitals a low-dose protocol was used, with an effective dose of 1 millisievert. Axial computed tomography was performed with a slice thickness of 0.5 mm and images were reconstructed at 1.0 mm to coronal and sagittal images. In both centres the scan ranged from the upper border of the frontal sinuses tot the lower part of the teeth in the maxilla. No intravenous contrast nor anaesthesia was used. In patients that objected to the CT scan after two attempts, the procedure was stopped because too much movement during the CT scan would interfere with the quality of the scan.

Opacification of the paranasal sinuses was analysed using a modified Lund–Mackay (L–M) score. The Lund–Mackay scoring system grades every sinus as 0: normal, 1: partial opacification, or 2: total opacification. With this system the opacification of the maxillary, anterior ethmoid, posterior ethmoid, sphenoid and the frontal sinus of both sides is graded. Moreover the ostiomeatal complex is scored as 0: patent and 2: occluded[15]. In case all sinuses are present, the L–M score can range from 0 to 24. However, in this study modification of the L–M system was necessary since the prevalence of non-developed sinuses in children is high. The total L-M score was divided by the amount of elements present, resulting in a score for opacification per element of the sinonasal system. Two observers (F.K. and M.C.B) who were blinded for genotype and previous outcomes of the study, analysed all CT scans.

Lund–Mackay scores on CT scans of patients with CF were compared with L–M scores on MRI-scans of children without CF. This group consisted of children in which a MRI of the head was performed for other reasons than rhinosinusitis. The MRI scans were collected from a database of the Radiologic Department of the Haga Teaching Hospital and personal data were removed before they were used in the analysis. MRI scans were included when the reason for MRI-scan was not otorhinolaryngologic pathology and the lower border of the maxillary sinus was depicted. The study group and the control group were matched for age.

In addition, the CT scans of CF patients were evaluated for the presence of anatomical variations, such as bulging of the lateral nasal wall, the anatomy of the uncinate process and the presence of osteitis/neoosteogenesis of the sinus walls. These anatomical variations could not be evaluated on the MRI scans of the

control group. The development of the sinuses however, was evaluated in the control group.

Symptoms of rhinosinusitis

Symptoms of rhinosinusitis were estimated using a Visual Analogue Scale (VAS), in which zero accounted for 'no problem' and a score of ten accounted for 'the problem is as bad as I can imagine'. Nine symptoms were questioned; nasal blockage, anterior nasal discharge, posterior nasal discharge, nose blowing, sneezing, headache, facial pain, decreased smell and decreased taste. A total score was calculated by adding up all VAS scores for every question leading to a minimum possible score of 0 and a maximum of 90. In subjects of 0 to 7 years the parents/caretakers filled out the questionnaire. Patients with the age of 8 to 17 years filled out the questionnaire themselves.

Microbiology

Upper (nasopharynx) and lower airway cultures were collected. For the upper airway culture, a flexible, thin swab was inserted in the nose until the nasopharynx was reached. No anaesthesia or decongestive drugs were used. Lower airway cultures consisted of expectorated sputum or a cough swab. Preferably the upper airway culture and the lower airway culture were collected on the same day, but a period of 7 days in between the two swabs was accepted for this study. Cultures were evaluated in the hospital where the patient was seen. In both hospital laboratories the samples were streaked on several agars; chocolate agar, Cystine-Lactose-Electrolyte Deficient agar (CLED agar) and Sabouraud agar. All samples were incubated aerobically at 37 °C and were analysed for growth of bacteria, fungi and yeast after 8–16 h and every 24 h during 7 consecutive days.

Data and statistics

Data collected in the present study were analysed with SPSS for Windows version 22. Lund–Mackay score and symptoms of rhinosinusitis were reported by descriptive statistics such as mean and standard deviation (SD). Comparison of paranasal sinus opacification between children with CF and without CF was performed with an unpaired t-test. Moreover, the correlation between L–M score and severity of CF was investigated with an unpaired t-test. The interobserver variability of the two observers of the CT scans was calculated with the intraclass coefficient.

RESULTS

Patients

A total of 58 children were included in the study. The patient characteristics are displayed in Table 1. There was a slight male preponderance and the distribution of mild versus severe CF was skewed towards severe CF. Only five patients had a history of sinus surgery and those were all attributed to the two older cohorts. Nasal steroids were more often used by children in the older cohorts, saline irrigations instead were more used by children in the younger cohorts. Systemic antibiotics were prescribed for pulmonary infections in all subjects.

Table 1.

Descriptive statistics of 58 children with Cystic Fibrosis separated in 6 age cohorts

Parameter	Cohort 1 0-2 yrs	Cohort 2 3-5 yrs	Cohort 3 6-8 yrs	Cohort 4 9-11 yrs	Cohort 5 12-14 yrs	Cohort 6 15-17 yrs
Number of patients	9	9	10	10	10	10
Mean age, years (SD)	1.3 (0.7)	3.6 (0.9)	7.1 (0.9)	10.4 (0.8)	13.2 (0.6)	15.9 (0.7)
Male, n (%)	3 (33)	4 (44)	4 (40)	8 (80)	7 (70)	8 (80)
Severe CF, n (%)	8 (89)	9 (100)	10 (100)	6 (60)	8 (80)	8 (80)
Conservative therapy						
Nasal steroids, n (%)	0 (0)	1 (11)	5 (50)	4 (40)	2 (20)	5 (50)
Systemic steroids, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (10)
Nasal saline irrigation, n (%)	4 (44)	3 (33)	5 (50)	1 (10)	1 (10)	3 (30)
Systemic antibiotics, n (%)	3 (33)	1 (11)	7 (70)	5 (50)	4 (40)	7 (70)
Surgical history						
FESS, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	2 (20)	3 (30)

Table 2 presents an overview of the genotypes of the participating subjects. One patient with one unknown genotype was allocated to a mutation class according to age of diagnosis and pancreatic function. Two patients carried one mutation that could not be attributed to a mutation class based on the literature. Characteristics of those patients are described below the table. (See Table 2)

Table 2.

Overview of genotypes and their mutation classes

Genotype	Frequency; n (%)	Mutation class
ΔF508/ΔF508	33 (56.9)	I-III
ΔF508/1653delCTT[a]	1 (1.7)	I-III
ΔF508/R1162X	1 (1.7)	I-III
ΔF508/A455E	2 (3.4)	IV-VI
ΔF508/5T13TG	3 (5.2)	IV-VI
ΔF508/R1066C	1 (1.7)	I-III
ΔF508/R553X	2 (3.4)	I-III
ΔF508/S1251N	1 (1.7)	I-III
ΔF508/Y849X	2 (3.4)	I-III
ΔF508/R117H	1 (1.7)	IV-VI
ΔF508/W1282X	1 (1.7)	I-III
ΔF508/2789+5G>A	1 (1.7)	IV-VI
ΔF508/1717-1G>A	2 (3.4)	I-III
ΔF508/3849+10kbC>T	1 (1.7)	IV-VI
ΔF508/c1725_1727[b]	1 (1.7)	I-III
ΔF508/N1303K	1 (1.7)	I-III
R117T/R117T	1 (1.7)	IV-VI
ΔF508/UNK[c]	1 (1.7)	I-III
A46D/A46D	2 (3.4)	I-III

[a] Patient diagnosed at age of 8 months with pancreatic insufficiency

[b] Patient diagnosed at age of 0 months with pancreatic insufficiency

[c] Patient diagnosed at age of 3 months with pancreatic insufficiency

Computed tomography

Interobserver variability in L–M score per element of the sinonasal system between the two observers was calculated with the intraclass coefficient (ICC). For the CT scan the ICC was 0.94 and for the MRI-scan the ICC was 0.78.

In total 54 CT scans were performed, because in the age cohort of 0–2 years one child objected and in two children the parents objected to the CT scan. One patient of 3 years old objected to the CT scan. In children that objected two attempts were taken, however ultimately they moved too much to gain CT scans with sufficient quality to evaluate. Mean L–M scores for the cohorts are depicted in Table 3. No specific trend was seen in the distribution between the cohorts.

Table 3.

Radiologic findings in children with CF and their age-matched controls

Parameter	Cohort 1 0-2 yrs	Cohort 2 3-5 yrs	Cohort 3 6-8 yrs	Cohort 4 9-11 yrs	Cohort 5 12-14 yrs	Cohort 6 15-17 yrs
CT scan (n/group)	6	8	10	10	10	10
Lund Mackay score in CF patients; mean (SD)	0.90 (0.7)	1.35 (0.5)	1.05 (0.5)	0.65 (0.5)	0.96 (0.4)	0.82 (0.3)
Lund Mackay score in control group; mean (SD)	0.10 (0.2)	0.40 (0.7)	0.20 (0.2)	0.07 (0.1)	0.19 (0.3)	0.10 (0.1)
Non-developed frontal sinus in CF patients; n (%)	6 (100)	8 (100)	9 (90)	6 (60)	4 (40)	4 (40)
Non-developed frontal sinus in control group; n (%)	6 (100)	7 (88)	6 (60)	2 (20)	2 (20)	1 (10)
Non-developed sphenoid sinus in CF patients; n (%)	2 (33)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Non-developed sphenoid sinus in control group; n (%)	3 (50)	1 (13)	0 (0)	0 (0)	0 (0)	0 (0)
Unilateral/bilateral bulging of lateral nasal wall in CF patients; n (%)	3 (50)	8 (100)	7 (70)	5 (50)	7 (70)	8 (80)
Abnormal anatomy of uncinate process in CF patients; n (%)	0 (0)	0 (0)	5 (50)	2 (20)	2 (20)	3 (30)
Osteitis/neoosteogenesis in CF patients; n (%)	0 (0)	1 (13)	3 (30)	3 (30)	7 (70)	5 (50)

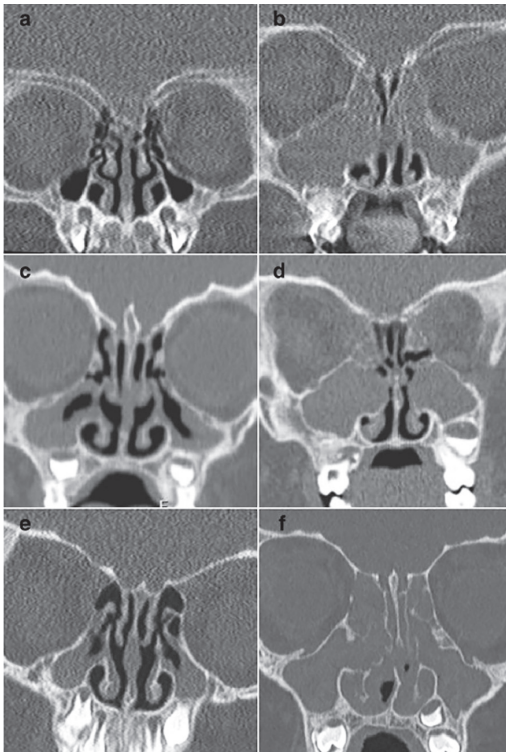
In total 54 MRI-scans of control patients were collected. The mean L–M score for the study group was 0.94 and for the control group was 0.17 (*p*-value = 0.000).

The mean L–M score for the 46 patients with severe CF was 1.02 (SD: 0.5) and for the 8 subjects with mild CF was 0.53 (SD: 0.3). These mean values differed significantly (*p*-value = 0.10).

For each cohort the L–M score was compared between controls and study patients. Results are displayed in Table 3. For every cohort the following *p*-values were calculated: for cohort 1 a *p*-value of 0.028, for cohort 2 a *p*-value of 0.005, for cohort 3 a *p*-value of 0.000, for cohort 4 a *p*-value of 0.001, for cohort 5 a *p*-value of 0.000 and for cohort 6 a *p*-value of 0.000. All L–M scores of CF patients were significantly higher compared to the control group in each cohort.

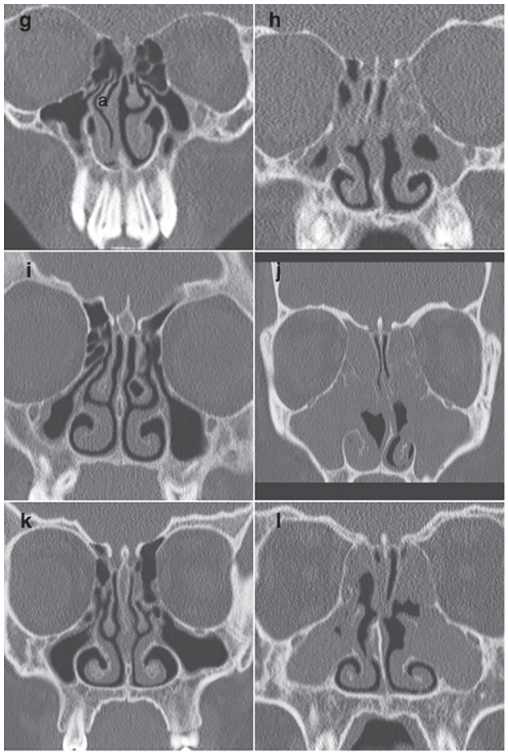
Table 3 presents an overview of the Lund–Mackay score and the development of the sinuses for each age cohort of both the study group and the control group. As expected the frontal and sphenoid sinus developed with age. Compared to the control group, the CF-patients showed less development of the frontal sinus in the older cohorts. Bulging of the lateral nasal wall appeared throughout the study population, whereas the uncinate process showed more abnormalities in the older cohorts. Osteitis/neoosteogenesis, was more frequent in the older cohorts of the study population. Fig. 1 and Fig. 2 show examples of the CT scans of the study group.

Figure 1.



Coronal CT scans of children with the lowest and the highest Lund-Mackay score for cohort 1 to 3. 1-year-old girl (a), 0-year-old girl (b), 5-year-old girl (c), 3-year-old boy (d), 7-year-old boy (e) and 6-year-old boy (f).

Figure 2.



Coronal CT scans of children with the lowest and the highest Lund-Mackay score for cohort 4 to 6. 10-year-old boy (g), 9-year-old boy (h), 13-year-old boy (i), 13-year-old girl who had sinus surgery in the past (j), 17-year-old girl (k) and 16-year-old girl (l).

In one patient the CT scan of the sinuses showed a mucocele in the ethmoid region. After confirmation of the mucocele with a MRI-scan, the surgeon and the parents decided to perform an ethmoidectomy to marsupialise the mucocele.

Symptoms of rhinosinusitis

Fully completed questionnaires were available from 53 of 58 patients. For the separate questions all available VAS scores were taken into account. Table 4 presents the total score and the complaint with the highest score for each cohort.

Table 4.

Overview of genotypes and their mutation classes

Age group	Complete questionnaires n (% of total)	Total VAS score Mean (SD) [0-90]	Highest score Complaint; mean (SD)
0-2 yrs	7 (77.8)	21.4 (15.6)	Nasal blockage; 2.9 (3.0)
3-5 yrs	6 (66.7)	24.0 (19.7)	Anterior nasal discharge; 4.3 (2.7)
6-8 yrs	10 (100)	20.8 (11.9)	Posterior nasal discharge; 3.8 (3.2)
9-11 yrs	10 (100)	13.6 (7.2)	Posterior nasal discharge; 2.4 (1.9)
12-14 yrs	10 (100)	18.0 (21.3)	Posterior nasal discharge; 3.7 (3.2)
15-17 yrs	10 (100)	23.7 (17.8)	Nasal blockage; 4.6 (3.5)

The mean total VAS score of the subjects with severe CF was 20.1 (SD: 16.7) and the total score of patients with mild CF was 18.8 (10.1) (*p*-value = 0.83). No correlation between symptoms and Lund–Mackay score was found in this study.

Microbiology

In total 51 nasopharynx cultures were collected; 7 in cohort 1, 8 in cohort 2, 10 in cohort 3, 10 in cohort 4, 9 in cohort 5 and 8 in cohort 6. Fifty lower airway cultures were performed on the same day or within 7 days of the nasopharynx culture and were included in the analysis; 6 in cohort 1, 8 in cohort 2, 10 in cohort 3, 8 in cohort 4, 9 in cohort 5 and 9 in cohort 6.

Unfortunately, 13 nasopharynx cultures and in 10 lower airway cultures were only analysed for the presence of *P.aeruginosa*.

Table 5 presents an overview of the most common microorganisms cultured from the upper and lower airways of the study patients. Microorganisms originating from the lower airways that were cultured less frequently, are described in the text below the table.

Table 5.

Most common cultured microorganism in upper and lower airways of children with CF

	Upper airway cultures						Lower airway cultures					
Microorganism	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Nasal/throat flora	6 (86)	3 (38)	6 (60)	6 (60)	7 (78)	7 (86)	5 (83)	7 (86)	7 (70)	4 (50)	8 (89)	8 (89)
Pseudomonas aeruginosa						1 (13)			1 (10)			
Staphylococcus aureus		1 (13)	1 (10)	1 (10)			1 (17)	3 (38)	1 (10)	3 (38)	1 (11)	2 (22)
Haemophilus influenzae			2 (20)		1 (11)					1 (13)		1 (11)
Moraxella catarrhalis			1 (10)									

The following microorganisms were cultured from the lower airways: *Nocardia* species (1 patient), *Escherichia coli* (2 patients), *Haemophilus parinfluenzae* (1 patient), coliform rods (2 patients), *Burkholderia multivorans* (2 patients), *Achromobacter xyloxidans* (1 patient), *Aspergillus fumigatus* (1 patient), *Streptococcus pneumonia* (1 patient), *Pseudomonas fluorescens* (1 patient), *Stenotrophomonas maltophilia* (1 patient) and yeasts (1 patient).

In one patient *P. aeruginosa* was cultured from the upper airways and was not found in the lower airway culture collected on the study day. However, in this patient several previous lower airway cultures did show *P. aeruginosa*.

DISCUSSION

In the present study, sinonasal pathology was evaluated in a group of CF patients 0 to 17 years of age. Almost all patients were diagnosed by postnatal screening, consisting of the most common CF causing mutations or by direct postnatal severe pathology. This resulted in a small amount of patients in the study group with mild CF. Generally, patients with one mild CF mutation present later in life. Comparison between patients with severe CF and with mild CF is therefore difficult. However, even with a small amount of mild CF patients, the opacification is significantly more in patients with severe CF.

Literature regarding CT analysis of the paranasal sinus system in CF patients showed that inflammation of the lining of the sinuses occurs early in life and does not progress over the years[16]. Our findings endorse that statement since the adjusted L–M score does not differ throughout the different age cohorts.

Moreover, a previous study in adult patients with CF using the same modified L–M score showed a mean of 0.87, which is in line with the mean of this study group of 0.94. Comparison of L–M scores between children with severe CF and mild CF revealed a significant difference, as in adults with CF[5]. These findings could imply that physicians should investigate severe CF patients more regularly or extensively for sinonasal pathology than patients with mild CF.

Osteitis/neoosteogenesis appears as irregularly thickened bony lining of the sinuses and is thought to be the result of chronic inflammation of the bone[15,17]. It is associated with increased severity of inflammation, longer duration of symptoms and previous surgery[17]. This could implicate that children with CF should express less osteitis than adults with CF. The mean percentage of osteitis in our study group was 35.2% with a range of 13 to 70% among the age cohorts. This percentage of osteitis increased with age. Our previous study in adults with CF reported a mean percentage of osteitis of 84.6%[5].

In this study the control group comprised MRI-scans of children without CF. We decided to use MRI scans instead of CT scans because almost all available CT scans of children on which the sinuses were completely visible are performed for sinonasal symptoms and would therefore be unsuitable for a control group. Indications for the MRI-scans were other reasons than otorhinolaryngologic pathology, such as epilepsy and cognitive abnormalities. Opacification of the paranasal sinuses can be estimated on MRI scans, however the Lund Mackay score in some cases was more difficult to apply. On T2 weighted MRI scans mucosa lining the ossal structures of the sinonasal system, that is rich in blood supply appears very distinct, suggesting opacification. The authors feel that this distinction would not have been that obvious on a corresponding CT scan and would not have been scored as partial opacification. Moreover, the slice thickness of MRI scans was larger than in the CT scans, making the partial volume effect more prominent in the control group. These could be reasons that the ICC of the two observers in the control group was evidently smaller compared to the ICC of the CT scans of the study group. The authors noticed that the L–M score on MRI scans can be overestimated in comparison to CT scans. Taking this overestimation in account we feel the conclusion that the L–M score in CF patients is significantly higher compared to the control group still holds.

Eggesbo and her colleagues argued for MRI to be superior to CT imaging in differentiation between mucosal thickening and pus-filled areas in the paranasal sinuses[18]. Other advantages of MRI are that it can guide the surgeon to the

collections of pus in the sinuses[18] and the absence of radiation, especially in children. Instead of radiation however, in many young children sedation is needed. In this study MRI scans of CF children would make comparison with the MRI scans of the control group more accurate. Despite the advantages the authors chose for a CT scan because of several reasons. First, the CT scan remains the golden standard for imaging the paranasal sinuses[1]. Also a CT scan is superior to MRI in depicting ossal structures, including important landmarks for surgery. Furthermore, imaging the sinuses with a CT scan is common practice and the authors aspired the study to be the utmost representative, in order to make a practice based advise for follow-up of these children. Ultimately, the authors were interested in the anatomical abnormalities and in osteitis/neoosteogenesis because this indicates the severity and chronicity of the sinonasal disease, which cannot be assessed with MRI.

According to the ALARA principle (As Low As Reasonably Achievable) radiation in children should be applied with caution. Risks of a CT sinus are damage to the eye-lens, which could eventually lead to cataract, damage to thyroid tissue and a small but significant increase in the lifetime risk of malignancy[19,20]. To minimize the radiation dose in the eyes, bismuth shields could be used[20], which we did not apply in this study. When using shields for the eyes, important artefacts due to the shields should be considered. Radiation doses of 10–50 mSv are associated with an increased lifetime risk for malignancy[21]. The effective dose of the CT scan used in this study was 1 mSv and although this is reasonably lower compared to the 10–50 mSv, the radiation dose is cumulative in life[22] and children with CF are expected to underdo more than one CT scan during life. For these reasons Cavel and colleagues suggest that in current practice the CT sinus for a child with CF is restricted to pre-operative use[23].

One inevitable aspect of questionnaires in very young children is that parents have to fill out the questionnaire and have to estimate subjective complaints such as headaches and decreased smell and taste. Symptoms such as discharge and nasal blockage are more easily objectified by parents. This can lead to an overestimation of these objective symptoms and an underestimation of less obvious symptoms[24]. Previous studies report that symptoms differ with age and that younger children with CF tend to complain more about nasal obstruction and discharge, as opposed to adolescents and adults who report headache more often[25,26]. This pattern is not seen in our population.

Unfortunately, in the beginning of the study a different routine in one microbiological laboratory resulted in 13 upper airway and 10 lower airway cultures that were only examined on the presence of *P. aeruginosa*. This resulted probably in an underestimation of other common microorganisms. Previous literature however, confirm that *S. aureus*, *P. aeruginosa* and *Haemophilus influenzae* are the most common cultured microorganisms from the upper airways in children with CF[27,28]. This is in line with the results of this study.

The accent of this article is on the imaging of the paranasal sinuses and the microbiology is addressed lightly. Since 2009 an increasing amount of articles showed evidence for similarity between micro-organisms in the upper airways and micro-organisms in the lower airways of patients with CF[12,29]. One study showed evidence for infection starting in the upper airways and subsequently progression to infection of the lower airways[2]. Eradication of pathogens in the paranasal sinuses is possible and results in better pulmonary condition[4]. With this fundament in mind it is important to know when to start searching for pathogens in patients with CF, especially in children.

In the Netherlands children with CF are generally examined for sinonasal pathology every year and from 4 years of age. This examination often comprises history taking and examination of the ears, nose and throat including rhinoscopia anterior. Upper airway cultures and nasal endoscopy are often omitted in children. Results of the present study however, show substantial opacification in children from 0 to 5 years of age. On the contrary this was not seen in the control group without CF and of the same age. This finding was surprising, because the literature suggests that children often show opacification on CT scans of the sinuses. Approximately 6–8 episodes of common colds in a year, with opacification lasting up to 4 weeks for a common cold will result in a high prevalence of opacified sinuses in children[30,31]. However, results of this study did not support this. This could mean that the CT scans of CF children are even more different from their age matched controls, than originally was thought. The authors feel that the results advocate for more extensive examination from birth. This could be every year for children with mild CF and every half a year for children with severe CF. Ideally the examination will consist of questionnaires, since 10% of patients with CF are tend to report complaints themselves[32]. Furthermore, there are reasons to include nasal endoscopy and upper airway cultures in the regular examination, since the paranasal sinuses can function as bacterial reservoir and contaminate the lungs. Moreover, the authors feel that when a pulmonary exacerbation doesn't react to the general treatment with for

example antibiotics and corticosteroids, it could be considered to refer a patient to the ORL department for examination.

In conclusion, this observational study is one of the few studies to include children with CF of 0–4 years. It shows substantial opacification in CF patients from birth until 17 years of age and this opacification was significantly higher compared to age matched children without CF. Moreover, the degree of opacification in patients with severe CF was significantly higher compared to subjects with mild CF. Abnormalities indicating a chronic course of rhinosinusitis were found in the study group as early as 3–5 years. Cultures from the upper airways in children with CF showed predominantly *S. aureus*, *H. influenzae* and *P. aeruginosa*. When questioned with questionnaires children and their parents report symptoms of rhinosinusitis, predominantly nasal obstruction and posterior/anterior nasal discharge. The authors feel these results advocate for regularly monitoring of the sinonasal system of children with CF from birth, especially children with severe CF.

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Chapter 6

Systemic absorption of nasally administered tobramycin and colistin in patients with Cystic Fibrosis

M.C. Berkhout, A.J. van Velzen, D.J. Touw, B.M. de Kok, W.J. Fokkens, H.G.M. Heijerman

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6 Systemic absorption of nasally administered tobramycin and colistin in patients with Cystic Fibrosis

ABSTRACT

Objectives

In Cystic Fibrosis (CF) patients the paranasal sinuses can constitute a niche for bacteria, which can migrate to the lungs. Nasal administration of antibiotics may be effective, but safety of this treatment has to be established first. The objective of this study was to investigate the systemic absorption of nasally administered tobramycin, colistin (administered as colistin sulfomethate sodium; CMS) and a combination of both drugs using systemic absorption as surrogate for safety. In addition, tolerability of the nasal irrigations was examined.

Methods

Ten adult CF patients performed three different nasal irrigations: 300 mg of tobramycin; 160 mg of CMS; and 300 mg of tobramycin combined with 160 mg of CMS. Serum concentrations of tobramycin and colistin A and B (the main components of colistin) were analysed. Tolerability was measured using a visual analogue scale. Dutch Trial Register: NTR 4008.

Results

Following the tobramycin and the combined irrigation, only two patients had detectable tobramycin serum levels, with the highest being 0.054 mg/L. Serum levels of colistin A and B were not detectable. All three nasal irrigation solutions were well tolerated with a higher tolerability for CMS compared with tobramycin.

Conclusions

Nasal irrigations with tobramycin, CMS and a combination of tobramycin and CMS resulted in safe serum levels and were well tolerated.

INTRODUCTION

Cystic Fibrosis (CF) is a genetic disorder affecting multiple organs. The most important cause of morbidity and mortality in CF patients is infection of the respiratory tract[1]. Treatment of CF patients focuses mainly on preventing or controlling lung infections with intravenous and inhaled antibiotics[2].

Similar microorganisms have been cultured from the upper and the lower airways[3,4]. Previous research has shown that *Pseudomonas aeruginosa* was cultured from the upper airways in 48%–57% of CF patients[3,5]. Infections in the paranasal sinuses can result in rhinosinusitis accompanied by complaints such as headache, blocked nose and loss of smell[6]. These complaints often compromise a patient's quality of life. Even more important, recent studies have shown that the paranasal sinuses can constitute a niche for *P. aeruginosa*, which can intermittently spread to the lungs and initiate or facilitate chronic lung infection[7–9]. Unfortunately, accurate treatment against pathogens in the upper airways is not available yet.

Tobramycin and colistin are the most frequently used antipseudomonal antibiotics for lung infection in patients with CF and therefore seem good candidates for local treatment of *P. aeruginosa* in the upper airways. Also, combining tobramycin and colistin might be better than single drug therapy[10]. Herrmann et al.[11] have shown that inhalation of this combination was more efficient than single tobramycin or colistin inhalation therapy in killing *P. aeruginosa* in biofilms in vitro and it also reduced *P. aeruginosa* cell counts significantly in rats and CF patients.

The present study is the first study to examine the safety of nasally administered tobramycin and colistin sulfomethate sodium (CMS). In addition, the tolerability of nasal irrigation with antibiotics was examined.

METHODS

The study protocol was conducted in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee South-West Holland. Informed consent was obtained from all subjects. From June 2013 to January 2014, adult patients (≥18 years) treated in the Cystic Fibrosis Centre of Haga Teaching Hospital, The Netherlands with a diagnosis of CF, based on a positive sweat test and/or genotype, were considered eligible for this study. Impaired renal (estimated glomerular filtration rate <50 mL/min) and liver (aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, lactate dehydrogenase and/or alkaline phosphatase ≥3× normal value) function, acute pulmonary exacerbation,12 allergy or intolerance for aminoglycosides or polymyxins,

recurrent epistaxis, ear/nose/sinus surgery 3 months prior to study entry and/or participation in another clinical trial 30 days prior to study entry were exclusion criteria.

Dutch Trial Register: NTR 4008.

Investigational drugs
In the present study, the investigational drugs were tobramycin (TOBI®, Novartis) and CMS (Colistin®, Forest Laboratories). Tobramycin peak serum concentrations >30 mg/L and trough levels >0.5 mg/L must be avoided.13 For colistin, toxic levels are not known.

Nasal irrigation
Nasal irrigation of the drugs was performed using a plastic squeeze bottle (NeilMed® Sinus Rinse™).

Subjects had to bend forward above a sink and slightly tilt their head in one lateral direction. The bottle was placed against the most superior nasal passage and the subject had to hold their breath. The bottle was squeezed until the solution started draining from the opposite nasal passage. When half of the bottle was empty, the procedure was transferred to the other nasal passage. The irrigation time was ~ 1 min.

Three irrigation solutions were used: (I) 300 mg of tobramycin; (II) 160 mg of CMS; and (III) 300 mg of tobramycin plus 160 mg of CMS. Approximately 214 mL of isotonic saline was added to all three drug solutions.

Subjects who were on tobramycin or colistin inhalation therapy had to stop this therapy ≥120 h before nasal irrigation was applied. Intravenous tobramycin or colistin therapy had to be terminated ≥48 h before nasal irrigation.

Subjects performed two irrigations with each solution with a time gap of 24 h between the two irrigations. One blood sample was taken just before the second irrigation and five more blood samples were taken at 0.5, 1, 2, 4 and 6 h after the second irrigation. The squeeze bottle was weighed before and after each irrigation to calculate the exact amount of solution that was administered to the nose.

Drug analysis

All tobramycin and colistin analyses were performed by the clinical pharmaceutical and toxicological laboratory of the Central Hospital Pharmacy, The Hague, The Netherlands. Drug serum concentrations were measured using validated HPLC–tandem mass spectrometry (MS/MS) assays[14,15].

For tobramycin, the lower limit of quantification (LLOQ) was 0.015 mg/L, the intra-assay coefficient of variation was 2.0% and the assay was linear from 0.25 to 5.00 mg/L. Colistin consists of multiple components, with the two main components colistin A (polymyxin E1) and colistin B (polymyxin E2) accounting for >75% of the compound. Since colistin was administered as CMS, which is hydrolysed in vivo into colistin, colistin A and B serum concentrations were measured before and after hydrolysis and CMS was indirectly determined. The LLOQ was 0.010 mg/L for both components while the intra-assay coefficient of variation was 6.1% and 5.2% and the assay was linear from 0.010 to 0.46 mg/L and from 0.010 to 0.34 mg/L for colistin A and B, respectively.

Tolerability

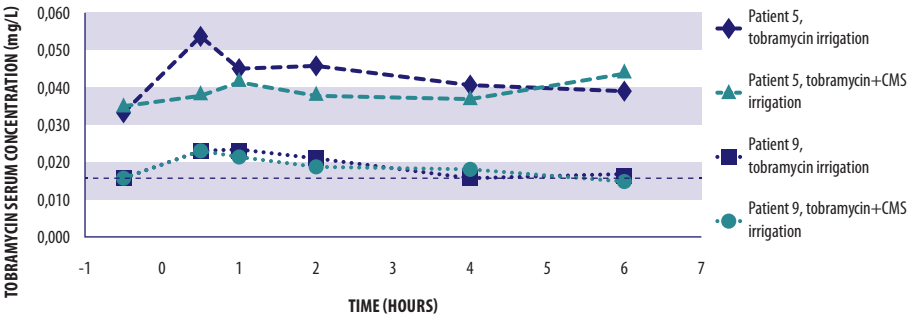
Subjects' tolerability of nasal irrigation was measured using a visual analogue scale (VAS), in which a score of 0 stood for 'no inconvenience' and a score of 10 stood for 'as much inconvenience as I can imagine'.

RESULTS

Ten patients were enrolled in the study with a mean age of 33.5±9.4 years and 50% were male. Seven out of 10 subjects reported at least one sinus surgery in the past.

All patients completed the tobramycin irrigations. The mean administered dose of tobramycin was 280.65±5.54 mg. Only two patients, Patients 5 and 9, had detectable tobramycin serum levels (Figure 1). Peak and trough serum concentrations were 0.054 and 0.034 mg/L for Patient 5 and 0.023 and ≤0.015 mg/L for Patient 9, respectively. The mean VAS score of all 10 patients for the tobramycin irrigations was 2.1±2.62.

Figure 1.



Tobramycin serum concentration–time curves for Patients 5 and 9 following nasal irrigation of 300 mg of tobramycin and 300 mg of tobramycin combined with 160 mg of CMS. The LLOQ was 0.015 mg/L for the tobramycin assay (broken horizontal line at 0.015 mg/L).

All patients completed the irrigations with CMS. For CMS, a mean of 151.20±3.44 mg was administered. In all 10 patients the serum levels of colistin A as well as colistin B were below the LLOQ of 0.010 mg/L. The mean VAS score for nasal administration of CMS was 0.12±0.13.

One patient could not stop his colistin inhalation therapy because he was clinically unstable; therefore, we collected data from nine patients for the combined irrigation. The mean administered doses for these nine patients were 284.09±15.61 mg of tobramycin and 154.40±2.72 mg of CMS. In the same two patients as with the single tobramycin irrigation, serum levels of tobramycin were above the LLOQ following the combined irrigation (Figure 1). Tobramycin peak and trough serum concentrations were 0.044 and 0.035 mg/L for Patient 5 and 0.023 and ≤0.015 mg/L for Patient 9, respectively. The mean VAS score for irrigations with a combination of tobramycin and CMS was 0.50±0.87.

DISCUSSION

To our knowledge, this is the first reported study in which the systemic absorption of tobramycin and colistin after nasal irrigations with tobramycin and CMS solutions was studied. Only 2 out of 10 patients had detectable, though very low, tobramycin serum levels, which were well below the toxic limits. Serum levels of colistin A and B, the two main components of colistin, were below the LLOQ for all patients.

Potential pathways of systemic absorption following nasal irrigation are absorption through the nasal mucosa, via the gastrointestinal tract after swallowing the irrigation solution or through the middle ear mucosa[16]. However, tobramycin and colistin are not effectively absorbed by the gastrointestinal tract[17,18] and research showed no nasal irrigation fluid in the middle ear, suggesting that the systemic uptake of the nasally administered antibiotics is through the nasal mucosa[19].

The two patients with detectable tobramycin serum levels showed similar levels after irrigation with solely tobramycin and with the tobramycin/CMS combination. This suggests that no interaction in drug absorption between colistin and tobramycin exists, which is advantageous since combined administration might be more effective in killing *P. aeruginosa*[13].

Tobramycin was less tolerated by the study population compared with CMS. However, this result could be limited because of the lack of a randomization scheme in the order of the different irrigations. Habituation to irrigation in general could lead to lower VAS scores as the study proceeds. However, the mean VAS score of the combination solution, which was administered last in the study, was higher (VAS=0.50) than the VAS score of the CMS solution (VAS=0.12).

In conclusion, systemic absorption of tobramycin was very low with non-detectable serum levels or levels well below the toxic or therapeutic levels. Nasal irrigation with CMS and with a combination of tobramycin and CMS resulted in non-detectable colistin A and B serum levels. Overall, nasal irrigations were well tolerated. However, patients tolerated the CMS irrigations the best and the tobramycin irrigations the least. Further research into the effects of nasal antibiotic irrigations on bacteria in the sinonasal area, on the symptoms of sinonasal disease and on pulmonary disease in CF patients is needed.

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Chapter 7

Ivacaftor and sinonasal pathology in a Cystic Fibrosis patient with genotype deltaF508/S1215N

C.L. Vreede, M.C. Berkhout, A.J. Sprij, W.J. Fokkens, H.G.M. Heijerman

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7 Ivacaftor and sinonasal pathology in a Cystic Fibrosis patient with genotype deltaF508/S1215N

ABSTRACT

In patients with Cystic Fibrosis and a type III mutation, ivacaftor (Kalydeco®, Vertex) can increase the opening time of the CFTR channel and improve chloride transport. Research showed significant improvement of lung function and increase in weight following ivacaftor use. However, ivacaftor showed to have adverse events on the sinonasal system as well, such as upper respiratory tract infections, nasal congestion and headaches.

This case report showed a positive effect of ivacaftor on the sinonasal pathology in a 17 year old patient with CF. After 5 months of ivacaftor use, the CT-sinus showed complete resolution of the opacification of the paranasal sinuses and a decrease in symptoms of sinonasal disease. This positive effect of ivacaftor on sinonasal pathology seems promising, therefore more research is needed to evaluate the effect of ivacaftor on the upper airways in CF.

INTRODUCTION

Since the discovery of the Cystic Fibrosis (CF) gene in 1989, attempts have been made to develop a new therapeutic approach by targeting the underlying CFTR protein defect. Ivacaftor (Kalydeco®, Vertex Pharmaceuticals) is the first of a new class of drugs known as CFTR protein potentiators. This drug is functional in "CFTR gating" or type III mutations, in which a dysfunctional CFTR protein is present at the apical membrane. Ivacaftor facilitates improved chloride transport by increasing the opening time of the CFTR channel[1].

Research has shown that treatment with ivacaftor can significantly improve lung function (FEV1 % predicted) by about 10%[1]. Other effects described were decreased pulmonary exacerbation rate and increased weight in patients with CFTR gating mutations[1]. Besides these promising improvements, adverse events of ivacaftor have been reported as well. Adverse events included upper respiratory tract infections, nasal congestion and headaches[2].

Here we report the positive effect of ivacaftor on chronic rhinosinusitis in a patient with CF.

CASE REPORT

A 17 year old female patient with CF was included in a compassionate use programme for treatment with ivacaftor. During this programme an unexpected clinical improvement of sinonasal pathology was observed.

She was diagnosed with CF shortly after birth. CF genotype showed a heterozygous deltaF508/S1251N mutation. The deltaF508 mutation is a class II mutation, which leads to the absence of CFTR protein on the apical membrane. The S1251N mutation is a class III mutation resulting in a defective CFTR protein on the apical membrane. Her condition remained clinically stable until in 2008 deterioration in both health and lung function occurred. Her predicted FEV1 declined from 80% in 2009 to 36% in 2013. During this period she had been hospitalised 17 times due to pulmonary exacerbations. She was chronically infected with strains of *Pseudomonas aeruginosa* and Nocardia. Due to insufficient pulmonary function a pre-transplant evaluation was started.

In addition to her pulmonary symptoms, she chronically suffered from headaches and nasal obstruction, most likely caused by chronic rhinosinusitis. During these chronic complaints of rhinosinusitis two CT-sinuses were performed in 2011 and 2012, (Fig. 1A and 1B), showing opacification of all paranasal sinuses, and thereby confirming the diagnosis of chronic rhinosinusitis. From 2011 nasal irrigations with saline and nasal steroids were initiated. Since one month, xylitol was added to the nasal irrigation solution.

Figure 1.

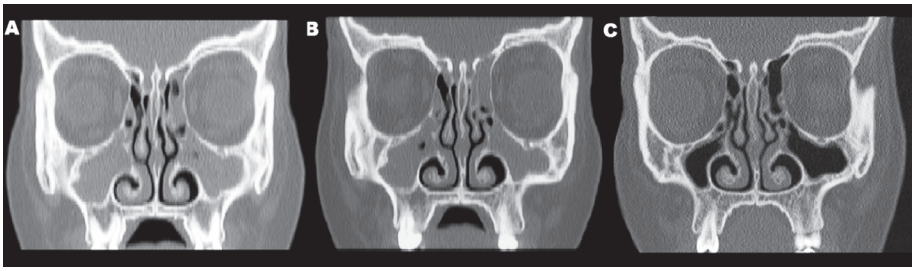

Figure 1 a and b: Coronal CT-sinus in 2011 (a) and 2012 (b) showing opacification of all shown paranasal sinuses (maxillary and ethmoid sinuses). c: Coronal CT-sinus in 2014, five months after treatment with ivacaftor was started, showing no opacification of all shown paranasal sinuses (maxillary and ethmoid sinuses).

Genistein and Curcumine were prescribed in July 2013, ivacaftor was started in October 2013 in compassionate use, as it is currently not registered for this mutation in Europe. Following the introduction of ivacaftor she did not have any pulmonary exacerbation in the past five months and FEV1 increased to 73% predicted. Lower airway cultures after the initiation of ivacaftor showed no change in *P. aeruginosa* and Nocardia colonization. Her sinonasal symptoms were investigated using a Visual Analog Scale (VAS) from 0 to 10 and showed a VAS-score of 3.9 for headache and 1.5 for nasal obstruction. After the initiation of ivacaftor no new therapy was started.

In April 2014, she was invited to participate in a clinical trial studying sinonasal pathology in children with CF, during which a CT-sinus was made. Whereas both CT-scans in 2011 and 2012 showed opacification of all paranasal sinuses, the current CT-scan showed complete resolution of the opacification of all sinuses (Fig. 1C).

DISCUSSION

This is the first report describing the positive effect of ivacaftor on sinonasal pathology in patients with CF. In contrast to the adverse upper airway events in patients using ivacaftor reported by Ramsey and colleagues, the use of ivacaftor in this patient resulted in a complete resolution of the opacification of the paranasal sinuses and a decrease in symptoms of sinonasal disease[2].

The sinonasal system is gradually receiving more attention in the treatment of the multi-organ disease CF. Previous studies have shown a prevalence of rhinosinusitis ranging from 63 to 100% in patients with CF[3,4]. This rhinosinusitis is often chronic and recalcitrant. Current therapies frequently fail to improve the pathology[3]. Moreover, paranasal sinuses potentially constitute a protected niche of adapted clones of *P. aeruginosa*, causing subsequent chronic lung infections[5].

More research is needed to evaluate the effect of ivacaftor on the paranasal sinuses in patients with CF. However, the evident improvement of the sinonasal pathology in this case report appears promising.

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Chapter 8

Summary

8 Summary

This thesis describes the pathology of the mucosa of the nose and the paranasal sinuses in patients with Cystic Fibrosis (CF). CF is a genetic disorder with a high morbidity and mortality in relatively young patients. The genetic defect encodes for a defective Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein, a chloride channel present in many epithelial cells. As a consequence, the secretions of these epithelial cells are higher in viscosity compared to patients without CF. This leads to different pathology in different organs: lung infection and bronchiectasis, pancreatic insufficiency, intestinal obstruction, biliary cirrhosis and absence of the vas deferens. Often these disorders co-exist. Surprisingly, pathology of the upper airways (UAW) in patients with CF has received little attention from researchers in the recent decades. This changed approximately 20 years ago with the gradual acknowledgement of the impact of infections of the nose and the paranasal sinuses on quality of life and on pulmonary pathology.

Over the last four decades the life expectancy of a patient with CF has increased enormously from approximately 10 to 40 years of age, due to the introduction of several innovative therapies. Improvements in life expectancy have allowed a shift in focus to quality of life factors and disorders impacting this.

One of the factors impacting quality of life in CF patients is sinonasal pathology. This thesis explores sinonasal pathology in cohorts of adults and children with CF. In chapter 2 to 4 different aspects of sinonasal disease in a group of 104 adult patients are described.

Chapter 2 focuses on the microbiology of the UAW, its correlation with the microbiology of the LAW and with clinical characteristics of sinonasal disease. Cultures from the UAW of CF patients were collected by nasal lavage and middle meatal swabs. Because no consensus is defined on the optimal culturing method of the UAW, these two methods were both performed to allow comparison. Although the results of both techniques did not diverge materially, they were slightly in favour of the endoscopically guided swabs from the middle meatus. Cultures from the UAW showed a great diversity in microorganisms, with *Pseudomonas aeruginosa* as the most prevalent in 48,1% of the patients. The outcome of the UAW cultures was also compared with cultures from the LAW. Similarity was determined in 50% of the patients with a positive culture from the UAW. Importantly, in 3 patients *P. aeruginosa* was cultured from the UAW after treatment which had previously been thought to have eradicated this pathogen

from the. This finding could suggest persistence of *P. aeruginosa* in the UAW after eradication therapy, indicating the importance of investigating and treating this compartment of the airways during such therapy.

Other aspects of sinonasal disease in adult patients with CF are described in **Chapter 3**. The same study group as in chapter 2 was used. In each patient a disease specific quality of life questionnaire (RSOM-31), nasal endoscopy and a CT scan of the paranasal sinuses were performed. With these instruments the prevalence of rhinosinusitis according to the EPOS guideline was determined. The prevalence of rhinosinusitis in this group was 63% and the prevalence of nasal polyps was 25%. Since a variety in mutations in the CFTR gene, cause a heterogenic range of phenotypes, the study group was divided into two groups with severe CF mutations (class I-III) and mild CF mutations (class IV-V). No difference was present in the prevalence of rhinosinusitis or nasal polyps. However, the characteristics on the CT scan did show differences between patients with severe CF and mild CF. Patients with class I-III mutations had significantly smaller frontal sinuses, sphenoid sinuses, more opacification in the sinonasal area and more often osteitis/neoosteogenesis of the maxillary sinus wall compared to patients with class IV and V mutations. Results from the RSOM-31 indicated that despite considerable sinonasal pathology, patients do not consider these problems to be particularly troublesome, relative to other symptoms.

An unexpected finding on the temporal bones of patients with CF is presented in **Chapter 4**. Through examination of the CT scans of the paranasal sinuses of patients with CF, we observed increased pneumatization of the temporal bones in patients with CF. Since the paranasal sinuses and the temporal bones both are subjected to pneumatization during childhood, one might have expected decreased pneumatization of the temporal bones. In chapter 4 the temporal bone pneumatization is investigated in the study population of 104 adult patients with CF and this pneumatization is correlated with genotype and paranasal sinus volume. Whereas paranasal sinus pneumatization is correlated with CF genotype among other influencing factors, no correlation between temporal bone pneumatization and CF genotype was seen in this study. Moreover, temporal bone pneumatization was not correlated to paranasal sinus volume. Hypothetically, in CF pneumatization of the temporal bone is caused by other influences than paranasal sinus pneumatization.

Because the results of our studies in adult patients with CF showed extensive pathology and reflected the chronic nature of sinonasal disease, information on the onset of sinonasal disease and the extent of pathology in children with CF became important to us. Extant literature was inconclusive and did not include children from birth. In **Chapter 5** we present an observational, cross-sectional study in 58 children with CF. Results showed that children and their parents most often reported nasal obstruction and posterior/anterior nasal discharge as symptoms of sinonasal disease. Cultures showed that *Staphylococcus aureus*, *Haemophilus influenzae* and *Pseudomonas aeruginosa* were the predominant microorganisms in the UAW of children with CF. CT scans showed substantial opacification from birth until 17 years of age. To account for the normal recurrent UAW infections in children without CF an age-matched control group was included in the study. This group consisted of children in which a MRI scan of the head was performed for other reasons than rhinosinusitis. Opacification of the paranasal sinuses was evaluated and compared with opacification on the CT scans of the patients with CF. This showed significantly more opacification in the patients with CF. The study group was also divided into two groups based on their CF genotype. The degree of opacification was found to be significantly higher in children with severe CF (class I-III mutations) compared to children with mild CF (class IV-V mutations).

In chapter 2 to 5 the importance and the extent of sinonasal disease in patients with CF is addressed. To date, unfortunately, no accurate treatment for sinonasal disease is available. Many options exist, ranging from topical medication to surgery. However, none of these therapies is sufficient to adequately treat rhinosinusitis with or without nasal polyps. In **Chapter 6** we explore the safety of another treatment option; topical antibiotics. The safety of nasally administered antibiotics had never been investigated in extant literature. Our study on the systemic absorption of nasally administered tobramycin, colistin and a combination of tobramycin and colistin in 10 adult patients is described. Systemic absorption is taken as a surrogate for safety and analyses showed very low values of tobramycin serum concentrations in only two of the 10 patients following the tobramycin and the combination irrigations. The detectable tobramycin serum levels were 0.054 mg/L, which is far below the toxic serum levels. Serum levels of colistin were undetectably low after the colistin and the combined irrigations. The study shows that tobramycin, colistin and a combination of tobramycin and colistin can be safely administered to the nose in patients with CF. Moreover, the irrigations were tolerated well by all patients.

8 Summary

In the course of the research outlined in this thesis, several new drugs targeting the molecular defect in CF were introduced. One 17-year-old girl who participated in the study described in chapter 5 used one such drug during our study (ivacaftor, a CFTR protein potentiator). In type III mutations this drug facilitates improved chloride transport by increasing the opening time of the CFTR channel. The girl we describe in the case report in **Chapter 7** carries one type II mutation and one type III mutation. Besides an increase in lung function after 5 months of ivacaftor use, she also showed a remarkable improvement in her symptoms and signs of sinonasal disease. A CT scan of her paranasal sinuses showed complete resolution of opacification of the paranasal sinuses. We believe these new drugs to be promising in the treatment of sinonasal disease in patients with CF and consider them worthy of further investigation.

8 General discussion and future perspectives

This thesis describes the pathology of the mucosa of the upper airways (UAW) in patients with Cystic Fibrosis (CF). Analyses were performed in three study groups; one group of 104 adult patients, one group of 10 adult patients and one group of 58 pediatric patients with CF.

Cultures of the UAW in the adult group showed in 64% of the patients pathogenic microorganisms with *P. aeruginosa* being most prevalent. Similarity was seen between the microorganisms cultured from the UAW and the lower airways (LAW) in 50% of the patients with a positive UAW culture. In 3 patients the UAW showed *P. aeruginosa* after a treatment which had previously been thought to have eradicated this pathogen from the LAW.

In the same adult study group, the prevalence of CRS was 64% and of CRSwNP 25%. Patient with severe CF had significantly smaller frontal sinuses, sphenoid sinuses, more opacification in the sinonasal area and more often osteitis/ neoosteogenesis compared to patients with mild CF. Whereas this genotype-phenotype correlation holds for the paranasal sinus pneumatization, the temporal bone pneumatization was not correlated to genotype or to the paranasal sinus volume.

The safety of nasally administered tobramycin and colistin was investigated on 10 adult patients with CF, showing very low to non-detectable serum levels of the antibiotics. Moreover, this treatment was tolerated well by the study group.

The study in 58 children with CF showed abundant opacification of the paranasal sinuses from shortly after birth. This was significantly higher than a control group without CF. Also in children the difference between patients with mild CF and with severe CF was seen in opacification of the sinuses.

A case report showed the positive effect of ivacaftor, a CFTR-correcting drug, on the sinonasal pathology of a 17-year-old girl with CF.

Several subjects for discussion stemmed from these studies, which will be presented below.

CULTURE METHODS FOR THE UAW

In chapter 2 we analysed two methods of culturing the UAW, nasal lavage and a swab from the middle meatus. Our results were slightly in favour of the endoscopic samples targeting for the sinus ostia in the middle meatus. In current literature no consensus on the best technique of culturing microorganisms from the UAW is defined. Different techniques are used, in different study populations and with different aims. Mainz et al. suggested that nasal lavage was the preferable method for sampling the UAW of patients with CF[1]. For this method both nostrils are flushed with isotonic saline and cultures are performed on the fluid that is collected. This is a gentle method, however this does not culture the middle meatus or the sinuses exclusively. Microorganisms cultured from the nasal vestibule show no good correlation with microorganisms cultured from the sinuses[2-4]. Endoscopically guided cultures from the middle meatus and their correlation with the content of the maxillary or ethmoidal sinus, is much better investigated[5-7]. Concordance between cultures from the middle meatus and from the maxillary sinus was present in approximately 75-87% of the cases[6,7]. This is confirmed by Chalermwatanachai and colleagues who showed that the microbiology of the middle meatus correlates well with the pathogenic organism of CRS. In the literature the use of protective devices such as a sterilized Killian nasal speculum with long leaves or a sterile tube around the swab to avoid contamination by the microorganism of the nasal vestibule during insertion and retraction of the swab are suggested[2,8].

Of the above mentioned studies, the only study performed in patients with CF is the study of Mainz and colleagues. The other studies were performed in patients with CRS but without CF. One of the hallmarks in patients with CF is a decreased mucociliary function of the sinonasal mucosa[1,9,10]. This could mean that microorganisms from the paranasal sinuses do not migrate to the functional ostia of the sinuses and thereby a culture from the middle meatus would not be representative. Moreover, when the ostia of the sinuses are obstructed by mucosal edema, microorganisms in the sinuses could differ from those in the middle meatus. On the contrary, microorganisms could be inhaled and be spread throughout the nasal cavity and the paranasal sinuses. Then a culture from everywhere in the nose should be representative. Obviously, the best method to collect microorganisms from the sinuses is a swab or an aspirate from inside the sinuses[10], however this is an invasive and potentially painful procedure.

One can elucidate from these considerations that the best method of culturing the UAW depends on the question that one likes to answer. When the researcher is interested in the microorganisms in the sinuses, a sinus culture is necessary. But, when cross infection and eradication are the main concern, then a culture from the nasal cavity or nasopharynx should be sufficient. With this in mind, in the study which investigated children with CF we chose to perform a swab from the nasopharynx. Especially in very young children a swab from the middle meatus under endoscopic guidance, without anaesthesia would be challenging. Because the potential route of cross infection is via the nasopharynx to the larynx and consequently to the LAW[11-13], we chose to culture the nasopharynx. This could mean that a false positive culture is obtained and treatment for the UAW is started based on a microorganism coming from the LAW. However, no extant literature describes evidence of cross-infection from the LAW to the UAW. In the light of eradication this will not represent a problem and the potential benefit will outweigh any potential harm.

In conclusion, we feel that the purpose of the culture should be allowed to determine the method of culturing the UAW in patients with CF.

TIMING OF CULTURING THE UAW

In the above mentioned paragraph we discussed the method of culturing the microorganisms from the UAW in patients with CF. Another question that rises is when to culture the UAW. In the Netherlands every 3 months the LAW are cultured to explore the microbiome in the lungs of the CF patients. Especially microorganisms such as *P. aeruginosa*, which can cause a rapid decline in pulmonary condition[14,15], are important to identify. When discovered in an early stage, *P. aeruginosa* can be eliminated from the LAW by eradication therapy with systemic and inhaled antibiotics[15-17]. In chapter 2 we cultured *P. aeruginosa* in the UAW of three patients who successfully completed an eradication therapy. All of these patients fulfilled the criteria for eradication, that is three successive negative LAW cultures in 6 months[18]. These findings can be explained in two different ways: *P. aeruginosa* in the UAW can be a new acquisition of the pathogen in the UAW or *P. aeruginosa* in the UAW was never eradicated from this compartment of the airways. Several studies support the latter hypothesis, suggesting a reservoir for *P. aeruginosa* in the UAW[12,19-22]. Persistence of *P. aeruginosa* in the UAW after eradication therapy might lead to cross-infection to the LAW and recurrence of the pulmonary infection[12,23]. To categorize the pulmonary infection with

P. aeruginosa the Leeds criteria were drafted. These criteria are based on lower airway cultures at least every three months:

- never infected: there has never been growth of *P. aeruginosa*.
- non-infected: no growth of *P. aeruginosa* over 12 months.
- intermittent colonization: growth of *P. aeruginosa* in > 0% and 50% of samples.
- chronic infection: growth of *P. aeruginosa* in > 50% of lower-airway samples[24].

In current practice these criteria could also be used to classify infection with other pathogenic bacteria.

Chronically infected patients harbour the pathogen in both the upper and the LAW[10]. In these patients regularly culturing the UAW presumably will not lead to new treatment. However, we feel the patients that are in the never infected, non-infected and intermittently infected groups could benefit from early recognition of CF pathogenic bacteria in the UAW. In these patients for example every three months a swab from the UAW could be performed. Early colonization with microorganisms can be detected and treated. This is also important in patients who have completed an eradication treatment. In these patients the LAW and also the UAW should be cultured to monitor the success of the eradication. Finally, we feel that in patients with a pulmonary exacerbation without proper response to treatment with for example antibiotics and corticosteroids, an UAW culture is justified. In these cases, current cross-infection might cause the persistence of the exacerbation and the patient could benefit from local treatment of the upper airway. In conclusion, we advise to culture the UAW for example once every three months and especially in the following groups of patients:

- patients that were never infected with pathogenic bacteria based on LAW cultures
- patients in which no pathogenic bacteria were cultured over 12 months based on LAW cultures
- patients with intermittent colonization with pathogenic bacteria based on LAW cultures
- patients that have completed eradication therapy for pathogenic bacteria in the LAW
- patients with persistent exacerbation of pulmonary disease without proper response to treatment.

Obviously, this requires good collaboration between the pulmonary and ORL department in hospitals treating CF patients. Indeed, specially trained nurses could help in collecting UAW cultures in CF patients.

ERADICATION OF MICROORGANISMS FROM THE UAW

When pathogenic microorganisms are cultured in the UAW, the challenge of accurate treatment awaits. Eradication of *P. aeruginosa* in the LAW is generally performed with use of systemic ciprofloxacin and nebulized tobramycin or colistin[25]. Given the widely acknowledged reservoir function of the UAW for microorganisms such as *P. aeruginosa*, eradication of pathogens in the UAW is an important goal. A study group in Copenhagen, that performed important research in the treatment of sinonasal infections in patients with CF, showed that eradication of *P. aeruginosa*, *Achromobacter xylosoxidans* and *Burkholderia* multivorans from the UAW is possible[26]. The researchers used the following regimen for eradication:

- functional endoscopic sinus surgery (including uncinectomy, anterior ethmoidectomy, medial antrostomy and often opening of the frontal and sphenoidal sinus)
- 2 weeks of intravenous broad-spectrum antibiotics against expected bacteria
- a minimum of 6 months of topical nasal steroids (mometasone furoate)
- a minimum of 6 months of daily nasal irrigations with saline and colistimethate sodium (when the bacteria cultured were susceptible for this drug)
- regular endoscopic cleansing of nose and sinuses by an otorhinolaryngologist

With this regimen the research team managed to accomplish no regrowth of pathogenic bacteria 6 months postoperatively in 41% of the patients[26].

In the United Kingdom other researchers investigated eradication of *P. aeruginosa* and *Staphylococcus aureus* from the UAW. The eradication regimen for *P. aeruginosa* was:

- 3 weeks of oral ciprofloxacin
- 28 days of nebulized tobramycin via mouth and via nose twice a day (nasal nebulization was performed with a pulsating aerosol, Pari Sinus nebulizer)
- 2 months of nebulized colistimethate sodium via mouth twice a day

And the regimen for *S. aureus* was:

- oral antibiotics for 2 weeks
- mupirocin (nasal ointment) for 5 days

Their results show a success rate of 67% eradication after 3 months of follow-up for both pathogens[27].

A third group from Switzerland performed a study in eradication of *P. aeruginosa* in the UAW of CF patients that underwent lung transplantation. They used the following eradication regimen for the UAW:

- 2 weeks of at least two antibiotics against *P. aeruginosa*
- nebulized treatment with colistin
- sinus surgery (fronto-spheno-ethmoidectomy)
- nasal douching with isotonic saline

With this treatment they achieved eradication of *P. aeruginosa* from the nose and the lungs in 35% of the patients[28].

One case report of Mainz and colleagues described eradication of *P. aeruginosa* from the UAW with 4 weeks of nasally administered tobramycin using the Pari Sinus[29]. After this treatment the patient remained free from *P. aeruginosa* for the follow-up time of 4,5 years.

These studies describe different eradication treatments with different success rates. The research group of Copenhagen suggested failure of eradication of pathogens from the UAW could be due to formation of biofilms on the mucosal lining of the sinuses[30].

The definition of a microbial biofilm is; a structured consortium of microbial cells surrounded by a self-produced polymer matrix[31]. Biofilms can be present inside tissues or secretions or may adhere to surfaces and show increased tolerance to antimicrobial therapy and the host defence[31-33]. Biofilms in non-CF chronic rhinosinusitis are thought to result in recalcitrant disease and to worsen the outcome of sinus surgery[5,34,35].

Several suggested treatments for biofilms in CF are presented throughout the literature[30,34]: topical antibiotics, probiotics, mechanical removal, surfactants, photodynamic therapy, ultrasound therapy and high doses of topical corticosteroids. In treatment of biofilms involved in rhinosinusitis evidence for the efficacy of any of these therapies is limited[34].

Based on this literature Aanaes et al. suggest debridement of the collections of bacteria during sinus surgery and postoperative treatment with intravenous antibiotics for two weeks and nasal irrigations with colistin and tobramycin or ciprofloxacin. In a proposed future study they will examine different routes of nasal administration with different antimicrobial solutions[30].

Another topical treatment with potential benefit in patients with CF could be xylitol. Xylitol is a five-carbon sugar alcohol which is found in plants and can have antibacterial effects. In nasal application it can enhance the innate immune system of a patient by increasing the endogenous antimicrobials present in the airway surface liquid[36]. A study performed in rabbits, showed an increase in bacterial killing of *P. aeruginosa* bacteria after administration of xylitol, compared to saline[37]. In patients with chronic rhinosinusitis sinonasal symptoms also improved after nasal irrigation with xylitol, compared to saline irrigations[38]. We feel that, especially in patients with CF, the topical treatment of chronic rhinosinusitis should be examined extensively because this group of patients often show recurrence of disease shortly after sinus surgery[39,40].

From the above mentioned articles it can be concluded that eradication of pathogenic microorganisms from the sinonasal area is possible, however no optimal treatment has yet been determined. The rationale for adding sinus surgery to eradication treatment is widening the ostia to the sinuses, facilitating nasal irrigation fluid to enter the sinuses[26]. However, this is an invasive treatment and a decision to perform surgery purely based on a positive UAW culture, without the guarantee of eradication could be difficult to justify. In the study of Wilson et al. no sinus surgery was performed, which is in contrast to the other two studies. They achieved a high eradication rate of 67%, however this is just after 3 months and longer follow-up is lacking[27]. Future studies should aim at comparing invasive eradication treatment regimens, including sinus surgery, with non-invasive treatment.

TIMING OF SINUS SURGERY

It is difficult to draft an evidence based statement on the effect of sinus surgery in patients with CF. Since the study groups are often small and include children as well as adults, generalizations are difficult to make. Three reviews have been performed in the last 4 years evaluating intervention studies on sinus surgery in patients with CF until 2014[41-43]. From these reviews it can be concluded that endoscopic sinus surgery in patients with CF improves symptoms of chronic rhinosinusitis and disease specific quality of life. The majority of the data supports a decrease in the number of inpatient hospital days due to sinus surgery. Conflicting data is reported on the effect of sinus surgery on the need for intravenous antibiotics and on pulmonary function tests, with the final conclusion that it has no beneficial effect on these outcome measures[41-43]. Surprisingly,

the studies of the Copenhagen group were not included in these reviews, while they were published in 2013. They show evidence for an improvement of pulmonary disease following sinus surgery combined with antimicrobial therapy as previously described[26,44]. Although a beneficial effect of sinus surgery is underlined by many studies, the risk of recurrent surgery is high in patients with CF[39,40]. From our own studies we have encountered patients that have had more than 20 sinus surgeries in their adult lives. Risk factors for a frequent need of revision surgery in patients with CF are the preoperative extent of polyposis and the radiological extent of disease[40,45]. However, this is contradicted by a study that showed a preoperative difference in CT and endoscopic findings between patients with CRS and CF and patients with CRS without CF, but no postoperative difference in endoscopic findings and quality of life between the two groups[46]. In summary, sinus surgery may improve endoscopic and CT findings and disease specific quality of life. However, CF patients are at risk for recurrent sinus surgery.

Aanaes and colleagues describe a novel purpose of sinus surgery in patients with CF. They suggest that it could facilitate topical treatment of the nasal cavity and paranasal sinuses[26]. Since CF is a disease affecting the mucosa, in theory sinus surgery would not be a permanent solution. After sinus surgery, pathologic mucosa will return in the sinonasal area. Therefore, topical maintenance treatment with for example antibiotics could be a good addition to current therapy. From their studies the Copenhagen group show good results with their regimen[26,44]. However, the timing of sinus surgery is difficult. Aanaes et al. suggest the following indications for sinus surgery[26,30,44]:

- Intermittently colonized patients with increasing frequency of Gram-negative bacteria cultured from the LAW or repeatedly declining lung function (>10% per year), despite antibiotic therapy
- Patients who recently received a lung transplantation
- Patients with severe symptoms of rhinosinusitis according to the EPOS criteria

Surprisingly, the outcome of an upper airway culture is absent in these indications. One could also argue that a positive UAW culture, for example with *P. aeruginosa*, is an indication for eradication, as is a positive LAW culture. If sinus surgery is a part of the eradication treatment of the bacteria from the UAW, then one could argue for sinus surgery following the first positive UAW culture. And is a positive UAW culture the only argument for surgery or should it be accompanied by symptoms of rhinosinusitis?

No comparative studies have been performed between eradication of microorganism with sinus surgery and without sinus surgery. The groups of Mainz and Wilson have demonstrated that eradication is possible without sinus

surgery[27,29]. It is important, but challenging, to perform such studies because it would have serious clinical implications. In theory sinus surgery to widen the ostia and facilitate topical treatment would be superior to topical treatment in non-operated patients. However, since evidence is absent, this question remains unanswered.

ANTIMICROBIAL TREATMENT FOR THE UAW

In chapter 5 we showed how nasally administered tobramycin and colistin do not result in high systemic concentrations in 10 patients with CF. With these results we could conclude that the nasal irrigations with tobramycin and colistin are safe. What we did not investigate was the efficacy of these irrigations.

Many patients with CF receive impressive amounts of antibiotics during their lives. These could be orally administered or intravenously administered. In their article Hansen and colleagues suggest that partially obstructed sinus cavities result in reduced access for systemically administered antibiotics. Using such, sub lethal antibiotic concentrations could lead to the development of antibiotic resistance and, more importantly, lead to the survival of the targeted bacteria in the sinonasal area[12]. Recently Doht and colleagues investigated the effect of systemic antibiotics on the inflammatory markers in the UAW in patients with CF[47]. They showed that intravenously administered antibiotics had a substantially lower effect on the sinonasal inflammatory markers, compared to the inflammatory markers of the LAW. The possible reasons they suggest are the difference in immune response in the UAW versus the LAW. Moreover, they suggest the anatomical character of the paranasal sinuses limits systemic antibiotics to reach the content of the sinus. Because the sinuses are often filled with purulent secretions and/or polyposis, antibiotics cannot reach the centre of the sinus from the mucosa of the lining of the sinus.

For these reasons topical antibiotics could be a good alternative. Among CF patients pulmonary administered antibiotics are an important cornerstone of the antimicrobial treatment. With aerosol antibiotics high concentrations can be achieved in the pulmonary system, without the disadvantage of high systemic levels and their side effects[48-50]. For topical treatment of the nasal cavity and paranasal sinuses the challenge remains getting the antibiotics inside the paranasal sinuses. To overcome this problem, new methods of delivery have been investigated. A pulsating aerosol increases the drug delivery in the

sinuses of healthy controls to 4-6%, compared to no delivery in other delivery methods. In patients with chronic rhinosinusitis limited deposition of the drug into the paranasal sinuses was found before sinus surgery. After sinus surgery this deposition increased to levels comparable with the deposition in healthy controls[51,52].

In chapter 5 we tested nasally administered tobramycin and colistin. For treatment of *P. aeruginosa* these two antibiotics could be combined to create a synergistic effect. Herrmann and colleagues showed that inhalation of this combination was more efficient than single tobramycin or colistin inhalation therapy in killing *P. aeruginosa* in biofilms in vitro[53]. Since nasally administered drugs are relatively new in CF, obviously more research needs to be initiated to identify and gather evidence for optimal nasal treatment.

PNEUMATIZATION OF PARANASAL SINUSES

Research on the paranasal sinuses of patients with CF has shown that pneumatization is often decreased compared to healthy controls. Moreover, some studies display a different pneumatization of the sinuses between patients with CF and patients with chronic rhinosinusitis[54,55]. In our study in chapter 3 we showed that the pneumatization also differs between patients with a mild form of CF and patients with a severe form of CF. Woodworth and colleagues have already demonstrated that pneumatization is correlated to specific mutations in the CFTR gene[56].

The pneumatization mechanism of the paranasal sinuses is still barely understood and it is debated whether it is influenced environmentally or genetically[57]. For a long time, it has been thought to be influenced by the degree of infection in the paranasal sinuses. Chronic rhinosinusitis during development decreases sinus pneumatization, leading to smaller sinuses[58,59]. Considering this hypothesis, one could speculate that patients carrying severe mutations might develop chronic rhinosinusitis earlier in life than patients with mild mutations. Consequently, pneumatization would be reduced or not even initiated, resulting in hypoplastic or aplastic sinuses respectively. Indeed, in chapter 3 we demonstrated that patients with severe CF had smaller frontal and sphenoidal sinuses compared to patients with mild CF. In chapter 6 we studied children with CF and found higher degrees of opacification in children with severe CF compared to children with mild CF. In the study in chapter 3 we demonstrated no difference in

the pneumatization of the maxillary sinuses between the two groups. This may be explained by the timing of pneumatization. The maxillary sinuses pneumatize prenatally, while the frontal and sphenoid sinuses develop after birth. Since rhinosinusitis can only develop postnatally, it can interfere with the pneumatization of the frontal and sphenoid sinuses to a greater extent than with the pneumatization of the maxillary sinus.

The theory of infection impeding pneumatization is contradicted by Chang et al. who state that sinus hypoplasia precedes sinus infection in a porcine model of CF[60]. They suggest a direct influence of the CFTR protein on sinus development. The residual CFTR function in patients with mild mutations as opposed to no CFTR function in the severe mutation group could explain our observation of smaller sinuses in severe CF. Patients with primary ciliary dyskinesia could help in differentiating between these two hypotheses, because they do not reflect a defective CFTR protein, but they do suffer from chronic rhinosinusitis. One study compared the incidence of frontal and sphenoidal hypoplasia or aplasia between patients with primary ciliary dyskinesia and CF and found a significant difference between these two groups[61]. Frontal and/or sphenoidal aplasia or hypoplasia was significantly more pronounced in patients with PCD compared to patients with CF. This could mean that the hypothesis of chronic infection impeding pneumatization may be more accurate. However, this is just one study and more research is needed to thoroughly investigate pneumatization of the paranasal sinuses. Comparison between patients with PCD and CF could be useful in future studies.

DIFFERENCE BETWEEN THE MIDDLE EAR AND PARANASAL SINUSES

To gain more knowledge on the pneumatization process in patients with CF, we investigated another part of the UAW that is subjected to pneumatization, the temporal bone. A few studies on this matter describe an increased pneumatization of the temporal bone instead of a decreased pneumatization in patients with CF compared to healthy controls[62,63]. In chapter 4 pneumatization of the temporal bone in patients with CF was measured and analyzed. No difference was found between patients with severe CF and patients with mild CF. Moreover, no correlation between paranasal sinus pneumatization and temporal bone pneumatization was found.

Our results and the previous described results are quite surprising and no good explanation is found in extant literature. A few hypotheses have been identified, which are described below.

The UAW, including the auditory tube, middle ear and the mastoid cells are lined with respiratory epithelium. This epithelium is ciliated pseudostratified columnar epithelium composed of ciliated cells, goblet cells and basal cells. In theory, defect CFTR protein function results in viscous mucus in the middle ear and the mastoid, causing chronic otitis media. Like the maxillary sinus and the ethmoid sinus, the temporal bone pneumatization starts prenatally, but primarily occurs during postnatal growth. The pneumatization starts at around 2 years of age and is completed between 10 and 15 years of age[64]. Chronic middle ear infections can impede this pneumatization process[65]. Surprisingly, the incidence of otitis media in patients with CF is comparable with patients without CF[66-68].

One explanation for the difference between the temporal bone and the paranasal sinuses, could be found in research describing the histologic characteristics of the auditory tube, middle ear and temporal bone. Todd and Martin studied temporal bone pneumatization (TBP) in patients with CF and in the general population and hypothesize that the mucosa of the auditory tube and the middle ear in CF patients is different in, for example, composition of goblet cells compared to non-CF patients[63]. Yildirim and colleagues performed histologic examination of the temporal bone of patients with CF and found lower densities of goblet cells in the mucosa of the auditory tube and middle ear of patients with CF compared to the middle ear mucosa and auditory tube of a non-CF population[65]. This could explain the low prevalence of ear pathology in CF patients. Additionally, they suggest a role for expression of mucin genes. Mucins are proteins that are produced by epithelial tissues and have the ability to form gel-like secretions. Differences between mucin gene expression in the epithelium of the sinuses and in the epithelium of the middle ear might explain the discrepancy between sinonasal pathology and middle ear pathology in CF patients.

Histologic examination on epithelium of the auditory tube, the middle ear and the temporal bone and comparison with histologic examination of the paranasal sinuses could help in answering this question.

DRUGS TARGETING THE CFTR DEFECT AND SINONASAL DISEASE

In chapter 7 we described the effect of one of the new class of drugs targeting the underlying CFTR protein defect in CF. Many of these drugs have been developed recently and the clinical effects have been investigated with so far promising results[69,70]. Ivacaftor specifically is a drug targeting class III mutations or also called 'gating' mutations. These mutations cause a defective CFTR channel regulation. Ivacaftor facilitates improved chloride transport by increasing the opening time of the CFTR channel[69,70]. Very good results have been observed in many of the studies investigating the effect of ivacaftor in patients with the G551D mutation. Forced expiratory volume increased approximately 17% from baseline, frequency of pulmonary exacerbations decreased and adults report a general improvement in quality of life[71]. Adverse events have been described as well, with upper respiratory tract infections, dizziness, tinnitus and headaches specifically to the UAW[72,73]. However, these adverse events were also described in the placebo group[73]. We report a complete resolution of opacification of the paranasal sinuses and a decrease in symptoms of sinonasal disease after the use of ivacaftor in a patient with CF. This was the first case report describing a positive effect of ivacaftor on sinonasal pathology in a patient with CF. In 2015 a second case report emerged with similar results. Chang and colleagues reported reversed CT findings of CF sinus disease in a patient who used ivacaftor for 10 months[74]. In their case report they describe a patient with mild CF carrying one G551D mutation. The patient had suffered from chronic sinusitis for many years and twice undergone sinus surgery without the desirable effect. After 10 months of ivacaftor use, nasal biopsies showed increased nasal voltage and pH, increased pH of airway surface liquid and decreased viscosity of the airway surface liquid. These results substantiate the clinical findings of decreased opacification on the CT scan and reduced symptoms.

These two case reports are important for the future of the treatment of sinonasal disease in patients with CF. The emergence of new drugs targeting the molecular defect in CF could mean that in the future chronic sinonasal disease no longer exists in patients with CF. When children are accurately treated with these new drugs they might not develop chronic rhinosinusitis and cross-infection between the UAW and the LAW may no longer be an objective for treatment. However, while these new drugs are promising and the arsenal of these drugs is rapidly expanding, today the prevention of chronic rhinosinusitis is not yet possible. Nonetheless, these drugs challenge the current approach of treating sinonasal

disease in CF. For example, it could become important to prevent chronic colonization of the paranasal sinuses in expectation of new drugs that would reverse the mucosal pathology. Since it is not expected that the new drugs will dissolve chronic colonization, eradication of encountered pathogenic micro-organisms in the UAW could become an important objective of therapy. Because of this development, it is very important that studies investigating such new drugs include the UAW. This could be accomplished by including disease-specific questionnaires, UAW cultures, nasendoscopy or even computed tomography of the paranasal sinuses.

In conclusion, we expect the near future to contain many rapid developments leading to an innovative approach to sinonasal disease in CF.

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Chapter 9

Appendices

Summary in Dutch (Nederlandse samenvatting)
Authors and affiliations
Portfolio
Curriculum Vitae
Dankwoord

9 Appendices

NEDERLANDSE SAMENVATTING

In dit proefschrift wordt de pathologie van de slijmvliezen van de neus en de neusbijholten bij patiënten met Cystic Fibrosis (CF) beschreven. CF is een genetische ziekte die gepaard gaat met een hoge morbiditeit en mortaliteit in relatief jonge patiënten. Het genetische defect leidt bij CF patiënten tot een defect Cystic Fibrosis Transmembrane conductance Regulator (CFTR) eiwit, een chloride kanaal in het apicale membraan van epitheliale cellen. Deze epitheliale cellen bevinden zich in exocriene klieren, welke aanwezig zijn in meerdere organen. Het defecte CFTR eiwit heeft tot gevolg dat de secreties van deze epitheliale cellen viskeuzer zijn dan bij mensen zonder CF. In verschillende organen leidt dit tot verschillende afwijkingen: longinfecties en bronchiëctasieën, insufficiëntie van de pancreas, intestinale obstructie, biliaire cirrose en agenesie van het vas deferens. Opvallend is dat de bovenste luchtwegen (BLW) lange tijd weinig aandacht ontvingen in het wetenschappelijk onderzoek bij patiënten met CF, ondanks dat de BLW bekleed zijn met hetzelfde respiratoire epitheel als de lagere luchtwegen (LLW). Ongeveer 20 jaar geleden kwam hier verandering in en werd de rol van sinonasale pathologie op de kwaliteit van leven en op de pulmonale conditie van patiënten met CF steeds duidelijker.

Als gevolg van innovatieve behandelingen is de levensverwachting van patiënten met CF de afgelopen 40 jaar enorm gestegen van 10 jaar tot ongeveer 40 jaar. Dit heeft tot gevolg dat behandelingen niet alleen meer overleving als doel hebben, maar dat ze ook steeds meer gericht zijn op het verbeteren van de kwaliteit van leven.

Sinonasale pathologie is één van de factoren met een negatieve invloed op de kwaliteit van leven van een patiënt. In dit proefschrift wordt sinonasale pathologie bij kinderen en volwassenen met CF onderzocht.
Hoofdstuk 2 tot en met 4 beschrijven verschillende aspecten van sinonasale pathologie bij een groep van 104 volwassenen met CF. **Hoofdstuk 2** beschrijft de microbiologie van de BLW, de relatie tussen de microbiologie van de BLW en de LLW en de relatie met symptomen van sinonasale pathologie. Op dit moment bestaat er geen consensus over de beste methode om micro-organismen in de BLW te kweken. Daarom werd in het onderzoek een uitstrijkje van de middelste neusgang genomen en een neusspoeling verricht. De resultaten van beide kweekmethoden waren licht in het voordeel van de uitstrijkjes van de middelste

neusgang. De kweken uit de BLW toonden een grote diversiteit aan micro-organismen, met *Pseudomonas aeruginosa* als meest voorkomende bacterie (48% van de patiënten). In 50 % van de patiënten met een positieve kweek uit de BLW, werden dezelfde micro-organismen gekweekt uit de LLW. Een belangrijke bevinding was dat bij drie patiënten waarvan werd verondersteld dat *P. aeruginosa* succesvol was geëradiceerd uit de LLW, de BLW kweken positief waren voor deze bacterie. Deze bevinding kan suggereren dat *P. aeruginosa* achterblijft in de BLW tijdens eradicatie therapie en dit benadrukt het belang van onderzoek van de BLW tijdens eradicatie therapie.

In **hoofdstuk 3** worden andere aspecten van sinonasale pathologie bij volwassenen met CF besproken. In dezelfde studie groep van 104 volwassenen werd bij patiënten een ziekte-specifieke kwaliteit van leven vragenlijst (RSOM-31) afgenomen, werd nasendoscopie verricht en werd een CT-scan van de neusbijholten gemaakt. Met deze instrumenten werd vastgesteld dat de prevalentie van rhinosinusitis, gedefinieerd volgens de EPOS richtlijn, in deze groep 63% was en de prevalentie van neuspoliepen 25%. De studie groep werd verdeeld in een groep met ernstige CF mutaties (klasse I-III) en milde CF mutaties (klasse IV-V), omdat bekend is dat bij CF patiënten een genotype-fenotype relatie bestaat. In de prevalentie van rhinosinusitis en neuspoliepen werd geen verschil aangetoond tussen de twee groepen. De CT-scan liet echter wel verschillen zien tussen de groep met ernstige CF en milde CF. Patiënten met klasse I-III mutaties hadden een kleinere sinus frontalis en sphenoidalis, de CT-scan toonde meer sluiering van de neusbijholten en vaker osteitis/neo-osteogenese van de wand van de sinus maxillaris, dan patiënten met klasse IV-V mutaties. Ondanks deze afwijkingen bij objectieve onderzoeken, gaven de CF patiënten weinig klachten van sinonasale pathologie op de RSOM-31 aan.

Hoofdstuk 4 beschrijft een onverwachte bevinding met betrekking tot het os temporale van patiënten met CF. Bij het analyseren van de CT-scans van de neusbijholten, viel een ruime pneumatisatie van het os temporale op. Omdat zowel de neusbijholten als het os temporale op kinderleeftijd pneumatiseren, zou men net zoals bij de neusbijholten, een verminderde pneumatisatie van het os temporale verwachten. In hoofdstuk 4 wordt in de groep van 104 volwassen patiënten met CF de pneumatisatie van het os temporale met de pneumatisatie van de neusbijholten vergeleken. Waar de pneumatisatie van de neusbijholten gerelateerd was aan het CF genotype, was de pneumatisatie van het os temporale bij patiënten met CF niet gerelateerd aan het CF genotype. Daarnaast werd in deze studie geen relatie gevonden tussen de pneumatisatie van de neusbijholten

en de pneumatisatie van het os temporale. Wellicht wordt bij patiënten met CF de pneumatisatie van de neusbijholten door andere factoren beïnvloed dan de pneumatisatie van het os temporale.

De studie bij volwassen patiënten met CF toonde uitgebreide pathologie van de slijmvliezen van de neus en de neusbijholten, vaak met tekenen van een chronisch karakter. Gegevens over het begin van deze pathologie waren echter schaars en beschikbare onderzoeken includeerden vaak geen kinderen vanaf de geboorte. In **hoofdstuk 5** wordt een onderzoek beschreven naar sinonasale pathologie bij 58 kinderen met CF. Kinderen van 0 tot en met 17 jaar werden geïncludeerd en bij hen werd een vragenlijst naar klachten van sinonasale pathologie afgenomen, een neuskweek werd afgenomen en CT-scan van de neusbijholten werd verricht. Neusobstructie en posterieure of anterieure rhinorroe werd het meest gerapporteerd door de kinderen en hun ouders/ verzorgers. *Staphylococcus aureus*, *Haemophilus influenzae* en *P. aeruginosa* waren de meest prevalente micro-organismen uit de BLW van de kinderen. Op de CT-scans werd uitgebreide sluiering van de neusbijholten gezien op de leeftijd van 0 tot en met 17 jaar. Om te corrigeren voor recidiverende BLW infecties met de bijbehorende sluiering van de neusbijholten bij gezonde kinderen, werd gebruikt gemaakt van een controle groep. Deze controle groep bestond uit kinderen zonder CF waarbij een MRI-scans van het hoofd was gemaakt om andere redenen dan rhinosinusitis. Vergelijking van beide groepen toonde significant meer sluiering van de neusbijholten bij de kinderen met CF dan bij de controle groep. Daarnaast werd ook deze studie groep van 58 kinderen verdeeld op basis van hun CF mutatie in ernstige (klasse I-III mutaties) en milde CF. Patiënten met ernstige CF toonden meer sluiering van de neusbijholten dan patiënten met milde CF.

In hoofdstuk 2 tot en met 5 wordt de omvang en de impact van sinonasale pathologie bij patiënten met CF beschreven. Ondanks het feit dat sinonasale pathologie bekend staat om het verslechteren van de kwaliteit van leven en de algemene gezondheid van de CF patiënt, is de optimale behandeling nog niet beschikbaar. Therapeutische opties variëren van nasale medicatie tot chirurgie, maar aanvullende behandelingen worden onderzocht. In **hoofdstuk 6** wordt een nieuwe therapeutische optie onderzocht, namelijk de nasale toediening van antibiotica. De veiligheid van nasaal toegediende antibiotica was tot op heden nooit onderzocht. De studie in hoofdstuk 6 beschrijft de systemische absorptie van nasaal toegediende tobramycine, colistine en een combinatie van tobramycine en colistine bij 10 patiënten met CF. De antibiotica werd toegediend door het toe te voegen aan neusspoelingen met zoutoplossing. In het onderzoek

diende systemische absorptie als surrogaat maat voor veiligheid, waarbij analyses bij slechts 2 patiënten zeer lage spiegels van tobramycine in het serum lieten zien na de nasale applicatie. De detecteerbare spiegels van tobramycine in het serum waren 0.054 mg/L, wat ver beneden de toxische grens is. De spiegels van colistine waren zo laag dat ze niet te detecteren waren. Samenvattend toonde de studie aan dat tobramycine, colistine en een combinatie van tobramycine en colistine veilig kan worden toegevoegd aan spoelwater en toegediend kan worden in de neus. Daarnaast rapporteerden patiënten dat ze de neusspoelingen met antibiotica goed verdroegen.

Tijdens de uitvoering van de onderzoeken die beschreven worden in dit proefschrift, werden nieuwe medicijnen ontwikkeld die aangrijpen op het moleculaire defect bij CF. Een zeventienjarig meisje dat deelnam aan het onderzoek beschreven in hoofdstuk 5, gebruikte één van deze nieuwe middelen (ivacaftor, CFTR potentior). Ivacaftor zorgt ervoor dat het defecte CFTR eiwit langere tijd open blijft, waardoor het chloride transport over de apicale membraan van epitheliale cellen verbetert. Het meisje dat wordt beschreven in **hoofdstuk 7** droeg een type II en een type III CF mutatie. Na 5 maanden van ivacaftor gebruik verbeterde niet alleen haar longfunctie, maar verminderden ook haar klachten en haar symptomen van sinonasale pathologie. Een CT-scan van haar neusbijholten toonde complete opheldering van de neusbijholten, waar eerdere CT-scans nog volledige sluiering lieten zien. Deze nieuwe medicatie is veelbelovend voor de behandeling van sinonasale pathologie bij patiënten met CF en wij adviseren dan ook wetenschappelijk onderzoek te verrichten naar deze therapeutische optie.

AUTHORS AND AFFILIATIONS

- **R.C. Aalbers**, MD. Department of Otorhinolaryngology, Haga Teaching Hospital, The Hague, the Netherlands
- **W.M.C. van Aalderen**, MD, PhD. Department of Pediatric Respiratory Diseases, Emma Children's Hospital AMC, Amsterdam, the Netherlands
- **L.H. el Bouazzaoui**, MD. Department of Pulmonology, Haga Teaching Hospital, The Hague, the Netherlands
- **R.W. Brimicombe**, MD, PhD. Department of Microbiology, Haga Teaching Hospital, The Hague, the Netherlands
- **W.J. Fokkens**, MD, PhD. Department of Otorhinolaryngology, Academic Medical Center, Amsterdam, the Netherlands
- **H.G.M. Heijerman**, MD, PhD. Department of Pulmonology, Haga Teaching Hospital, The Hague, the Netherlands
- **F. Klerx-Melis**, MD, PhD. Department of Radiology, Haga Teaching Hospital, The Hague, the Netherlands
- **B.M. de Kok**, MD. Department of Otorhinolaryngology, Haga Teaching Hospital, The Hague, the Netherlands
- **M. Nuijsink**, MD, PhD. Department of Pediatrics, Haga Teaching Hospital, The Hague, the Netherlands
- **E. Rijntjes**, MD, PhD. Department of Otorhinolaryngology, Haga Teaching Hospital, The Hague, the Netherlands
- **C.J. van Rooden**, MD, PhD. Department of Radiology, Haga Teaching Hospital, The Hague, the Netherlands
- **A.J. Sprij**, MD. Department of Pediatrics, Haga Teaching Hospital, The Hague, the Netherlands
- **D.J. Touw**, MSc, PhD. Department of Clinical Pharmacy and Pharmacology, University Medical Center Groningen, Groningen, the Netherlands
- **A.J. van Velze**n, MSc. Central Hospital Pharmacy, The Hague, the Netherlands
- **C.L. Vreede**, MD. Department of Pulmonology, Haga Teaching Hospital, The Hague, the Netherlands

PORTFOLIO

PhD student: M.C. Berkhout
Name PhD supervisor: Prof. W.J. Fokkens, Dr. H.G.M. Heijerman, Dr. E. Rijntjes

Courses

2011 Basic methods and reasoning in biostatistics (ECTS 1)
2011 Regression analysis (ECTS 1)
2011 Good Clinical Practice (ECTS 0.5)

Presentations

2011 Sinonasal pathology in adult patients with Cystic Fibrosis. *Cystic Fibrosis Young Investigators meeting*. August 2011, Lille, France. (ECTS 0.5)
2012 Sinonasal pathology in adult patients with Cystic Fibrosis. *35th European Cystic Fibrosis Conference*. June 2012, Dublin, Ireland. (ECTS 0.5)
2012 Failure of eradication therapy of *Pseudomonas aeruginosa* in Cystic Fibrosis: Watch the nose. *35th European Cystic Fibrosis Conference*. June 2012, Dublin, Ireland. (ECTS 0.5)
2013 Importance of bacteriology in upper airways of patients with Cystic Fibrosis. *Symposium 'New Scientific Horizons in the Treatment of Cystic Fibrosis'*. March 2013, Barcelona, Spain. (ECTS 0.5)
2014 Sinus and temporal bone in Cystic Fibrosis. *25th Congress of the European Rhinologic Society*. June 2014, Amsterdam, the Netherlands. (ECTS 0.5)
2014 Pharmacokinetics of nasally administered antibiotics in Cystic Fibrosis. *25th Congress of the European Rhinologic Society*. June 2014, Amsterdam, the Netherlands. (ECTS 0.5)
2015 Neusbijholtenpathologie bij volwassenen met Cystic Fibrosis. *227e KNO-vergadering*. November 2015, Nieuwegein, The Netherlands. (ECTS 0.5)
2016 Characteristics of the CF patient. *26th Congress of the European Rhinologic Society*. July 2016, Stockholm, Sweden.

Supervising

2013 B.M. de Kok, 1 month of scientific internship (ECTS 1)
2014 C.L. Vreede, 3 months of scientific internship (ECTS 2)

Awards and Prizes

2015 Poster prize 'Ivacaftor and sinonasal pathology in a Cystic Fibrosis patient with genotype delta F508/S1212N'. *10th symposium of experimental rhinology and immunology of the nose, SERIN 2015*. March 2015, Stockholm, Sweden.

2015 Dr. J.L. Chanfleury van Ijsselsteinprijs 'Cystic Fibrosis en bovenste luchtwegproblematiek'. Hagaziekenhuis, Den Haag, the Netherlands.

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CURRICULUM VITAE

Maaike Berkhout was born in Beverwijk, the Netherlands, on April 16th, 1985. After she graduated the Gymnasium at the Kennemer College in 2003, she started her medical training at the Leiden University Medical Centre in Leiden. During the last months of her medical study she worked at the Otorhinolaryngology department and the Pulmonary department of the Haga Teaching Hospital, The Hague, where she initiated the research in sinonasal pathology in patients with Cystic Fibrosis. In September 2010 she obtained her medical degree and in October 2010 she continued the research in a PhD project (Prof. dr. W.J. Fokkens, Dr. H.G.M. Heijerman and Dr. E. Rijntjes). After 3,5 years of full-time research, she started her training in Otorhinolaryngology at the Academic Medical Centre in Amsterdam (prof. dr. S. van der Baan, prof. dr. W.J. Fokkens and dr. A.M. König). During the first two years of her residency at the AMC she completed this thesis.

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