Monitoring cystic fibrosis lung disease in children

Clinical utility and associations between functional and structural methods

Marcus Svedberg

Department of Paediatrics

Institute of Clinical Sciences

Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG

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marcus.svedberg@vgregion.se

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This PhD thesis is dedicated to my parents, Alf and Gunhild Svedberg. For their endless support and encouragement.

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Department of Paediatrics, Institute of Clinical Sciences
Sahlgrenska Academy, University of Gothenburg
Gothenburg, Sweden

ABSTRACT

Background: Cystic Fibrosis (CF) is an inherited progressive disease that causes severe damage to the airways and other organs of the body. Many methods are available to track CF lung disease but longitudinal data are needed to better understand the clinical utility and associations between different methods.

Objectives: To analyse and compare longitudinal data from lung function tests and image tests in children with CF.

Methods: In **study I** children aged 6–17 years attending Gothenburg's CF clinic underwent multiple breath washout examinations, spirometry tests and a clinical stability assessment every 3rd month over a period of 1 year. Variability of the outcome parameters were analysed in clinically stable patients. In **study II–IV** 75 children aged 0–17 years underwent multiple breath washout examinations, spirometry tests, chest computer tomography (CT) and chest x-ray examinations between 1996–2016 at the Gothenburg CF centre. Longitudinal trends and associations between the outcome measures were analysed together with the effect of respiratory infections and other confounding factors.

Results: A total of 25 children completed a total of 107 visits of which 104 visits had complete data available in **study I**. The relative change in lung clearance index and FEV_1% was +-17% (95th percentile) at clinical stable visits. In **study II–IV** a total of 75 participants with CF were included together with a healthy cohort of 140 children aged 0–17 years. Children with CF underwent lung functions tests and image tests and the healthy cohort only

underwent multiple breath washout examinations. **Study II** demonstrated that intermittent and chronic infections were associated with an increased progression rate of structural lung disease measured with chest CT. **Study III** demonstrated associations between longitudinal lung clearance index and the extent and progression rate of structural lung damage assessed with chest CT. The Lung clearance index was more sensitive than chest x-rays to detect early CF lung disease in **study IV.** The combined results from a normal chest x-ray and a normal lung clearance index were associated with low extent of lung damage assessed with chest CT.

Conclusions: Multiple breath washout is a sensitive method to detect early CF lung disease. Lung function and imagine tests captured different dimensions of CF lung disease. The use of multiple methods in clinical practice provides a more robust assessment of CF lung disease than using either measure alone.

Keywords: children, cystic fibrosis, lung disease

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SAMMANFATTNING PÅ SVENSKA

Bakgrund*:* Cystisk Fibros (CF) är en allvarlig, ärftlig, progressiv multiorgansjukdom som ofta leder till en för tidig död. Barn och ungdomar med CF har ofta symtom från luftvägarna och magtarmkanalen. Främst är det lungsjukdomen som är orsaken till morbiditet och mortalitet hos personer med CF. Lungsjukdomen börjar ofta vid mycket tidig ålder och kan fortskrida utan att en person med CF behöver uppvisa några tydliga symtom som tecken på att lungsjukdomen försämras. För att tidigt upptäcka och förstå hur lungsjukdomen fortskrider över tid genomgår personer med CF regelbundet olika objektiva undersökningar på sjukhuset. Lungfunktionen kan mätas antingen med hjälp av en spirometriundersökning eller inert gasutsköljning. Det vanligaste utfallet vid en spirometri är hur mycket luft en person kan blåsa ut på 1 sekund och det vanligaste utfallet vid en inert gasutsköljning är lung clearance index. Lung clearance index är ett mått på hur bra vi omsätter luften i våra lungor vid vanlig andning (tidalandning). Strukturella skador på lungorna uppstår förr eller senare vid CF och skadorna kan upptäckas vid olika röntgenundersökningar. Vanliga lungröntgenundersökningar är konventionell lungröntgen och datortomografi av lungorna. Vi behöver bättre förstå hur vi kan använda oss av alla dessa metoder för att följa och försöka bromsa lungsjukdomen och hur resultaten från metoderna förhåller sig till varandra.

Syfte: Att analysera och jämföra longitudinella resultat från lungfunktionsundersökningar och lungröntgenundersökningar hos barn med cystisk fibros i syfte att skapa en förståelse över hur CF lungsjukdomen ändras över tid och möjligheter att bromsa sjukdomsprocessen.

Metod: I **studie I** inkluderades barn från Göteborgs CF center som var mellan 6–17 år gamla. Vid varje fysiskt besök på sjukhuset var 3e månad genomförde de en spirometriundersökning och en inert gasutsköljning. Deltagarna skattade också sina luftvägssymtom och genomgick en läkarundersökning vid samma tillfälle. Utfallet från lungfunktionsundersökningarna inert gasutsköljning och spirometri analyserades för att förstå hur resultatet från båda undersökningarna varierade över tid hos individer med CF som är kliniskt stabila. I **studie II-IV** inkluderades 75 barn tillhörande Göteborgs CF center som var 0–17 år gamla. Barnen genomförde regelbundet lungfunktionsundersökningar, lungröntgen- och datortomografiundersökningar av lungorna över en period av 20 år. Lungskadorna som respektive röntgenundersökningarna kunde påvisa omvandlades till siffror motsvarande typen av skada och utbredningen av lungskadan. Resultatet från röntgenundersökningarna analyserades tillsammans med resultatet från lungfunktionsundersökningarna för att förstå

hur lungsjukdomen förändrades över tid mätt med respektive metod. De longitudinella trenderna av sjukdomsförloppet för respektive mätmetod jämfördes med varandra och hur de påverkades av olika infektioner i lungor och andra faktorer för att förstå vad som påverkar progressen av lungsjukdomen.

Resultat: I **studie I** inkluderas totalt 25 barn med CF som genomförde sammanlagt 107 besök under 1 års tid. Variationen över tid för lung clearance index och spirometriutfallet hos barn med CF som bedömdes kliniska stabila var +-17% för båda parametrarna. I **studie II-IV** inkluderades total 75 barn med CF tillsammans med 140 friska barn som endast genomförde en inert gasutsköljning. I **studie II** visade vi att bakterier som infekterade luftvägarna hade stor negativ påverkan på hur lungsjukdomen förändrades över tid. Att använda sig av upprepade mätningar av metoden inert gasutsköljning visade sig i **studie III** kunna prediktera både utbredningen och progressionen av strukturella lungskador mätt med datortomografi. I **studie IV** visade vi att inert gasutsköljning är en känsligare metod än konventionell lungröntgen att upptäcka tidig CF-sjukdom. En kombination av inert gasutsköljning och lungröntgen ökade förståelsen för att uppskatta de strukturella skadorna mätt med datortomografi vid tidig skolålder jämfört med att använda respektive metod var för sig.

Slutsats: Lungfunktionsundersökningar och röntgenundersökningar bidrar med olika sorts information om hur lungsjukdomen vid CF fortskrider. Genom en ökad förståelse om vilka faktorer som påverkar lungsjukdomens framfart och hur resultaten från de olika undersökningarna förhåller sig till varandra, skapar vi en bättre förutsättning för hur vi kan använda oss av olika mätmetoder för att tidigt upptäcka, följa och bromsa CF-sjukdomen.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Svedberg M**, Gustafsson PM, Robinson PD, Rosberg M, Lindblad A. Variability of lung clearance index in clinically stable cystic fibrosis lung disease in school age children. *Journal of cystic fibrosis. 2017:17:236-241.*

II. **Svedberg M**, Gustafsson PM, Tiddens H, Imberg H, Piovic A, Lindblad A. Risk factors for progression of structural lung disease in school-age children with cystic fibrosis. *Journal of cystic fibrosis. 2020;19:910-916.*

III. **Svedberg M**, Imberg H, Gustafsson PM, Tiddens H, Davies G, Lindblad A. Longitudinal lung clearance index and association with structural lung damage in children with cystic fibrosis. *Thorax. 2022 Mar 11;thoraxjnl-2021-218178.*

IV. **Svedberg M**, Imberg H, Gustafsson PM, Mela Brink, Håkan Caisander, Lindblad A. Chest x-rays are less sensitive than multiple breath washout examinations when it comes to detecting early cystic fibrosis lung disease. *Acta Paediatr. 2022;00:1–8.*

CONTENTS

ABBREVIATIONS

Acronym	Definition
BMI	Body Mass Index
CF	Cystic Fibrosis
CFCS	Cystic Fibrosis Clinical Score
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CT	Computed Tomography
CXR	Chest X-ray
FEV_1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
ICC	Intra-class Correlation Coefficient
LCI	Lung Clearance Index
LCI_{adj}	Lung Clearance Index adjusted to age
MBW	Multiple Breath Washout
MRI	Magnetic Resonance Imaging
NS	Northern Score
PEx	Pulmonary exacerbations
PRAGMA-CF	Perth-Rotterdam Annotated Grid Morphometric Analysis
SF_6	Sulphur hexafluoride

1 INTRODUCTION

Cystic Fibrosis (CF) is a chronic life-shortening genetic disease. The disease CF is progressive and most morbidity and mortality comes from damage to the lungs. Although life expectancy has increased over the last decades, many people with CF pass away in young adulthood due to chronic CF lung disease with respiratory failure. In Sweden about 750 individuals are living with CF and of these 750 individuals, 250 of them are children.

The lung disease in CF is characterised by thickened mucus in the airways. The altered mucus in the airways makes a person with CF more prone to infections and chronic inflammation in the lungs. Structural and functional impairments within the lungs of children with CF are detectable soon after birth and at pre-school age most children have pathological findings in the lungs.[1-4] Symptoms from the airways usually begin in early childhood and vary from child to child but the condition sometimes progresses without any symptoms.[5-7] In the early stages of CF lung disease the symptoms are often are mild or absent which is why objective outcome measures that can capture and monitor the progress of lung disease are needed.[8] The optimal strategy for monitoring CF lung disease in children still remains unclear.

There are many different methods available to detect and track CF lung disease. Imaging includes methods such as chest x-ray, computed tomography, chest magnetic resonance imaging (MRI) and functional methods such as spirometry, multiple breath washout (MBW) and oscillometry. The methods spirometry and chest x-ray have been implemented within clinical practice over a long period of time and are still recommended by the CF guidelines to track CF-lung disease.[9-11] As CF care has improved over the years and new effective medicines are becoming increasingly available to CF patients, both spirometry and chest x-ray are considered by many to be insensitive to detecting and tracking early CF disease in children.[12-14]

The challenge is to find a feasible and sensitive objective outcome measure to track and detect early CF lung disease. The method MBW has proven in several studies to be both a more sensitive method than spirometry in detecting early CF-lung disease as well as feasible in all paediatric ages.[4,7,15-17] The method MBW has been used at the Gothenburg paediatric CF centre since 1999 even

though the method was not commercially available until about 10 years ago. The Lung clearance Index (LCI) is the most common outcome measure derived from MBW examination and reflects the total ventilation inhomogeneity of the lungs. LCI is now an established outcome measure in clinical trials in children with CF.[18-27] The clinical use of MBW in clinical practice is limited to a few tertiary CF centres and there are still questions that need to be answered before MBW is ready for use in clinics. More studies are needed to understand how both the short and longer trends of LCI could be implemented into daily clinical practice. At Gothenburg paediatric CF-centre we have used MBW together with other methods over a period of two decades which provides us with the opportunity to study how the longitudinal trends from different outcome measurements are related to each other. All examinations and procedures represent a major burden for the affected child and their families. As health-care providers we must always balance the benefits and risks associated with each procedure and decide whether all the methods, applied separately or in combination, add information about CF-lung disease.

2 BACKGROUND

2.1 CYSTIC FIBROSIS

Cystic Fibrosis is a monogenetic condition caused by a mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene, located on chromosome 7.[28] The *CFTR* gene provides instruction for the *CFTR* protein, a protein involved in maintaining the balance of salt and water of the airways, intestine, and pancreas, among other organs.[29]

Today there are more than 2000 known variants in the *CFRT* gene (http://www.genet.sickkids.on.ca), of which approximately 400 mutations have been confirmed to be disease causing. A loss of CFTR function leads to an accumulation of thick, adherent mucus in mucus producing organs. The mucus stagnation in the affected organs plays a critical role in the disease pathogenesis by compromising airflow and nutrient digestion.

2.2 CF LUNG DISEASE

The lung disease CF is generally characterized by repeated infections and an intense inflammation resulting in a progressive lung disease. The lining of the airways is coated with a thin mucus layer. The properties of the mucin gel lining of the airways are necessary for proper clearance of pathogens.30 The pathophysiological mechanisms of how the CFTR dysfunction causes chronic inflammation in the CF airway is not yet fully understood, but the lack of a functional CFTR causes dehydration of the airway surface liquid and an impaired mucociliary clearance of the airways.[29,31,32] As the mucociliary clearance is an important defence mechanism of the airways, individuals with CF are more prone to infections of the lungs. The inflammatory response to an infection is very intense and prolonged in CF airways.[33] A chronic inflammation of the CF airways is established at an early age, driven by a dysregulated recruitment of immune cells into the airways and repeated airway infections.[34-36] Pulmonary inflammation in children with CF was associated with poor nutritional status and a faster deterioration of the lung disease.[37-39] By regular clinical check-ups and close monitoring of lung structure, lung

function, symptoms and infections, we will get a chance to understand and thus prevent or slow down the progression of CF lung disease.

2.2.1 AIRWAY PATHOGENS

The understanding of how airway pathogens affect the lung disease progression are of great importance as recurrent pulmonary infections are the leading cause of morbidity and mortality in people with CF.[40] Cystic Fibrosis standards of care recommend regular screening of airway pathogens every third month.[11] At Gothenburg paediatric CF centre the aim was to obtain a respiratory secretion sample (sputum sample or laryngeal suction in non-sputum producing individuals) from each CF-patient at least every third month. Bronchoalveolar lavage was only performed on clinical indications and not performed as a surveillance program at our CF-centre as little evidence supports or is recommending of this invasive approach.[41-43]

At the perinatal period the lungs are considered sterile but soon after birth they become colonised with microbes. The airway microbiome changes over time and is dependent on a number of factors, such as genetics, geographic location, age, antibiotic exposure and the presence of other airway pathogens.[44] The complexity of the composition of the airway microbiome and how it affects CF lung disease is far from understood. By getting more insight about how the CF microbiome affects CF lung disease progression, it may help us to develop interventions that can halt or reverse the course of progressive pulmonary damage and improve the quality of life in CF patients.

Staphylococcus aureus is a common and prevalent cultured airway pathogen in children with CF.[45] The pathogenicity of methicillin-sensitive *Staphylococcus aureus* in is still unclear but individuals with CF colonised with methicillin-resistant *Staphylococcus aureus* were more hospitalised and had a faster lung function decline.[46,47]

Pseudomonas aeruginosa is a commonly recognized bacteria associated with chronic lung infection in CF. As colonization with Pa has been associated with a more rapid lung function decline and earlier mortality.[48,49] Therefore, early detection and aggressive antibiotic therapy are advocated to eradicate the organism from the airways.[11]

There are several other bacterial species, such as *Haemophilus influenzae*, *Burkholderia cepacian complex*, *Achromobacter species* and *Mycobacteria* species that can be cultured from CF airways. Viral and fungal infections are

also associated with increased risk of a pulmonary exacerbation as well as lung function decline, but how they impact the CF airway disease is still not well understood.[50-54]

2.2.2 TREATMENT OF CF LUNG DISEASE

Gothenburg paediatric CF has about 65 patients that are regularly assessed every 6 weeks at the CF-centre or at their local hospital. New born screening for CF was (and remains) not implemented within Swedish health care during the study period. The cohorts included in this thesis were born between 1990–2009 and some of the included participants were observed until 2016. As well as improvements and changes in CF therapy over the years, there have also been changes in treatments for these cohorts over time. Mucolytics therapy was normally advocated twice daily followed by airway clearance techniques. Isotone saline (0.9% NaCl) was inhaled in infants and pre-schoolers and at school age hypertonic saline (3–6% NaCl) was introduced. As hypertonic saline (6% NaCl) has been shown to be tolerable in infants and more effective than isotone saline. All patients at our CF-centre now inhale 6% hypertonic saline.[26] Inhalation of dornase alpha (Pulmozyme) hasn't been used on regular basis since it was introduced but has been used as an additive mucolytic therapy if clinically indicated. Both hypertonic saline and dornas alpha have demonstrated an improvement in ventilation inhomogeneity (an improvement in LCI) in school children with CF with normal lung function measured with spirometry.[18,55]

Prophylactic antibiotic (Flucloxacillin) was normally used until the age of 5 years at our CF centre till 2015, and after 2015 prophylactic antibiotic is only prescribed on demand. The evidence as to whether anti-staphylococcal prophylactics therapy was beneficial in infants and pre-schoolers with CF still remains unclear and there is a possibility for a risk of higher prevalence in *Pseudomonas aeruginosa* infections.[56] The first line of treatment for mild pulmonary exacerbation (defined clinically by new or worsening airway symptoms over a shorter period of time) was usually oral antibiotics (e.g., amoxicillin/clavulanic acid or trimethoprim sulphate). The length of the antibiotic treatments was normally 10–14 days and was initiated at home after contact with the CF-clinic or after a visit to the CF clinic. Chronic infections with *Pseudomonas aeruginosa* were treated with a combination of iv antibiotics, inhaled antibiotics, and azithromycin.

Over the last decade *CFTR* modulators have been added to therapeutic regimens.[57] The *CFTR* modulator drug can enhance or partially restore the expression, function, and stability of a defective *CFTR* protein of eligible patients with a specific *CFTR* mutation. Treatment with *CFTR* modulators have proven to provide a positive impact on lung function, ventilation inhomogeneity, quality of life and nutritional status.[23,25,27,58] To date, four *CFTR* modulators exist on the global market and about 85% of Swedish individuals with CF are eligible for *CFTR* treatment. In the cohorts in this thesis a few individuals over a short period of time were treated with *CFTR* modulators.

2.3 MONITORING CF LUNG DISEASE IN CHILDREN

Structural and functional lung impairments in children with CF are detectable soon after birth and progress throughout childhood sometimes without clinical signs or symptoms.[1,59,60] Therefore, objective methods are needed to monitor CF lung disease progression as well as to guide therapies and interventions. Currently, CF centres use different multiple modalities to detect and track CF-lung disease.[61] At Gothenburg paediatric CF centre regular chest CT was added at the beginning of 2000 to chest x-rays to track structural lung disease. MBW together has been used since 1999 together with spirometry to monitor functional abnormalities of CF lung disease over time. There are other objective methods available to monitor CF lung disease but they will not be further discussed in this chapter. Imaging and functional tests of the lungs are regarded complementary markers to detect lung abnormalities in children with CF.[62] It is important for health care providers to understand that all examinations and procedures represent a burden for the affected child, and sometimes the procedures may involve a potential risk for the child.[63,64] Health-care providers must always balance the pros and cons associated with each examination and decide whether the methods, will add substantial information about CF lung disease.

2.3.1 SPIROMETRY

Dynamic spirometry is the most common pulmonary function test in individuals with CF. The CF guidelines recommended CF-centres to perform spirometry every 3rd month when the child is old enough to perform spirometry.[11] Most children can perform a correct spirometry around the age of 6 years. The most commonly used parameters from spirometry are Forced

Expiratory Volume in one second (FEV_1) and Forced Vital Capacity (FVC). The measurements FEV_1, FVC and FEV_1/FVC are considered to primarily capture changes in disease progression of the larger airways.[13] As CF lung disease is believed to start locally in the smaller airways the outcome measurements FEV_1, FVC and FEV_1/FVC are questioned in their ability to detect early CF lung disease.[13,65,66] The spirometry measurements Forced Expiratory Flow, FEF_{25-75} and FEF_{75} are argued to better reflect and capture changes in the smaller airways.[13] The problem with using FEF_{25-75} or FEF_{75} is the high coefficient variability for both measurements and that none of the measurements have proved to offer any significant advantage over the FEV_1, FVC or FEV_1/FVC measurements.[67]

As the pulmonary health has improved over the last decades about 80% of the children with CF (age 6–17 years) in Sweden currently have a lung function ($FEV_1\% \geq 80$) within the normal range.[68] Chest CT and MBW indicate both structural and functional abnormalities present in most of the children with normal lung function. Even though dynamic spirometry is not as sensitive in detecting early lung disease it is still the best validated method to track and evaluate CF lung disease after interventions in children.[69,70] Over the recent years mobile home spirometers are available which gives the CF population new options and possibilities to understand and track CF lung disease in between physical visits. The mean annual progression rate in children with access to good CF care is 1-2 FEV_1%-units.[70] The minimal clinical important change between FEV_1% measurements is still not known, but a decrease in FEV_1% with 10% between visits is often regarded as a sign of an exacerbation.[71]

2.3.2 MULTIPLE BREATH WASHOUT

Multiple breath washout is a lung function test that measures the degree of ventilation inhomogeneity of the patient's airways. Although the method was introduced already back in the 1940s, it didn't gain popularity until the beginning of 2000. Recent advances in technology and equipment have made the method more accessible for both researchers and clinicians. The method has proven to be feasible in all paediatric ages and a sensitive test to detect early CF lung disease.[17,66,72] MBW assesses ventilation distribution by measuring how efficiently the lungs clear a tracer gas during tidal breathing. The most common tracer gases used are nitrogen or sulphur hexafluoride (SF_6).

As SF_6 is an exogen gas it must first be washed-in into the lungs. For the inert gas nitrogen, no wash-in phase is needed. The wash-out phase is performed using 100% oxygen or medical room air until the end-tidal tracer gas concentration reaches 1/40th of its starting concentration (Figure 1 and 2).

Figure 1. Schematic overview of the procedure multiple breath washout using nitrogen (left picture) and sulphur hexafluoride (SF6) as an inert gas (right picture). No washout phase is needed when using nitrogen as an inert gas. Reprinted with permission of the American Thoracic Society. Copyright 2022 ATS. Subbarao et al. Ann Am Thorac Soc. 2017 Jan;14(1):145.

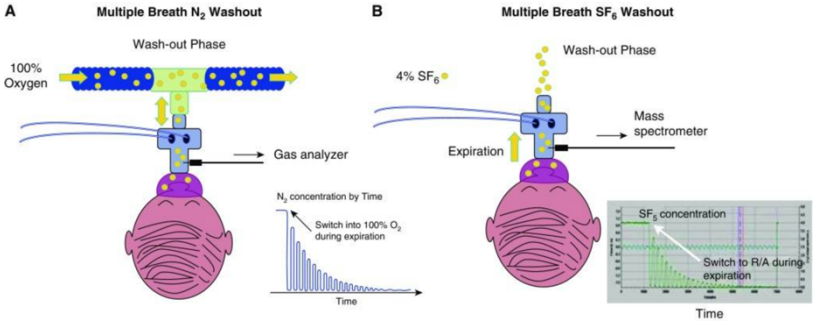
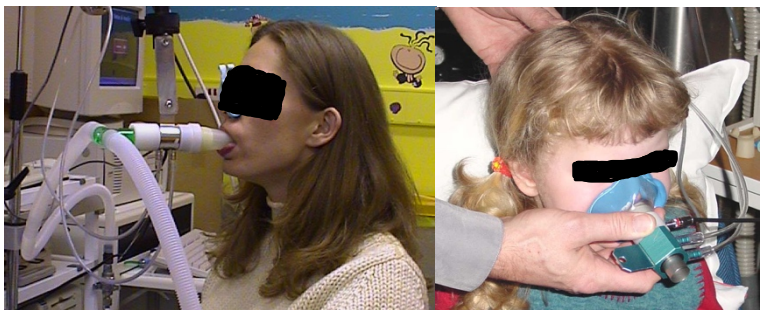


Figure 2. Picture of a young women (A) and a young child underdoing a multiple breath washout examination. Published with permission.



The most common reported outcome measure of the MBW test is lung clearance index (LCI). LCI is calculated as the number of lung volume turnovers required to clear the lung of the tracer gas to a 1/40 of the starting concentration (se equation 1).

Equation 1: $LCI=V_{ce}/FRC$

Equation 2: FRC= net volume of the inert gas exhaled (Cet_{start} - Cet_{end})

V_{CE} is the cumulative net expired volume (sum of tidal breath volumes minus current dead space) during the washout phase. FRC is calculated as showed in equation 2 and Cet is the concentration at end-tidal volume at start (Cet_{start}) and the end (Cet_{end}).

LCI reflects the overall ventilation inhomogeneity of the airways as the LCI results consists of information from the whole airway tree. As the peripheral airways (airway <2mm in diameter) represent about 90% of the total lung volume, LCI is regarded as a measurement primarily of the peripheral airways. An inhaled gas is distributed evenly throughout the lungs in a healthy child. The more obstructed or narrowed the airways are the longer it takes to wash out the tracer gas, which results in an increasing LCI-value. LCI reference values are relative constant throughout childhood except in infants and pre-school children.[73]

The MBW examination has been shown in several studies to be a more sensitive test than spirometry to detect early CF disease.[15,66,74] As CF lung disease is already present at infancy and pre-school ages we need other methods than spirometry to track early CF lung disease. Abnormalities in ventilation distribution and structural lung damage are both present in infants with CF and make it possible to detect and track CF lung disease as well as to intervene.[3,6] There is a correlation between cross sectional LCI and SLD measured with chest CT and the association appears to grow stronger with age.[75] Current opinion is that both methods should be regarded as complementary markers of CF lung abnormalities.[17,75] Regional changes in SLD or ventilation/perfusion might not be captured using a global functional measurement such as LCI. LCI might also not respond adequately to completely obstructed/non-ventilated airways due to severe mucus plugging, whereas other methods might be more sensitive or suitable. Even though chest

CT and MBW are considered complementary methods to track CF lung disease, longitudinal studies are needed to understand the potential use of LCI as a predictor of the extent and the progression of SLD. Several studies have demonstrated that LCI responds to interventions and LCI is today an important endpoint in interventional trials in children with CF.[21,23,26,76] Recent studies have progressed our knowledge of how to use MBW as a monitoring tool for CF lung disease in daily clinical practice.[24,26,77] Important questions that we still need to understand include the clinically important difference between LCI measurements and how to approach longitudinal trends of LCI in clinical practice.

As clinicians, we need to consider that the MBW examination is both time consuming and technically challenging to perform, especially in younger ages. Reducing the treatment burden and time spent at the hospital is a priority for the CF community.[78] This will be a major challenge for the health care providers in terms of balancing the pro and cons with each examination performed. Today, there are commercially available MBW-systems. The MBW-systems uses different inert gases and software, and the LCI results are unfortunately not interchangeable or comparable between MBW equipment.[59,79] Although recent refinements have been made in the MBW software to improve the agreement between different inert gases, the LCI results from different MBW systems are still not comparable.[80,81]

2.3.3 CHEST X-RAY

Chest x-ray (CXR) has been used for more than 50 years to evaluate CF lung disease. [82,83] Several scoring systems have been developed over the years to facilitate the comparison of chest radiographs as well as to objectify the progression of lung disease.[84]

Chest x-rays are still recommended by international guidelines to track early cystic fibrosis lung disease even though more sensitive methods are available.[9-11,85] Computed tomography (CT) of the chest is far more sensitive than CXR to detect early lung disease and also provides a more detailed information of the proceeding structural lung damages.[86] Chest MRI of the lungs has evolved over the last decade and is an interesting alternative to track CF lung disease, but chest MRI examinations are often limited to research settings and will not be discussed in detailed in this thesis.[3,14,87,88]

The advantages of chest x-rays are that the method is accessible world-wide, affordable and easy to use in all paediatric ages. The scoring systems for CXR

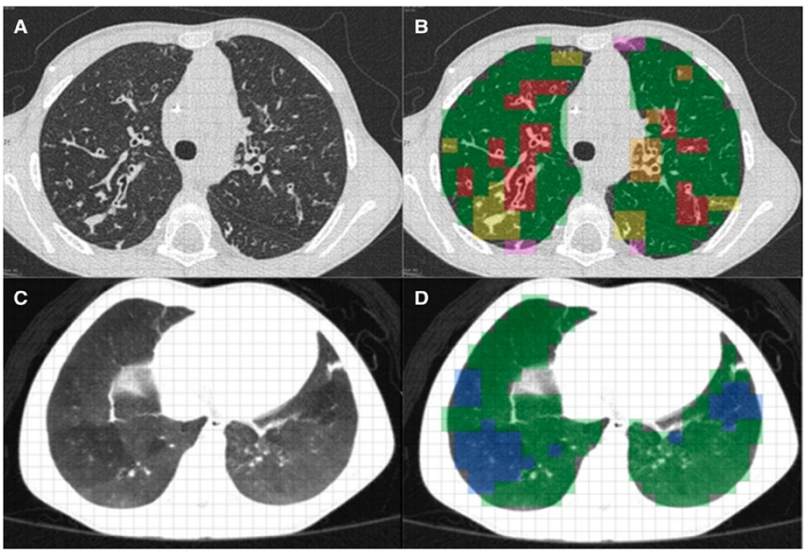
are fairly easy to learn and to perform for the radiologist and the scoring systems are considered to have a relatively good reproducibility.[84,89] The radiation dose is low and is of less concern in comparison to chest CT. As the CF health has rapidly improved over the last decades we need to understand if chest x-rays are still a useful method to track CF lung disease when more sensitive methods are available. A question that needs to be addressed is whether annual CXR, separately or in combination with other methods, can add information about early CF-lung disease progression on an individual level.

2.3.4 CHEST COMPUTED TOMOGRAPHY

One of the most sensitive image modalities to assess structural lung damages is chest CT. Chest CT scans yield a variety of common CF structural markers, such as bronchiectasis, bronchial wall thickening, mucus plugging, atelectasis and trapped air. Bronchiectasis is a common well described structural lung pathology in patients with CF and is associated with a reduced quality of life and higher risk of pulmonary exacerbations.[90,91] SLD starts early in life and at 1 year of age bronchiectasis was present in 1/3 of the children with CF. At the age of 3 years bronchiectasis was present in almost 2/3 of the children.[1] Infections of the airways play an important role in neutrophil-related inflammation in the lung, which is associated with faster progression of SLD.[6,38] Still, little is known if permanent and reversible airway damage occurs/arises directly with an airway infection or how intermittent and chronic airway pathogens influence the yearly SLD progression rate.

To quantify the extent and severity of CF lung disease and to understand the longitudinal trends of SLD, a scoring system needs to be applied to the image data. Several scoring systems are in use, and the most sensitive chest CT scoring system today is the Perth-Rotterdam Annotated Grid Morphometric Analysis for CF (PRAGMA-CF, Figure 3) PRAGMA-CF which is the only fully quantitative CT scoring system to track minor longitudinal changes in SLD over time.[2] Unfortunately, PRAGMA-CF is not commercially available and requires special software as well as extensive in-house training in order to fully comprehend.

Figure 3. (A) A slice from inspiratory chest CT showing common CF structural lung pathology. (B) Inspiratory slice annotated with PRAGMA-CF: red =bronchiectasis; yellow = mucous plugging; orange = bronchial wall thickening; pink = atelectasis; green = normal lung tissue. (C) Expiratory slice from a chest CT (D) Expiratory slice annotated with PRAGMA-CF: blue = trapped air; green = not trapped air. Reprinted with permission of the American Thoracic Society. Copyright 2022 ATS. Rosenow et al. Am J Respir Crit Care Med. 2015 May 15;19.



The other existing scoring systems for chest CT are only semi quantitative and are considered too insensitive to detect smaller changes of the progression of CF lung disease, which makes it less useful in clinic practice.[92] The intra and inter-observer reliability of the existing scoring systems are considered good although there are differences especially between observers and for outcomes measures such as mucus and bronchial wall thickening.[2,81,92,93] CT protocols have been standardised across multiple CF centres and across different scanners.[63]

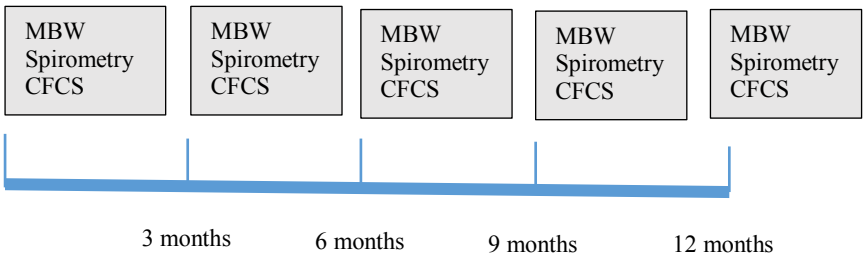
The radiation is one of the major drawbacks with chest CT. The accumulation of radiation limits the use of surveillance CF in clinical care. At Gothenburg paediatric CF centre chest CT has been performed since the beginning of the year 2000 from the age of 6 years and then every third year at the annual assessment. We need to better understand how cross sectional and longitudinal outcome measures from different methods are associated with an each other in

order to optimize/rationalize the frequency of surveillance and minimize the negative impact on CF patients and their families.

3 MATERIAL AND METHODS

Study I was a single centre, prospective observational study. The study protocol involved 3 monthly assessments of MBW, spirometry and Cystic Fibrosis Clinical Score (CFCS) at Gothenburg's paediatric CF centre over a period of 12 months (Figure 4.). All children aged 6–17 years obtaining complete data for at least two test visits were included in the analysis.

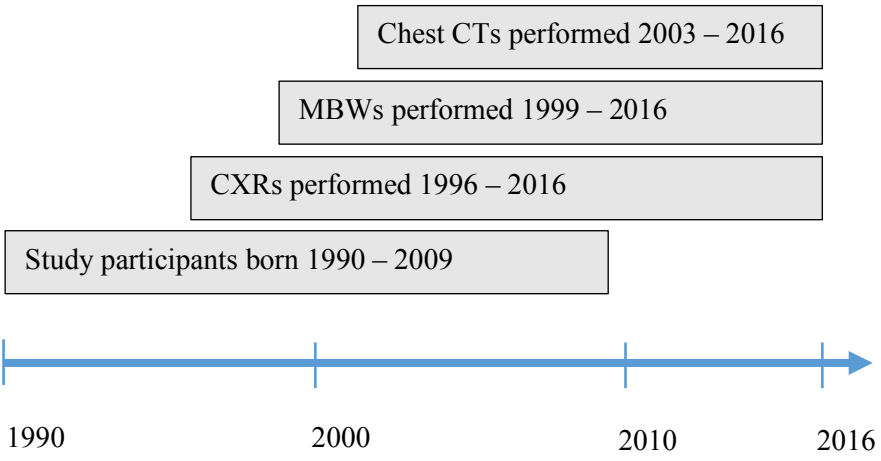
*Figure 4. Overview of the study protocol for **study I***



Abbreviations: CFCS, Cystic Fibrosis Clinical Score; MBW, Multiple breath washout

Study II–IV are single centre retrospective observational studies. All studies used the same cohort of children born 1990–2009 attending Gothenburg paediatric CF centre (Figure 5).

*Figure 5. Overview of when different methods were performed in relation to the study participants for **study II-IV**.*



Abbreviations: CXR, chest x-ray; MBW, Multiple breath washout

Study II included children who had performed at least one chest CT at annual assessment in a clinically stable condition.

Study III included children who had performed at least one MBW-examination and one chest CT at annual assessment in a clinically stable condition. This study also included a MBW-reference group aged 0–17 years.

Study IV included children who had performed at least one chest X-ray, one MBW-examination and one chest CT at annual assessment in a clinically stable condition. This study also included a MBW-reference group aged 0–17 years.

3.1 PROCEDURES AND ANALYSIS

3.1.1 SPIROMETRY

Spirometry (Jaeger AG, Würzburg, Germany) was performed according to ERS and ATS recommendations at the time being.[94,95] The outcome measure FEV_1 was used in **studies I-IV** and the Global Lung Initiative reference equation was used and FEV_1 were expressed as z-score and percent predicted.

3.1.2 MULTIPLE BREATH WASHOUT ACQUISITION AND ANALYSIS

In **study I**, multiple breath washout was performed using Exhalyzer D (Ecomedic AG, Switzerland, software version 3.1.6) and nitrogen as an inert gas in accordance with ERS/ATS consensus recommendations.[79] During the MBW examination the participants were sitting in an upright position wearing a nose clip and breathing through a mouthpiece interface. The CF physician was blinded to the LCI results.

MBW examinations in **study III–IV** was performed using a mass spectrometer AMIS 2000 (Innovision A/S, Odense, Denmark). Sulphur hexafluoride (SF_6) was used as an inert gas, with a start concentration of 4%. Children aged 0–2 years performed MBW sedated with chloral hydrate in a supine position using a face mask. Pre-schoolers and school-aged children with CF performed MBW awake in a sitting position using a facemask (3–4 years) or a mouthpiece (from age 5) together with a nose clip. A minimum of two technically acceptable MBW tests were considered acceptable.

The MBW examinations assessed as acceptable between 1999–2016 were available for re-evaluation with the software LabVIEW. Updates of the software LabView and changes in the settings have affected the MBW outcomes parameters over time. A re-analysis of all MBW examinations was performed by Marcus Svedberg using the same settings and procedures in LabVIEW and in accordance to the MBW consensus statement[79].

A total of 140 healthy children (0–17 years) with no earlier known history of lung disease performed one MBW examination with the same software, equipment, and procedures as described for the participants with CF. The MBW examinations for infants and pre-schoolers (0–6 years) were re-analysed by Marcus Svedberg and the healthy school-children (7–17 years) were re-analysed by Per Gustafsson, using the same software and settings as in the children with CF.

3.1.3 CHEST X-RAY ACQUISITION, ANALYSIS AND REPRODUCIBILITY

Chest X-ray (Adora; NRT X-RAY A/S, Hasselager, Denmark) was used in **study IV** and the CXR included a posteroanterior view during inspiration. The Northern Score (NS) system was used to analyse the chest imaging.[89] NS is assessed by dividing the lungs into four zones, and each zone is assigned a score of 0–4 (0=no pathology, 4= most pathology). A further 0–4 points can be assigned according to the radiologist's perception of the severity of the existing structural lung damage. The total score is between 0–20, with a higher score reflecting more severe SLD. NS=0 is defined as a normal CXR and a NS ≥1 is abnormal.

Three paediatric radiologists evaluated 30 radiographs each (Total 90 radiographs) to assess the observer reliability of the scoring system NS. All radiographs were anonymised and stratified according to SLD severity. One of the paediatric radiologists re-analysed the same 30 radiographs 1 month later in randomised order to limit the risk of a memorization bias.

3.1.4 CHEST CT ACQUISITION, ANALYSIS AND REPRODUCIBILITY

Chest CTs were performed in **study II–IV** using two different CT scanners: Light Speed Ultra (GE Healthcare Inc., Chicago, USA); and Discovery CT750 HD (GE Healthcare Inc., Chicago, USA). All CT scans used the same scanning protocol and scanning procedures. The scans were performed during voluntary breath hold at end inspiration and at end expiration. A discontinuous scanning protocol were used to reduce the cumulative radiation. The inspiratory scans generated on average 10–15 slices and the expiratory scan on average 3–4 slices.

All chest CT images were assessed with the PRAGMA-CF scoring system. The PRAGMA-CF image scoring system allows quantification of bronchiectasis, mucus, bronchial wall thickness, atelectasis, and normal lung structure on the inspiratory scans. On expiratory scans, trapped air and normal lung structure were annotated. In PRAGMA-CF, 10 equally distant slices are normally annotated, but due to the use of a discontinuous chest CT protocol, all slices on the scans were annotated. The primary outcomes of the chest CT scans were total airway disease, a composite score of bronchiectasis,

mucus, bronchial wall thickening, expressed as the volume proportion of the respective pathology on the scan divided by the total volume of the scan.

All CT-scans were unidentified and randomised and analysed by Marcus Svedberg. Marcus Svedberg has been trained in different chest CT scoring systems including PRAGMA-CF. To determine the intra-rater reproducibility 20 CT scans were randomly selected and re-analysed after one month by Marcus Svedberg.

3.1.5 CYSTIC FIBROSIS CLINICAL SCORE

The Cystic Fibrosis Clinical Score (CFCS) is one of many scoring systems to evaluate CF disease severity and the progression of CF lung disease. [96,97] The CFCS consists of five subjective questions reflecting the patient's pulmonary symptoms and five objective questions related to the physical examination. Each question is given a score between 1–5 and a higher score reflects a worse clinical status (total score range were 10–50). The CFCS assessment was performed every third month by the CF physician in **study I**. The CFCS results was not used in clinical practice and the physician was blinded to previous CFCS results.

3.1.6 PULMONARY EXACERBATIONS

Pulmonary exacerbation (PEx) are frequent events in children with CF. They can cause permanent loss of lung function, a faster lung disease progression and worse quality of life.[98] The pathophysiology of a PEx is not fully understood and to date there is no consensus about how to define a PEx in individuals with CF.[99] The different existing definitions generally combine subjective symptoms from the airways (e.g. cough, mucus), objective measurements (e.g. drop in lung function), microbiology and the physician's decision to treat.

A PEx in **study I** was defined with the following criteria: 1. Worsening in three or more parameters in CFCS with a minimum change of five points in CFCS, compared to baseline value and 2. ≥10% decrease in FEV_1% compared to baseline value. Baseline CFCS and FEV_1% for each participant was defined from the visit with the lowest (best) CFCS during the study period. This definition resembles Fuchs criteria of a PEx and made it possible to enter the study even if the participant was considered clinically instable.[71]

3.1.7 WEIGHT, LENGTH AND BODY MASS INDEX

In **study I** weight and height were obtained at each visit when the participant underwent MBW examinations and spirometry. In **study II–IV** weight, height and body mass index (BMI) were obtained from the Swedish CF registry. Weight, height and BMI were all reported as z-score.

3.1.8 SPUTUM CULTURES

In **study I** chronic *Pseudomonas aeruginosa* and chronic *Staphylococcus aureus* were retrieved from the clinical journal and the Swedish CF registry. Chronic *Pseudomonas aeruginosa* and *Staphylococcus aureus* were both defined according to Leeds criteria.[100]

The results from sputum cultures and cultures from laryngeal suction in **study II–IV** were available from 1995–2016 by the department of Clinical microbiology in Gothenburg. The following pathogens were used: *Staphylococcus aureus*, *Haemophilus influenzae*, *Mycobacterium abscessus*, *Burkholderia cepacia* complex, *Achromobacter xylosoxidans*, Aspergillus species and *Pseudomonas aeruginosa*. Complementary information about intermittent and chronic infections was also collected from the Swedish CF Registry, where there was information from the year 1990 and onwards.

Participants were defined as having an intermittent respiratory infection if they had a positive sputum culture for *Staphylococcus aureus*, *Haemophilus influenzae* and Aspergillus species. *Pseudomonas aeruginosa* was categorised according to the Leeds criteria with the except that the categories of 'never having had *Pseudomonas aeruginosa* '' and 'free of *Pseudomonas aeruginosa* were pooled together[100]. *Mycobacterium abscessus*, *Burkholderia cepacia* and *Achromobacter xylosoxidans*, were pooled together under the same criteria as described above for *Pseudomonas aeruginosa*, due to the low numbers of patients who were infected with the above mentioned airway pathogens.

3.2 STATISTICS

The statistics performed in this thesis are sometimes of a relatively complex nature and are all performed by a statistician. In this section the statistics are

greatly simplified and described on a more general level. For the person who is more interested in the statistical procedures please read the statistic chapter for respective paper and the appendix for respective paper online.

3.2.1 GENERAL STATISTICS

For descriptive purposes, the data in **study I-IV** are presented as mean, median and range for continuous variables and as numbers (%) for categorical variables. In **study I-V** SAS ver. 9.4 software (SAS Institute, Cary, NC, USA) were used in all statistical calculations except a few statistical calculations in **study I** which were performed using SPSS version 23 software (IBM Corp, Armonk, NY, USA).

3.2.2 LCI VARIABILITY

In **study I** LCI variability was calculated using all available LCI data and the LCI measurements when the participants were defined as clinically stable. LCI variability was expressed as 1. within- and between-subject variability. 2. Standard Deviation and the Coefficient of Variability for both within- and between-session. 3. Upper Limit of Normal (ULN) of LCI. ULN was calculated as the relative percentage change in LCI (former vs. latter LCI) between visits. The relative percentage change in LCI-values had a normal distribution and the following formula was used to calculate ULN of LCI, where LCI1 and LCI2 represents the former and the latter LCI-values.

$$\sum_{n}^{n} Average\left(\frac{LCI2 - LCI1}{LCI1}\right) + SD\left(\frac{LCI2 - LCI1}{LCI1}\right) x1.64)$$

The ULN for LCI variability was defined as the 95th percentile.

3.2.3 LONGITUDINAL OUTCOME MEASUREMENTS

Analysis of longitudinal outcome measurements in **study I-IV** were performed using mixed effects models, including both random and fixed effects. Different types of models were considered, depending on the longitudinal distributions and trends for respective outcome measurements analysed. Robust standard errors were used to account for the skewed distribution if needed. Comparisons between sub-groups were performed by the inclusion of sub-group variables as fixed effects in the models. To assess whether disease progression differed between the sub-groups, a sub-group with age interaction

was included. The effect of single airway infections and chronic infections were calculated and adjusted in the models.

3.2.4 ASSOCIATIONS BETWEEN OUTCOME MEASUREMENTS

To evaluated correlations between outcome measures we used Pearson correlation analysis, Spearman's rank correlations, Cohen's kappa coefficient and Mann-Whitney U test. Associations between longitudinal measurements in **study I-IV** were analysed using different statistical models (e.g. mixed effect model, generalised estimating equations, joint modelling), or by omitting the slope of respective outcome measures from the model.

3.2.5 LCI IN HEATHY CHILDREN

To account for natural age-trends in **study III–IV** in LCI between infants, pre-schoolers, and school-age children, all LCI values in children with CF were age adjusted (LCI_{adj}). The following equation was derived from cross sectional measurements in the healthy reference population.

$$LCI_{adj} = \begin{cases} LCI - 0.12 \times age, & if\ age < 6.0 \\ LCI, & if\ age \geq 6.0 \end{cases}$$

3.2.6 REPRODUCIBILITY

In **study II and IV** the inter- and intra-class correlation coefficient (ICC) and by units' agreements was calculated to describe the reproducibility of respective parameters.

3.2.7 ETHICS

Study I: registration number 337-09 ethical board in Gothenburg.

Studies II–IV: registration number 206-18, ethical board in Gothenburg.

4 SUMMARY OF RESULTS

4.1 STUDY I

This included 25 children with CF aged 6–17 years old over a median period of 12 months. A total of 107 of 109 visits were included in the study, whereas 2 visits were excluded due to missing CFCS data at visits. The demographics of the study participants are summarised in Table 1.

*Table 1. Demographics of the 25 participants in **study I**. The median study period was 12 months.*

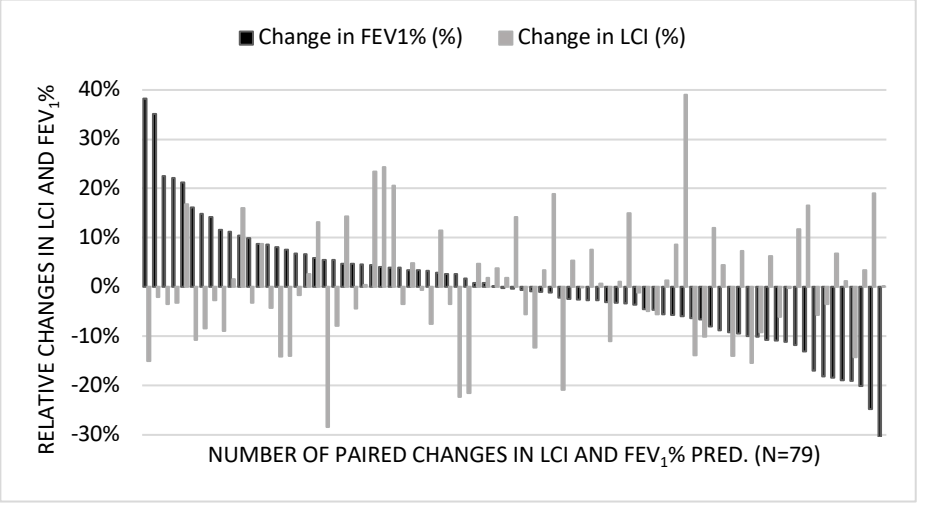
Variable	Start	End
Participants	25	
Male (%)	15 (60%)	
Age (years)	10.1 (6.5;17.4)	
ΔF508 homozygot (%)	14 (56%)	
Pancreatic insuffiency (%)	24 (96%)	
Pa colonised (%)	5 (20%)	
Sa colonised (%)	3 (12%)	
Hypertonic saline (%)	23(92%)	
Pulmozyme (%)	8(32%)	
Allergic asthma (%)	4 (16%)	
Height (z-score)	-0.54 (-2.14–0.65)	-0.32(-2.40–0.94)
Weight (z-score)	-0.38 (-2.18–0.39)	-0.46 (-2.18–0.56)
CFCS	15 (12–23)	15 (11–26)
FEV_1%	91.5 (55.0–121.9)	91.5 (41.7–120.8)
LCI	9.06 (6.74–17.44)	9.11 (6.37–16.92)

Results are presented as n, n (%) and median (range). Abbreviations: LCI, Lung Clearance Index; Pa, *Pseudomonas aeruginosa*; Sa, *Staphylococcus aureus*; CFCS, Cystic Fibrosis Clinical Score.

Only 7 visits (7%) across 4 participants fulfilled PEx criteria. The physician only detected objective pathological finding in 4 participants using the CFCS, whereas most pathological findings were respiratory symptoms in patients. The participants experiencing a PEx had a mean (range) LCI of 13.60 (10.12–18.63) which was significant higher compared to a mean LCI of 8.91 in the clinical stable participants ($p<0.001$). No significant correlation between changes in CFCS and LCI between visits was observed ($r=0.041$, $p=0.71$) but

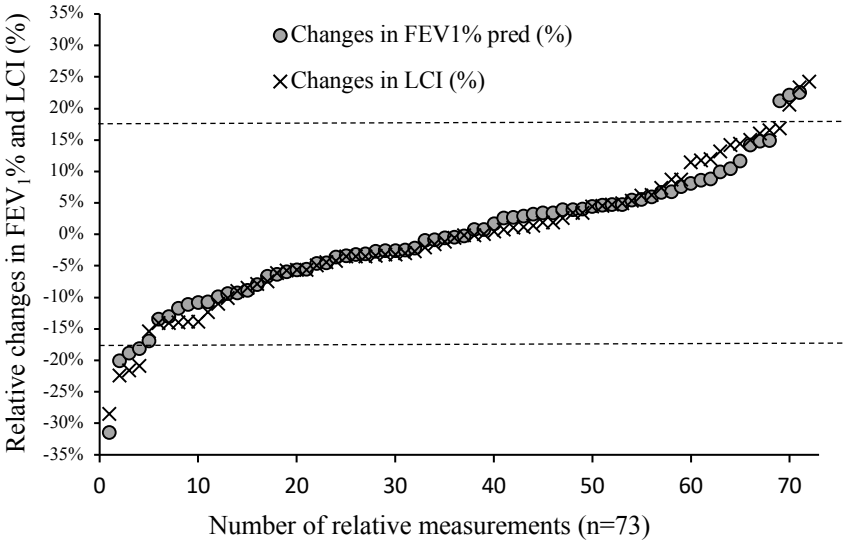
there was a weak significant correlation between higher CFCS and worsening in FEV_1% between visits (r=-0.399, p<0.001). No associations between changes in LCI and FEV_1% between visits were observed (r=-0.012, p= 0.30, Figure 6).

Figure 6. The relative changes FEV_1% and lung clearance index (LCI) between visits illustrated as pairs. There were in total 79 pairs and no significant correlation was observed between relative changes in FEV_1% and LCI between all visits.



The within-subject variability of both LCI and FEV_1% was low with no significant difference when all data were included or if only stable visits were included. There was a positive correlation between both within- and between-session variability of LCI expressed as SD with an increasing mean LCI (r=0.41, p<0.001). The ULN of LCI was 19% (95^{th} percentile) when all visits were included and 17% when only clinical stable visits were included. LLN of FEV_1% was -19% (95^{th} percentile) when all visits were included and -17% when only clinical stable visits were included (Figure 7).

Figure 7. Relative changes in LCI and FEV_1% pred. compared to former visit in all clinical stable visits plotted independently of each other in an increasing order. Using the 95th percentile as Upper Limit of Normal LCI was +17% and Lower Limit of Normal was -17% for FEV_1% pred.



4.2 STUDY II

This study included 75 participants with CF who had performed a total of 200 chest CTs and 820 spirometry measurements over a mean (range) period of 12.0 (3.0–18.0) years. For demographics characteristics of the participants in study II (Table 2).

*Table 2. Demographics characteristics of the participants in **study II***

Variable	Results
Participants	75
Study period per participants* (years)	12.0 (3.0–18.0)
Pancreatic insufficiency	67 (89%)
CF diagnosis <1 year	39 (51%)
Shared care	24 (32%)
Homozygous dF508 / heterozygous dF508 / other mutations	38 (51%) / 34(45%) / 3(4%)
Total FEV_1 measurements performed	820
FEV_1 measurements performed per participants	12.2 (116)
FEV_1 (z-score) at age of 7 years	0.12 (-3.14–2.70)
Total number of chest CTs performed	200
Weight at 7 years of age (z-score)	-0.42 (-3.01–1.75)
Height at 7 years of age (z-score)	-0.41 (-3.34–1.91)
BMI at 7 years of age (z-score)	-0.27 (-2.11–1.99)
Cultures/participants /year	4.23 (0.12–15.4)
Pa incidence/ participants /year	0.24 (0.00–0.93)
Age at first Pa infection (years)	7.4 (0.4–17.4)
Participants with chronic Pa infection	22 (29%)
Age at onset of chronic Pa infection (years)	12.6 (3.1–18.9)
participants with chronic Ma or Bc infection	7 (9%)
Age at onset of chronic Ma and Bc infection (years)	15.4 (13.2–18.5)
Sa incidence/participants t/year	1.94 (0.0–7.11)
Hi incidence/participants /year	0.39 (0.00–2.36)
Asp incidence/participants /year	0.61 (0.00–5.16)

Results are presented as: n, n (%) or mean (range). * Study period for each subject is defined as the time between the first and the last airway culture. Abbreviations: Pa, *Pseudomonas aeruginosa*; Ma, *Mycobacterium abscessus*; Bc, *Burkholderia cepacia* complex; Ax, *Achromobacter xylosoxidans*; Asp, *Aspergillus* species; Sa, *Staphylococcus aureus*; Hi, *Haemophilus influenzae*.

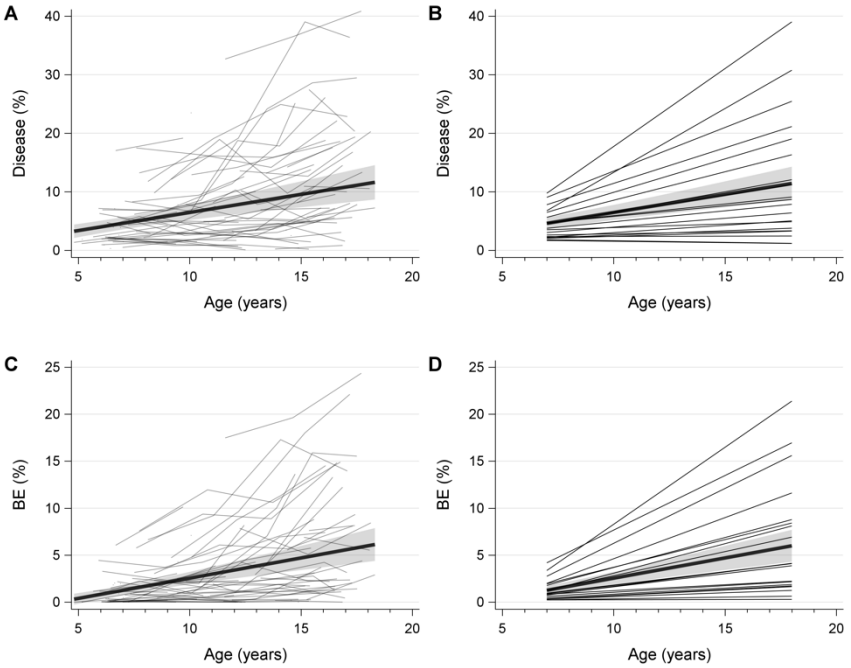
The statistical models for chest CTs and spirometry measurements demonstrated a good agreement between the observed and predicted values.

The intra observer variability calculated with ICC for total airway disease and bronchiectasis was 0.95 (95% CI: 0.85–0.98) and 0.96 (95% CI: 0.89–0.99), respectively.

4.2.1 PROGRESSION OF CF LUNG DISEASE

The mean annual progression rate of total airway disease and bronchiectasis was 0.62 (95% CI: 0.36–0.86) and 0.43 (95% CI: 0.28–0.58), respectively (Figure 8). The mean annual decline rate for FEV_1 measured as z-score was -0.16 (95%CI: -0.18 – -0.13). No significant differences in the annual mean progression rate or the decline rate were observed for chest CT or spirometry measurements with regards to gender, birth cohorts born 1990–1999 vs 2000–2009, pancreas insufficiency vs sufficiency, age at diagnosis or shared care. The mean difference for total airway disease and bronchiectasis was significantly higher in the participants diagnosed with CF after 1 year of age ($p=0.016$). No mean difference in FEV_1 was observed in the in the sub group with late CF diagnosis.

Figure 8. Panel A and C describe the observed longitudinal progression of total airway disease and bronchiectasis for all 75 participants. Panel B and D describe the relationship between the level of SLD at age 7 and the estimated progression rate of total airway disease and bronchiectasis. In panels B and D, 20 randomly selected subjects stratified for CF disease level are presented to avoid over-plotting. The thick black lines and grey-shaded bands represent the mean progression with 95% CI.



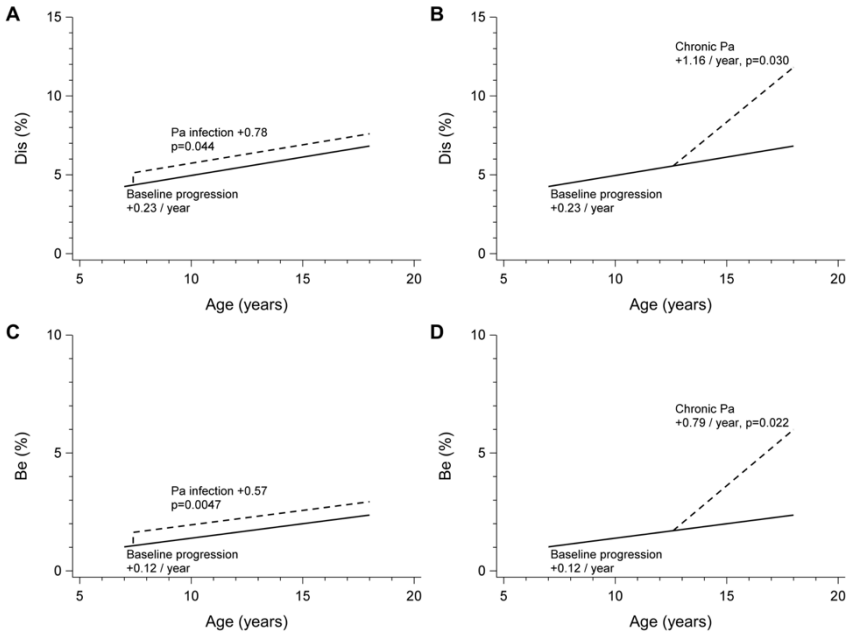
Abbreviations: %Dis, Total airway disease; %Be, bronchiectasis. Both of these parameters are expressed as percentage of the total lung volume.

4.2.2 THE EFFECTS OF AIRWAYS PATHOGENS ON CF LUNG DISEASE PROGRESSION

When adjusting for airway pathogens the annual mean progression rate for total airway disease was reduced to 0.23 per years (p=0.095) and for bronchiectasis 0.12 (p=0.057) respectively. The annual mean decline rate for FEV_1 (z-score) significantly decreased to 0.12 (p=0.001) after adjustments for

airway pathogens. A temporary airway infection with *P. aeruginosa* caused significant structural lung damage measured with chest whereas no change in FEV_1 could be detected after a *P. aeruginosa* infection when compared to respective baseline (Figure 9). The annual trajectory trends for chest CT and spirometry measurements significant increased after chronically infected with *P. aeruginosa.* (Figure 9).

Figure 9. Panel A and C demonstrate that a single infection with Pseudomonas aeruginosa was associated with significant mean lung damage, expressed as an increase in total airway disease and bronchiectasis. The annual progression rate for total airway disease was 0.23% and 0.12% for bronchiectasis, when adjusted for the airway pathogens. Panel B and D demonstrates the changes in SLD progression rate when the airways are colonised with Pseudomonas aeruginosa. When chronic infected by Pseudomonas aeruginosa, the mean annual rate of progression of total airway disease and bronchiectasis increased significantly to 1.16% (p=0.030) and 0.79% (p=0.022), respectively, as compared to the baseline progression rates.



Abbreviations: %Dis, Total airway disease; %Be, bronchiectasis. Both of these parameters are expressed as percentage of the total lung volume. Pa, *Pseudomonas aeruginosa*

4.2.3 OUTCOME MEASUREMENT ASSOCIATED WITH CF LUNG DISEASE PROGRESSION

At the age of 7 there was a good correlation between the extent of SLD and the subsequent progression of the total airway disease (r=0.61, p=0.0012) and bronchiectasis (r=0.89, p=0.010) when adjusted for airway pathogens (Figure 9). The was no significant association between lung function measured as FEV_1% at the age of 7 and further decline rate in FEV_1%. First at the age of 13 there was weak but significant correlation between the level of FEV_1% and FEV_1% annual decline rate (r=0.31, p=0.031).

4.3 STUDY III

A total of 75 children attending Gothenburg's paediatric CF centre were included in this retrospective study together with 70 healthy children. The children with CF had performed at least one chest CT and one MBW-examination during a median study period of 9 years. The healthy cohort performed one MBW examination each and served as reference population for LCI at different ages (Figure 10). The demographics and cross-sectional data of the CF disease of the cohort are presented in Table 3 and Table 4.

*Table 3. Demographics characteristics of 75 children with CF in **study III***

Variable	n (%) / median (range)
Female sex	24 (32%)
Pancreatic insufficiency	67 (89%)
dF508/dF508, dF508/other, other/other	38 (51%) / 34(45%) / 3(4%)
Age at diagnosis (years)	0.8 (0.0–9.0)
Children treated with CFTR modulators	1 (1%)
Children with CF-related diabetes mellitus	2 (3%)
Chronically infected with Pa during study period	22 (29%)
Age at onset of chronic Pa infection (years)	13.0 (3.1–18.9)
Children with ABPA	3 (4%)
Follow-up time[†] (years)	9.0 (1.0–14.1)
Number of MBW examinations/child	11 (1–18)
Number of chest CTs/child	3 (1–5)
Chest CTs at MBW examinations (+/- 3 days)	146 (73%)

[†]Time between the first and the last multiple breath washout or the last chest CT.
Abbreviations: CT, computed tomography; MBW, multiple breath washout; Pa, *P. aeruginosa*, CFTR, cystic fibrosis transmembrane conductance regulator, ABPA, Allergic bronchopulmonary aspergillosis.

*Table 4. A cross sectional overview of the CF disease of the cohort in **study III** including healthy references data LCI. Age adjusted LCI-values are presented as LCI_{adj}.*

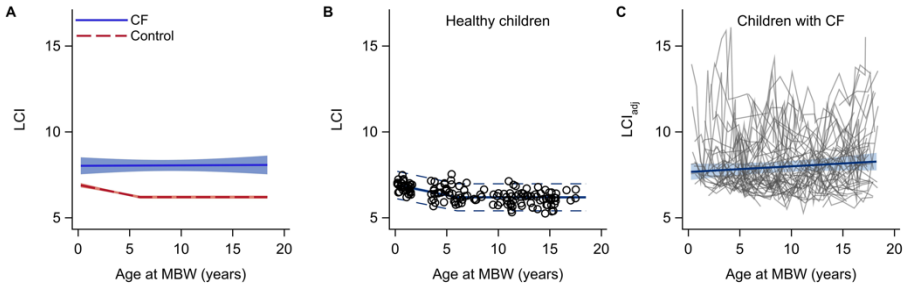
Variable	Age 2 years (n=27)	Age 5 years (n=41)	Age 7 years (n=44)	Age 12 years (n=44)
LCI	7.7 (6.3–13.8)	7.4 (6.2–12.2)	7.5 (5.7–12.4)	7.9 (5.6–11.0)
LCI_{adj}	7.2 (5.8–13.3)	7.3 (6.1–12.1)	7.5 (5.7–12.4)	7.9 (5.6–11.0)
ULN LCI	7.4	7.0	7.0	7.0
FEV_1 (z-score)	-	-0.1 (-2.0–2.1) †	-0.1 (-3.1–2.7)	-0.4(-3.8–2.6)
Chest CT (%Dis)	-	-	3.8 (0–17.5) ‡	5.4 (0.9–32.7) §
Chest CT (%Be)	-	-	0.8 (0–7.6)‡	2.0 (0–17.5) §
BMI (z-score)	-0.1 (-1.7–1.8)	-0.3 (-2.8–1.4)	-0.2 (-1.9–2.65)	-0.4 (-2.0–1.2)

Data are presented as median (range).
†30 of 41 subjects performed technically acceptable spirometry.
‡35 of 44 subjects had a chest CT between age 6–8 years.
§30 of 44 subjects had a chest CT between age 11–13 years.
Abbreviations: %Be, bronchiectasis (%); BMI, body mass index; CT, computed tomography; %Dis, total airway disease (%); FEV, forced expiratory volume; LCI, lung clearance index; LCI_{adj}, age adjusted lung clearance index; ULN, upper limit of normal.

4.3.1 PROGRESSION OF LCI IN CHILDREN WITH CF

In children with CF there was a slow, non-significant mean progression rate of age-adjusted LCI (LCI_{adj}) of 0.03 LCI_{adj}-units per year (95% CI: -0.01–0.08, p=0.13). In girls and in the children born between 1990-1999 there was a significant annual LCI_{adj} progression rate of 0.11 units (95% CI: 0.03–0.20, p=0.011) and 0.07 units (0.01–0.12, p=0.025), respectively.

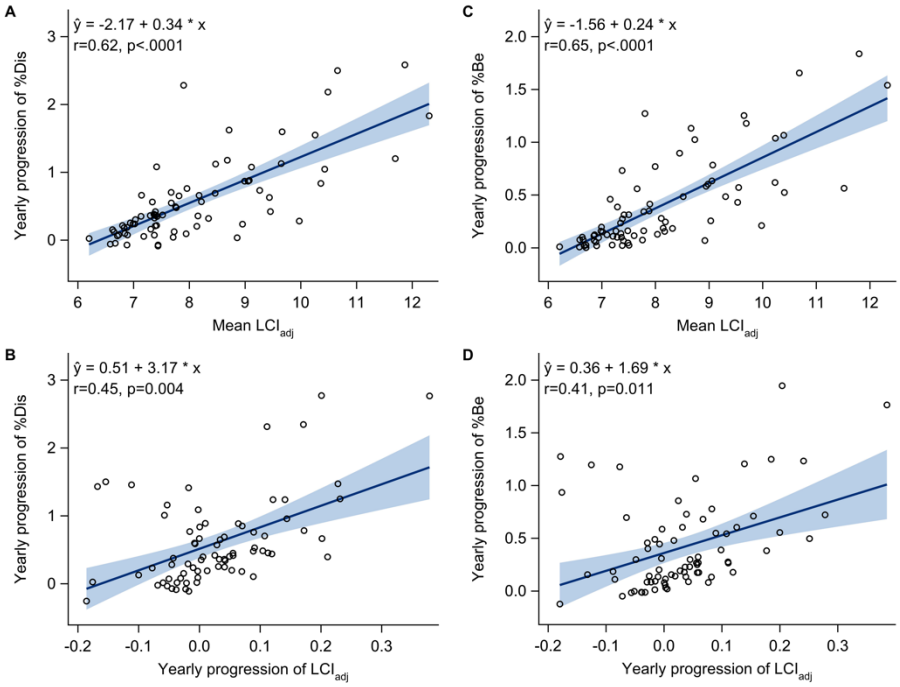
Figure 10. Panel A: Mean LCI vs age in 75 children with CF and 140 healthy children. Panel B: Cross sectional LCI-values for the healthy cohort are illustrated at different ages. Panel C: Longitudinal progression of age-adjusted LCI in the CF cohort is presented. Circles and grey lines are individual data. The blue lines with shaded bands show the mean trends with 95% confidence limits. The dashed lines in panel B are the 95% prediction limits.



4.3.2 ASSOCIATIONS BETWEEN LONGITUDINAL LCI AND SLD PROGRESSION RATE

There was a significant positive correlation between historical mean LCI_{adj} and progression rate of SLD ($p<0.0001$, Figure 11). An increase with 1 mean LCI_{adj}-unit was associated with 0.34 (95% CI 0.27–0.41) percentage points increased annual progression rate of total airway disease and 0.24 (95% CI: 0.19–0.29) percentage points increased annual progression rate of bronchiectasis.

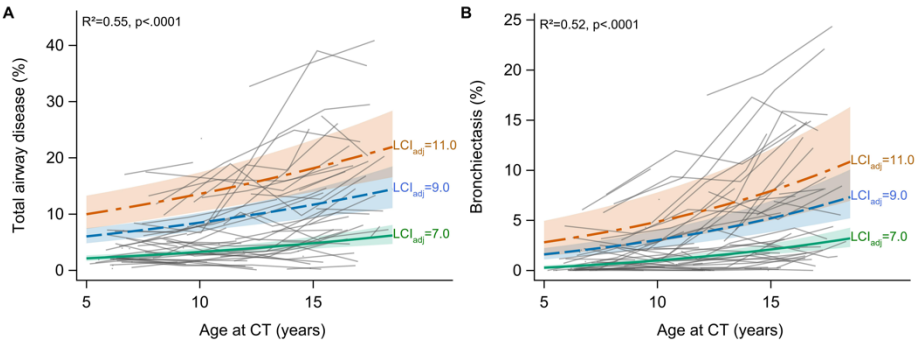
Figure 11. Annual progression rate of total airway disease (panel A and B) and bronchiectasis (C and D) versus mean age adjusted LCI and annual progression rate of age adjusted LCI. Circles represent the best linear unbiased predictions of each participants progression and mean, estimated from longitudinal chest CT and MBW examinations. The solid lines with shaded bands are fitted regression lines with 95% confidence limits.



4.3.3 ASSOCIATIONS BETWEEN LCI AND EXTENT OF SLD

Mean LCI explained 55% of total airway disease variability and 52% of bronchiectasis variability (Figure 12). LCI measured at chest CT that explained 49% of the variability of total airway disease and 35% of the variability of bronchiectasis. In higher LCI-values >9.0 there was a weaker association between LCI and SLD whereas longitudinal LCI measurements had a better correlation over the whole LCI spectra (Appendix article III). A normal mean LCIadj at the age of 6 years corresponded to median total airway disease of 2.3% (95% CI: 0.7–5.5) and bronchiectasis of 0.4% (95% CI: 0.0–1.8).

Figure 12. Associations between the level of longitudinal LCI and the progression of structural lung damages measured as total airway disease (panel A) and bronchiectasis (panel B). The coloured thick lines and shaded bands represent estimated median trends with 95% confidence limits, assuming a constant LCI_{adj} throughout childhood.



4.4 STUDY IV

All 75 eligible children with CF were included in the study with a median follow-up time of 11.9 years (3.0–18-0). A total of 941 CXR examinations and 777 MBW examinations of 785 eligible fulfilled MBW consensus statement were included in the study.[79] The major characteristics of the cohort are presented in Table 5. A cohort of 70 healthy children with no known lung disease who all had performed one MBW examination were also included as reference material.

*Table 5. Characteristics of the participants in **study IV**.*

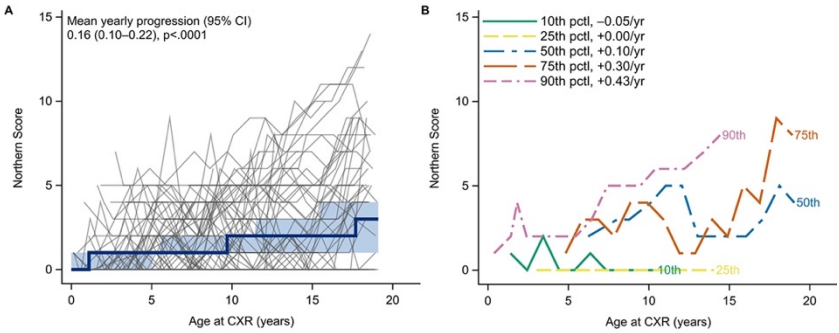
Variable	n (%) / median (range)
Female sex	24 (32%)
Pancreatic insufficiency	67 (89%)
dF508/dF508, dF508/other, other/other	38 (51%) / 34 (45%) / 3 (4%)
Children treated with CFTR modulators	1 (1%)
Children with CF-related diabetes mellitus	2 (3%)
Children with chronic infection of *P. aeruginosa*	22 (29%)
Age at onset of chronic *P. aeruginosa* (years)	13.0 (3.1–18.9)
P. aeruginosa incidence/participant/year	0.2 (0.0–0.9)
S. aureus incidence/participant/year	1.2 (0.0–7.1)
Aspergillus species incidence/participant/year	0.2 (0.0–5.2)
Follow-up time* (years)	11.9 (3.0–18.0)
Number of CXRs/child	13 (4–19)
Number of MBWs/child	11 (1–18)
Number of chest CTs/child	2 (1–5)

* Time elapsed between the first and the last chest X-ray.
Abbreviations: CT, computed tomography; CXR, chest x-ray; MBW, multiple breath washout

4.4.1 EXTENT AND PROGRESSION RATE FOR NORTHERN SCORE

The annual mean progression rate for NS was 0.16 NS-units (95% CI: 0.10–0.22) and when adjusting for airway pathogens the mean annual progression rate decreased to 0.10 NS-units (95% CI: 0.10–0.22). Children diagnosed with CF after 1 year of age had an average 1.1 NS-units higher mean Northern Score than the children diagnosed with CF before 1 year of age.

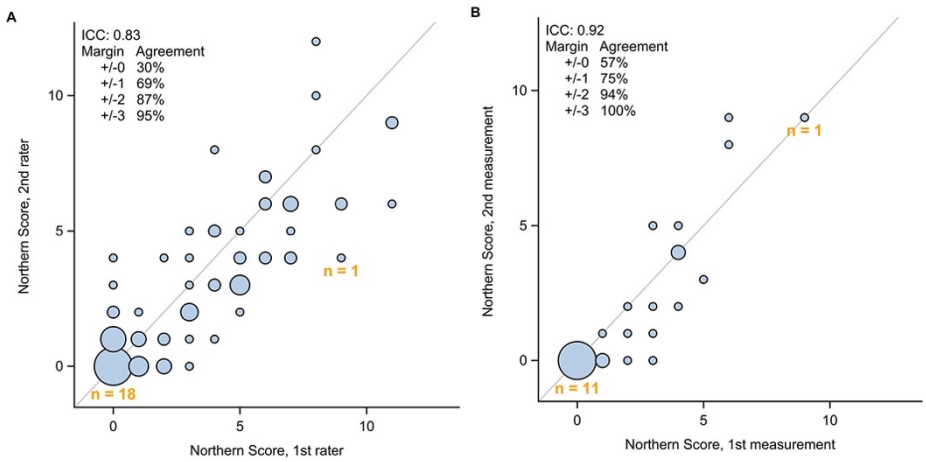
Figure 13. Longitudinal development of CF lung disease measured with Chest X-ray in 75 children with CF. A. Chest X-rays were analysed with Northern score and individual Northern score data are presented as grey solid lines and the estimated median progression curve is presented as a blue solid line with 95% confidence interval. B. The Northern score progression and variability in 5 different participants, corresponding to the 10th, 25th. 50th, 75th and the 90th percentiles of the Northern score progression rate.



4.4.2 WITHIN AND BETWEEN RATER FOR NORTHERN SCORE

The ICC between raters were 0.83 and the NS-values differed up to +-3 NS-units within the 95% confidence interval. The ICC within rater was 0.92 and perfect agreement was achieved for 57% of the re-evaluated CXRs and NS-values differed up to +-2 NS-units within 95% confidence interval (Figure 14).

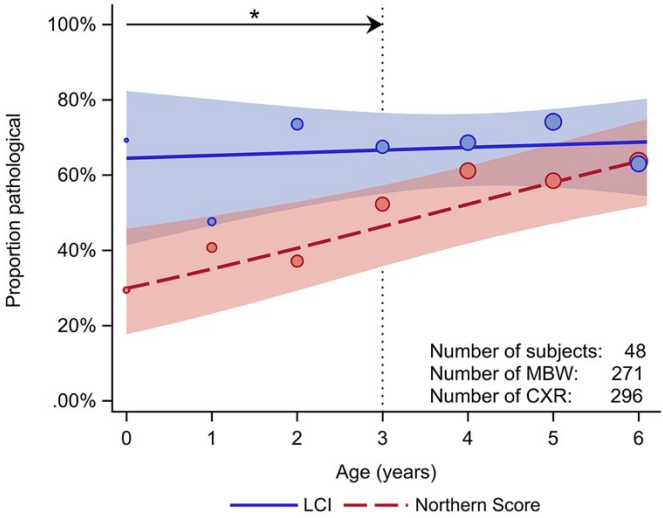
Figure 14. Reproducibility of the chest x-ray scoring system Northern Score. In panel A the inter-rater variability for 90 chest X-rays are illustrated and in panel B the intra-rater variability of 30 chest X-rays are illustrated. The circles sizes are proportional to total evaluated chest x-rays and represent the actual similarities and differences between raters.



4.4.3 SENSITIVITY OF MBW EXAMINATIONS AND CXRS TO DETECTS EARLY CF LUNG DISEASE

The method MBW had a significant higher proportions of early pathological examinations compared to chest x-rays (Figure 15).

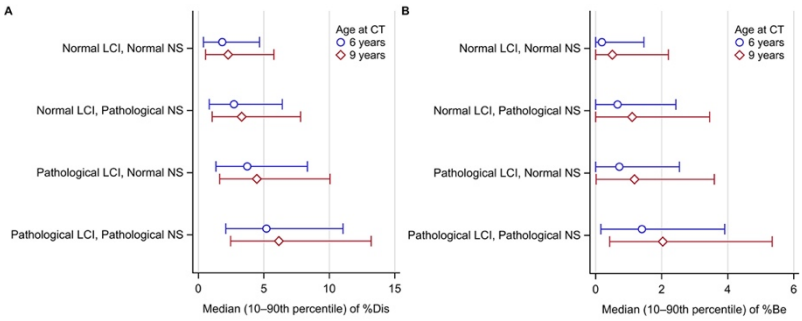
Figure 15. MBW-examinations with abnormal LCI values and abnormal chest x-rays (Northern Score ≥1) in young children with CF plotted over age. The coloured curves represent the mean trends (95% confidence intervals) and the circles represent the actual mean proportions every year and the sizes of the circles correspond to the number of measurements per year. The dotted line represents the age when there were a significant difference ($p<0.05$) between the proportions with pathological MBW examinations and abnormal chest x-rays.



4.4.4 ASSOCIATION BETWEEN MBW AND CXR IN COMPARISON TO CHEST CT

At the age of 6 years a normal NS or LCI was associated with median total airway disease of 2.9% (10–90th percentile: 0.8–6.9) and 2.3% (10–90th percentile: 0.5–6.0%), respectively. When combining the results from a normal CXR and MBW examination at the age of 6 years the median total airway disease decreased to 1.8% (10–90th percentile: 0.4–4.7%) and the median amount of bronchiectasis was 0.2% (10–90th percentile 0.0–1.5%, Figure 16).

Figure 16. Panel A and B illustrates the estimated percentiles of total airway damages (A) and bronchiectasis (B) at age 6 and 9 years in relations to the combined results from multiple breath washout examinations and chest X-rays.



5 DISCUSSION

5.1 PROGRESSION OF CF LUNG DISEASE

There was no mean lung disease progression in **study I** measured with spirometry, MBW examinations or CFCS score. The lack of disease progression might partly be explained by the relatively short study period of 12 months, low number of study-participants and the fact that only 4 participants experienced a PEx at the hospital visits during study period. The cohort in **study II–IV** revealed a significant mean annual decline in lung function measured with spirometry whereas the mean annual progression rate of LCI was very slow and non-significant. The SLD progression rate measured in the same cohort in **study II-IV** using chest CT and CXR examinations demonstrated a significant mean annual increase of SLD for both methods.

5.1.1 LCI PROGRESSION RATE

There are few other studies available that have assessed the progression of CF lung disease using both functional and structural methods over a longer period of time. A 2-year longitudinal study in Danish school-aged children demonstrated a significant mean LCI progression rate of 0.35 LCI-units per year, performed with Exhalyzer D.[81] Chest CT was performed at the beginning and the end in the same study revealed an improvement in total airway disease and bronchiectasis. Children with aged 5-10 years from North America underwent regular surveillance with MBW examinations (Exhalyzer D) over a period of 2-years, demonstrated no mean progression in LCI at all during the study period.[101] A retrospective study from a Swiss paediatric CF-cohort, also monitored with Exhalyzer D, revealed a slow significant increase of 0.1 LCI-units per year in the younger children and a more pronounced LCI progression rate in the older school children.[102] Results from children with CF in England, using the same MBW equipment as in our study revealed a deterioration of 0.18 LCI-units per year as well as a progression in SLD measured with chest CT.[103]

The results from above mentioned studies are conflicting but also not really comparable regarding LCI progression. The studies have used different MBW equipment, inert gases, as well as different treatment strategies. Some studies have only used the first and the last MBW results whereas others studies have used the results from MBW examinations more frequently. We need to

standardise the time points for the assessment and treatments protocol to better increase the understanding of real-world data from MBW- examinations.

One possible explanation that might have affected the lack of mean LCI progression in our study is that we do not have new born screening in Sweden. Most children in our cohort already had a pathological LCI at diagnosis and the already acquired ventilation inhomogeneity may not be reversible at that time. The inert MBW gas SF_6 might not be as sensitive as nitrogen to detect further progression of CF lung disease when ventilation inhomogeneity already was established. An increase in 1 LCI-unit was often followed by a course of antibiotic treatment which might have affected the slow LCI progression rate in **study III–IV**.[24,104]

There was a significant mean LCI progression in the participants born between 1990–1999, but not in the older sub cohort born between 2000–2009. Over the past 20 years knowledge has increased as to what interventions affect the course of LCI. The introduction of inhaled hypertonic saline and pulmozyme as well as the compliance to these medications have probably affected the patients LCI values over time.[18,26,55,76,105] Unfortunately, we do not have the information about the participants' medications or compliance to medication over time so we can only speculate that this finding most likely reflects the continues improvement in CF health care over time. We also observed a mean significant LCI progression in girls but not in boys. No significant gender differences in FEV_1% decline rate or differences in SLD progression measured with chest CTs or CXRs was observed in the same cohort. A gender disparity in CF disease progression has been described in earlier studies.[106,107] The results from our studies partly support the hypothesis that CF lung disease progression is greater in girls than in boys, but the studies was not designed to assess this finding or this question further.

5.1.2 CXR PROGRESSION RATE

There was a relatively slow significant annual mean progression rate of NS for the cohort in **study II–IV**. In the younger sub-group born 2000–2009 there was a significant slower progression rate compared to the older sub-group born 1990–1999. There was no difference in FEV_1 progression rate between the sub-groups but there was a significant difference in LCI progression rate and a trend towards a slower SLD progression rate in the older cohort born 2000–

2009. A comparison of mean annual FEV_1 decline rate with the Australian patient registry between 1988–2017 and the changes in FEV_1 decline rate between the sub groups born 1990–1999 and 2000–2009, reveals no significant change in mean FEV_1 decline rate for neither cohort.[108] These findings support the fact that we cannot just rely on a single method to track CF lung disease and emphasise the need to use different methods in clinical practice as well as in CF research studies.

After adjusting for airway pathogens, the mean NS progression rate decreased by almost 40%. This association was also seen for SLD measured with chest CT and indicates that airway pathogens play an important role in SLD progression rate. Interestingly, Sandvik and colleagues demonstrated no progression of SLD over a period of 2 years in children with CF and they have a much more aggressive antibiotic regime compared to Gothenburg CF-centre.[81]

5.1.3 CHEST CT PROGRESSION RATE

The first chest CTs in **study II–IV** were performed around the age of 7 years and demonstrated a highly variable extent of total airway disease. In some participants the lung parenchyma was hardly affected at all whereas a few participants demonstrated a far advanced lung disease with severe damage to the airways. Bronchiectasis was present in almost 80% of the participants and the mean lung function measured with FEV_1 was normal at the same age. This finding strongly supports that chest CT or other sensitive methods need to be performed at early age to identify individuals with a greater risk of faster CF lung disease progression.

In **study II** we demonstrated a significant mean annual progression rate of total airway disease and bronchiectasis. The Australian AREST cohort performed chest CT biannually from diagnosis and they described an annually mean increase of 0.82% in total airway disease and a mean increase of 0.18% in bronchiectasis in children 0–6 years.[109] A cross sectional study in school-aged children also from the Australian AREST cohort demonstrated relative similar extent of SLD indicating that the annual SLD progression rate was relatively similar to our cohort.[22] The few studies available that have used the same scoring system as in our study, all revealed a very variable progression rate of SLD except the study from Sandvik and colleagues.[81,103,109-111] The variable progression rate could be explained by the introduction of *CFTR*-modulator in some of the studies, different antibiotic regimes and also how experienced respective centres are with using chest CT in clinical practice.

Airway pathogens had a significant impact on the annual mean SLD progression rate measured with chest CT in **study II**. When adjusting for airway pathogens there was still an increasing mean annual trend of SLD progression, but the annual SLD progression rate was no longer significant. The progression rate of SLD was significant associated with chronic *P. aeruginosa* infections and intermittent infections with *P. aeruginosa*. Participants chronically infected with *P. aeruginosa* had almost a 7-fold increase of the annual progression rate for bronchiectasis, when compared to the adjusted baseline. These associations highlight the important of early detection and eradication of *P. aeruginosa* as well as to offer *CFTR* modulators to all persons with CF as *CFTR* modulators have proven to reduce the incidence of *P. aeruginosa*.[112,113] The statistical simplification in describing CF lung disease progression as linear, might not be in accordance how CF lung diseases evolve in real-life. The progression of the lung disease is an continuous and ongoing process, aggravated by both intrinsic and extrinsic factors.[1,2,109,114] Point damages to the lungs due to airway infections and chronic airway infections were the main risk factors of a faster SLD progression identified in **study II**.

5.2 VARIABILITY OF LCI

In **study I** we have demonstrated that LCI variability increased with a higher mean LCI. Our results support that changes in LCI between MBW measurements should be expressed as relative changes rather than absolute changes (e.g. 1 LCI units). Applying relative changes in LCI will lower the risk of bias when interpreting the clinically relevant changes in LCI between MBW-measurements. Oude Engberink and colleagues came to the same conclusion in pre-school children with CF that the relative change in LCI between two test occasions was independent of the magnitude of the LCI.[115]

The normal variability of LCI in clinical stable school-aged children was 17% (95^{th} percentile). Other comparable studies have demonstrated a biological variability of LCI between visits in clinical stable CF children to be +-25% (97.5^{th} percentile).[115,116] The relative high variability in LCI could be due to asymptomatic variations of the CF disease not captured by clinical symptoms

or spirometry. It is important to understand the biological variability of LCI doesn't tell us what relative changes in LCI that should be considered as abnormal in clinical practice. The lung function parameter FEV_1% demonstrated the same variability of +-17% as LCI in **study I,** but in clinical practice we often consider a 10% relative decline in FEV_1% as pathological.[71] The question about what clinical minimal change in LCI (or FEV_1%) to act on, is still not fully understood. The participants in **study I** who experienced a PEx were too few in number to draw any conclusions about the increase in LCI between MBW measurements. Other studies have demonstrated a relative mean increase in LCI between 9–23% during a PEx.[24,27] We do not know how LCI processes/changes throughout a PEx or when LCI reaches its highest level in relation to different airway infections or symptoms. There is probably not a clear distinction as to when an increase in LCI should be considered abnormal, but rather a matter of sensitivity and specificity to detect or evaluate the treatment of PEx. The higher relative increase in LCI between MBW measurements the more certain you can be that the increases in LCI is due to a deterioration of CF lung disease rather than the biological variability of LCI. The choice of using the 95th percentile for ULN for LCI variability instead of 97.5th percentile was because of several reasons. To increase the sensitivity to detect a Pex, the challenge in defining clinical stability in CF as well as the small population sample used in **study I**.

5.3 THE REPRODUCIBILITY OF A SCORING SYSTEM

In order to measure changes in SLD over time in clinical practice, a scoring system must be applied to the image data. The variability of the SLD measured with a scoring system ideally reflects the true changes of the lung disease over time, or the changes could be due to low accuracy of the scoring system. The reproducibility of a scoring system is often assessed by measures of intra- and inter variability. The ICC for both intra- and inter observer variability for NS demonstrated very good to excellent reproducibility in **study IV**. This data was in line with other studies. [117] On an individual level the reproducibility was +-3 NS-units (95th confidence interval) and a normal CXR (NS=0) was in 40% of the cases re-scored as pathological. This variability must be put into perspective of the low extent CXR pathology and the slow annual mean progression 0.16 NS-units of the CF cohort. These findings question the

usefulness of the Northern Score system and CXRs to track CF lung disease in clinical practice.

The PRAGMA-CF is the only fully quantitative scoring system available for chest CT that can discriminate smaller changes in SLD over time.[2,92] The intra observer reproducibility for PRAGMA-CF measured as ICC was excellent (>0.9) in **study II,** and in line with other studies[2,81]. The inter observer ICC for the most reproducible component bronchiectasis using PRAGMA-CF varied between 0.56–0.91.[2,81] The same reasoning as with the scoring system Northern Score for CXR, it is questionable how good PRAGMA-CF is on an individual lever to discriminate smaller changes in CF lung disease. It is essential for the health care provider to have good knowledge about the reproducibility of each method used in clinical practise to assess the longitudinal trends of CF lung disease. The hope is that automated image processing techniques and interpretations of chest CT/MRI scans and CXRs can solve this problem in the near future.

5.4 ASSOCIATIONS BETWEEN DIFFERENT METHODS TO TRACK CF LUNG DISEASE

There was a correlation between FEV_1% and LCI in **study I** but there was no correlation between the relative changes in FEV_1% and LCI between measurements every 3rd month. As most participants in **study I** were considered clinical stable this finding could be due to the normal fluctuations of LCI and FEV_1% over time. Studies comparing relative changes in LCI and FEV_1% after an intervention have been demonstrated a weak or no association between the two measurements.[118,119] Our results further strengthen that short term changes in LCI and

FEV_1% reflect information from different parts of the airways. In **study II** we have demonstrated that cross sectional FEV_1% data and the decline rate of FEV_1% were both poor predictors of the annual FEV_1% decline rate and the SLD progression rate measured with chest CT. Spirometry is still an important measurement to track CF lung disease and used together with other methods

provides the clinician with a more robust assessment than using either measure alone.

In **study I** there was a moderate association between airway symptoms measured with CFCS and LCI, but no correlation was observed between changes in airway symptoms and changes in LCI between visits. This latter observation might be explained by the normal variability of LCI or that changes in LCI is not initially captured by clinical symptoms. The mean LCI was higher in the few individuals that experienced a PEx in **study I** and this association between higher LCI and more frequent PEx have earlier been established in a paper from Vermeulen and colleagues.[120]

In **study III** we demonstrated an association between longitudinal LCI measurements and the extent of SLD and with the progression rate of SLD. A low mean LCI, in comparison to a higher mean LCI was associated with a much lower extent of SLD at different paediatric ages. Earlier studies have demonstrated an association between the magnitude of LCI and the degree of airway inflammation as well as the SLD progression rate. A possible explanation to this finding is that a high mean LCI reflects a greater inflammation of the airways over time that causes faster disease progression rate.[2,121,122] Cross sectional LCI-values performed at chest CT were less associated with the extent of SLD compared to longitudinal LCI values. A possible explanation to this finding may be the biological variability of LCI between MBW measurements, as well as sudden deteriorations/improvements in LCI caused by pulmonary infections or interventions.[24,26,102,115,123] Longitudinal and single LCI values may carries different information about CF lung disease whereas single LCI measurement are more useful to capture and potentially reverse ongoing airway damages. Our results strengthen the clinical use of MBW to pursue a low LCI children with CF. A low LCI throughout the paediatric ages may alter CF lung disease progression rate as well as limit the extent of SLD.[7,101,124]

In **study IV** we concluded that MBW examinations were more sensitive than CXRs to detect early deterioration of CF lung disease. CXR examinations may still be clinical useful to track early CF lung disease as they measure different aspect of CF lung disease. The sensitivity in detecting CF airway pathology with CXRs are low compared to chest CT, and CXR examinations were not associated with changes in treatment and/or diagnostic whereas chest CT examinations were.[125,126] The combined results from both CXR and MBW examinations improved the estimation of the extent of SLD measured with

chest CT, compared to each method used alone. The results from a normal CXR and MBW examination at 6 years of age were associated with a very low extent of SLD measured with chest CT. This information may be used as a surrogate to chest CT if you have a normal LCI and normal CXR. Chest MRI and ultra-low dose chest CT examinations are other promising alternatives to track early CF disease.[87,88,127,128] Both methods are more complicated and more time consuming than CXRs and to date there are no general guidelines as to how to perform chest MRI and ultra-low dose chest CT.

In **study II** demonstrated that the extent of SLD measured with chest CT at 7 and 12 years of age was a very good predictor to understand the subsequent/continues progression of total airway disease and bronchiectasis. This relationship was also unchanged when adjusting for airway pathogens.

5.5 STRENGTHS AND LIMITATIONS

Limitations of **study I** is that it is observational in nature and only included 25 participants from a single CF centre, which makes its findings less generalizable. The calculation of ULN of LCI, multiple LCI-values from each participant were used due to the small study sample, and therefore not independent. Even though the participants were assessed as clinical stable the variability of LCI may still have been affected by antibiotic treatment, lack of compliance to inhalation therapy or pulmonary exacerbation prior to the MBW-examinations. The study included the majority of school-aged patients at our CF-centre, minimizing the selection bias of participants. Our CF-centre has very long experience in how to perform and interpret MBW examinations. The MBW equipment now used is commercially available and, in most studies, including LCI as an outcome measure.

In the **studies II–IV** we acknowledge several limitations. All studies are retrospective, single centre studies including real world data. The results from lung functions test or imagines examinations were not blinded for the clinician. Information about changes in treatments or compliance to treatment are confounding factors that were not available and could have affected the results in all studies. Even though the participants were clinically stable at the annual

assessment, it is possible that a pulmonary infection close to the annual review might have affected the outcome of both pulmonary function's tests and x-ray examinations. A limitation is the lack of chest CTs during the pre-school ages, to compare the differences in progression and sensitivities of the methods. Another limitation is that the LCI results derived from the mass spectrometer are not fully interchangeable with outcome measurements from other MBW equipment. The studies included all available individuals at the Gothenburg CF centre and shared care hospitals, including repeated MBW examinations, chest CTs and CXR examinations and airway pathogens over a period of 20 years. The studies also included a healthy MBW reference cohort, analysed with the same software and settings as used in the CF cohort. All chest CTs and MBW examinations were analysed by one paediatrician with proper training and supervision from different experts in respective fields.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

Lung function and image tests are complimentary measures that capture different dimensions of CF lung disease. The use of multiple methods in clinical practice provides a more robust assessment of CF lung disease than using either measure alone. To understand of lung health in children with CF, we need to use multiple modalities that provide complementary information on lung structure, function and infection status. It is of utmost importance for the clinician to have knowledge about the pros and cons with each method used as well as knowledge about how the outcome measures from each method are associated with one other. There are associations between functional and structural measurements and this information can be used in clinical setting to better understand the progression rate CF lung disease as well to individualize the number of examinations needed for each child with CF.

In the future we need to address how we can intervene in and prevent the progression of CF lung disease and consider some key questions. What is the optimal combination of functional and image techniques to monitor CF disease progression and to assess responses to treatment? What is the minimal clinical difference for respective methods used to monitor CF progression? We need multicentre clinical and research collaborations to address these questions. The standardisation of techniques, both for functional and structural methods, are essential to compare data between CF-centres and CF registries. Radiation is still a concern, and we need to understand how radiation affects the risk of cancer as individuals with CF soon may reach 60+. Better chest CT scans are already available with lower radiation and improved quality, and the use of ultra-low dose chest CT protocols and MRI protocols are being developed and harmonised through the European Respiratory Society and the European Society of Cystic Fibrosis. Automated image analysis for both chest CT and chest MRI scans are getting better and better and will hopefully improve the reproducibility for respective methods. In our search to improve the CF surveillance and CF care we always need to address the impact of overall burden for CF patients and their families. The uncertainty of risks and the clinical benefits with novel and new techniques and procedures require careful management. The future is bright!

7 ACKNOWLEDGEMENTS

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