The evolution of lung function in newborn screened preschool children with cystic fibrosis

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A thesis submitted by

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For the Degree of MD(Res)

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Declaration of Originality

I, Julie Duncan confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Ethical and site research and development approval had been granted before my appointment (Principal Investigator Dr Paul Aurora, with assistance from Ms Jane Chudleigh). Data from 108 preschool children are presented in this study. All subjects were recruited equally by myself and Ms Emma Raywood, with some assistance from Ms Sarah Legg. Children were tested between June 2013 and June 2015. I undertook measurements (lung function testing and clinical examination) on 124 test occasions, and in 92 children whose results were included in the final dataset. Two people were required for each test occasion and I was assisted by a paediatric respiratory physiologist; most occasions by Ms Emma Raywood, and also Ms Sarah Legg, Ms Stephanie Rees, Ms Jane Kirkby and Ms Joanne Miles. The remaining children were tested by two of the above respiratory physiologists when I was not available, and clinical examination performed by Dr Gwyneth Davies (five subjects), Dr Victor Ambrose (four subjects), Dr Anjay Pillai (two subjects), Dr Paul Aurora, Dr Martin Samuels and Ms Jo Miles (one subject each). All clinical data (from diagnosis to preschool test age) was collected by the author with the assistance of clinicians or nurse specialists at each tertiary centre. Lung function interpretation was performed by myself, Ms Emma Raywood and Ms Sarah Legg. Spirometry and plethysmography data was over-read by a senior physiologist (Ms Jane Kirkby). I performed all the statistical analyses presented, with guidance from Professor Angie Wade and Ms Sooky Lum.

All the preschool subjects reported in this thesis were part of a longitudinal study and had undergone lung function testing as infants; attempted at three months, one year and two years of age. At one year of age, infants also underwent chest computed tomography and broncho-alveolar lavage. Chapters five and six present longitudinal data and include infant measurements collected not by myself, but as part of an earlier study (Principal Investigator Professor Janet Stocks). Chapter four reports a comparison of data I collected to a historical cohort of preschool children. Again, this data was collected in an earlier study (Principal Investigator Professor Janet Stocks), and not by myself. All longitudinal and comparative data collected as part of previous studies are reproduced with permission from the Principal Investigator.

Abstract

Most morbidity in cystic fibrosis (CF) is due to progressive pulmonary disease. Recently, small molecule therapies targeting the basic defect of the cystic fibrosis transmembrane conductance regulator protein have been developed, and newborn screening (NBS) for CF allows intervention before irreversible lung damage occurs. Unless the evolution of pulmonary function in young children is known, the optimal age of starting new therapies or enrolling CF NBS children into interventional trials is unclear.

The London Cystic Fibrosis Collaboration has studied CF NBS infants longitudinally from diagnosis, as well as a contemporaneous group of matched healthy controls, and reported lung function was normal in the NBS CF group at two years of age. This thesis reports the preschool follow-up of the same infants, and describes the evolution of lung function to six years of age. The primary hypothesis was that lung function would remain within the normal range. Secondary aims were to compare preschool pulmonary function to that measured in children diagnosed with CF a decade earlier, and to describe which measures in NBS infants could predict preschool lung function.

67 preschool children with CF and 41 healthy controls underwent multiple breath washout (MBW), specific airway resistance and spirometry measurements. Lung Clearance Index (LCI), measured by MBW, was abnormal in CF NBS preschool children, but was better than children diagnosed with CF a decade earlier. The most significant predictor of abnormal preschool lung function was LCI at two years of age.

Contrary to the hypothesis, compared to contemporaneous controls, lung function deteriorates at preschool follow-up in CF NBS children managed with standard UK care. As two year LCI was a significant predictor of later decline, implying that the root causes of decline were before this age, disease modifying therapies and interventional trials should be targeted before two years of age.

Impact statement

Identifying lung disease early in young children with cystic fibrosis

Cystic fibrosis (CF) is one of the most common inherited diseases, affecting over 10,500 people in the UK. The genetic defect underlying this disease results in thick, sticky mucus in many of the organs of the body. In the lungs, this abnormal mucus leads to repeated chest infections and progressive lung damage, which is the main cause of death in patients with CF.

The mainstay of therapy is antibiotics to treat infections, and assistance in clearing mucus from the airways with physiotherapy and medication. In the past, research has concentrated on improving infection management, such as identifying the most effective antibiotic, or designing a drug to improve mucus clearance. However, individuals may not become symptomatic from lung infections until early childhood, but we now know that inflammation and lung damage starts immediately from birth secondary to the genetic defect. This presents a challenge as disease is difficult to detect in young children as diagnostic tests are limited. A key question I aim to answer is what disease markers can we measure in infancy to predict which children deteriorate more rapidly? Can we identify CF patients in whom disease appears at an early age and prevent any further progression?

A revelation in CF treatment over the past decade has been the development of new 'modulator' therapies that go back to the root cause of the defect in CF. They act to correct or improve the function of the abnormal protein that occurs as a result of the faulty gene. These novel therapies provide the potential opportunity to intervene earlier in young children with CF and perhaps prevent the development of lung disease altogether. The question then is when should we start these modulator therapies in young children?

This work aims to define which tests are most effective at detecting early lung disease, and what are the future disease outcomes for a child with an abnormal test at a young age. It is hoped that this research will inform clinicians about which tests are useful in children, and which disease outcomes can be used in interventional trials. If lung disease is shown to occur early, and has an impact on future health, this advocates for starting new therapies at an early age and inclusion of young children in clinical trials. By detecting early disease and its consequences, we aim to improve longer term health in children with CF.

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List of abbreviations

ABPA:	Allergic bronchopulmonary aspergillosis
ATS:	American Thoracic Society
B:	Unstandardised coefficient
BAL:	Broncho-alveolar lavage
BMI:	Body mass index
BTPS:	Body temperature, pressure and water vapour saturated conditions
CEV:	Cumulative expired volume
CF:	Cystic fibrosis
CFTR:	Cystic fibrosis transmembrane conductance regulator
CO_2:	Carbon dioxide
CT:	Computed tomography
DVD:	Digital versatile disc
ELISA:	Enzyme-linked immunosorbent assay
ERS:	European Respiratory Society
$FEV_{0.4}$:	Forced expired volume in 0.4 second (mL)
$FEV_{0.5}$:	Forced expired volume in 0.5 seconds (mL)
$FEV_{0.75}$:	Forced expired volume in 0.75 seconds (mL)
FEV_1:	Forced expired volume in one second (mL)
FEF25-75:	Forced Expiratory Flow between 25% - 75% of expired FVC (mL·s-1)
FEFV:	Forced Expiratory Flows and Volumes
FRC:	Functional Residual Capacity (mL)
FRCpleth:	Functional Residual Capacity (mL) obtained using body plethysmography
FVC:	Forced Vital Capacity (mL)
GA:	General Anaesthesia
GORD:	Gastro-oesophageal reflux disease
He:	Helium
HC:	Healthy control
HI:	*Haemophilus influenzae*
HRCT:	High Resolution CT

IL:	Interleukin
IRT:	Immunoreactive trypsinogen
LCFC:	London Cystic Fibrosis Collaboration
LCI:	Lung Clearance Index
Log:	Logarithmic scale
M:	Month
MBW:	Multiple Breath Inert Gas Washout
MCP:	Monocyte chemoattractant protein
NBS:	Newborn Screening
NE:	Neutrophil elastase
PA:	*Pseudomonas aeruginosa*
PS:	Preschool
R_{aw}:	Airway resistance
rhDNase:	Recombinant DNase
RVRTC:	Raised Volume Rapid Thoraco-abdominal Compression
SA:	*Staphylococcus aureus*
SM:	*Stenotrophomonas maltophilia*
sR_{aw}:	Specific airway resistance
s*R*tot:	Specific total airway resistance
TO:	Turnover
TNFα:	Tumour necrosis factor alpha
URSO:	Ursodeoxycholic acid
Y:	Year
Z-s	Z-score (standard deviation score)

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This work would not be possible without the collaboration of all the centres of the LCFC, whose members attended numerous meetings to advise on the project, and I thank them for their ongoing input to this longitudinal study. I also thank Action Medical Research and the Cystic Fibrosis Trust for funding this project.

I was amazed by the dedication and enthusiasm of all the families that participated in this project; meeting you all was such a privilege, and I hope that this work will contribute in some way towards improving CF care.

Lastly I would like to thank my family, particularly my parents, for their never-ending love and support. I can't quite believe that my niece and nephew were not even born when I started my studies, it has been such a joy to watch them grow.

Unfortunately, my grandfather passed away towards the end of this work, always ready with a joke or unexpected compliment to brighten my day. I miss him greatly and therefore dedicate this work to him.

Summary of publications and awards related to this thesis

Peer reviewed publications:

- **Monitoring early lung disease in cystic fibrosis: where are we now?** **Duncan J**, Aurora P. 2014. *Breathe*. 10(1): pp 34-47. (Review)

Abstracts:

- **Calculation of conductive inhomogeneity in children with severe CF lung disease: which method works?**
  Verger N, Arigliani M, Raywood E, **Duncan J**, Bush A, Aurora P. Thorax. 2016;71(Suppl 3):A183-A4

- **The Validation of nitrogen washout (N2-WO) for measurement of lung clearance index (LCI) and functional residual capacity (FRC) in preschoolers**
  Raywood E, **Duncan J**, Viviani L, Bush A, Aurora P. European Respiratory Journal 48 (suppl 60):PA1232. Sept 2016.

- **Improved lung function in preschool children with CF over the last decade.**
  Duncan J, Raywood E, Lee S, Davies G, Wade A, Bush A, Stocks J, Aurora Pediatric Pulmonology 2015, 50: S193–S453. doi: 10.1002/ppul.23297.

Awards:

2016 **The Ella Roberta Foundation Research Award (1st Prize)** for best poster presentation at The John Price International Paediatric Respiratory Conference, King's College, London, UK

2015: **The University College London Graduate Student Conference Fund Award** for oral presentation at The North American Cystic Fibrosis Conference, Arizona, USA

2014 **2nd Prize** University College London Graduate School Poster Competition, UK

1 Introduction

1.1 Early lung disease in children with cystic fibrosis

1.1.1 Introduction

Cystic fibrosis (CF) affects multiple organ systems, but the major cause of morbidity and mortality is lung disease. The lungs are essentially normal at birth[1], but from early life, a cascade of infection and inflammation leads to progressive structural damage, loss of lung function and ultimately respiratory failure. Animal and human studies indicate that these pathological processes begin shortly after birth[2, 3] and it is likely that early insults determine the progression and severity of subsequent disease. The exact mechanisms of how infection and inflammation affect the developing lung are still being described. Furthermore, how early deficit in lung function and structure predicts later outcome is not fully understood.

CF used to be a disease of early childhood, and in the 1980s life-expectancy was less than 20 years of age. Children born today with CF are expected to live to at least their fifth decade[4]. There have been many advances in CF care over this time period, one of the most important being the implementation of newborn screening (NBS). This was adopted nationally in the UK in 2008[5] and most children with CF are now identified shortly after birth, before any clinical evidence of lung disease is apparent, and treatment started within the first few weeks of life. The opportunity to intervene in the early stages of disease therefore arises, but although in previous studies nutritional outcomes are clearly improved in NBS infants compared to those clinically diagnosed with CF, the evidence of benefit on lung health is less clear when the two groups are compared[6].

Our ability to detect and monitor early disease in children with CF has also improved over the last twenty years. Lung Clearance Index (LCI) measured by the multiple breath washout (MBW) technique is highly feasible and has been shown to be a sensitive marker of abnormality in children under six years of age[7]. Low radiation dose computed tomography protocols have been developed and these techniques could potentially serve as regular surveillance measures. As earlier studies were based on children with a clinical diagnosis of CF, and considering the earlier identification of NBS infants and associated differences in care between these two groups, we now need to

describe how lung disease evolves in NBS children to inform potential interventions in clinical care, develop appropriate disease surveillance techniques and to power trials of new treatments.

1.1.2 The importance of identifying early lung disease in young children with CF

The ultimate goal of NBS is that earlier diagnosis and introduction of treatment in specialist CF centres will lead to improved outcomes. However most respiratory treatments in NBS children under six years of age are based on agreed guidelines of best practice and expert opinion[8]. There are few completed randomised controlled trials of commonly used treatments which are recommended from diagnosis in the U.K. A ground-breaking development in recent years has been the development of small molecule therapies that target the basic defect of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, instead of tackling the consequences of its dysfunction. VX-770 (ivacaftor), a CFTR potentiator, corrects chloride transport in class III mutations, the commonest of which is p.Gly551Asp, with sweat chloride going from very abnormal to intermediate levels, improved lung function and less frequent pulmonary exacerbations[9]. Subsequently there has been huge interest in developing similar molecules for more common mutations in CF. The combination CFTR corrector and potentiator lumacaftor/ivacaftor, although not as strikingly effective as ivacaftor in class III mutations, showed a reduction in pulmonary exacerbation in patients with the more common p.Phe508del homozygous mutation[10]. Preliminary data on triple therapy show similar efficacy to ivacaftor, even in patients heterozygous for p.Phe508del[11, 12]. What follows therefore is the opportunity to identify CF shortly after birth by NBS, start these potentially curative treatments, and prevent irreversible lung damage from an early age.

We must, however, proceed with caution. If lung function remains relatively normal in infancy, these treatments could be delayed, avoiding potential adverse effects on the developing lung, risks of long-term toxicity and increased treatment burden to patients. If we can pinpoint when lung disease takes hold and in which children, the appropriate time to deploy new treatments can be better determined and targeted to susceptible individuals. Previous work from the London Cystic Fibrosis Collaboration (LCFC) has suggested that structure and function are so nearly normal in the first two years of life that these novel treatments could be delayed. Key to achieving these goals is first to

understand the natural evolution of lung disease in children with CF up to six years of age in order to adequately power studies of existing and new treatments.

1.1.3 Aim of the thesis

The overall aim of the thesis is –

> *To describe the evolution of lung function from diagnosis to six years of age in a NBS cohort of children with CF managed with standard UK care*

To develop the specific hypotheses, aims and objectives stated at the end of this chapter, it is first important to consider evidence for the following, addressed in the remainder of the introductory chapter:

- How does early pulmonary disease affect lung function in young children with CF diagnosed by NBS?
- What techniques are available to detect and monitor lung disease in children under six years of age?
- What have studies in clinically diagnosed and NBS children with CF shown to date?

1.2 The pathophysiology of early lung disease in CF

CF is a multi-system disease affecting around 1 in 2500-3500 live births with a carrier frequency of 1 in 25 among Northern Europeans[13]. It is an autosomal recessive disorder caused by mutations in a single gene (*CFTR)* on the long arm of chromosome 7, resulting in a defect in CFTR protein. More than 1900 mutations in the *CFTR* gene have been described, and at least 1500 are disease-causing[14]. Amongst other actions, this multifunctional protein regulates ion transport across epithelial cells lining the airways, hepatobiliary system, sweat glands and reproductive tract. As well as progressive lung damage, manifestations of disease include pancreatic insufficiency, liver disease and male infertility.

The specific mechanism by which defects in *CFTR* lead to progressive lung damage are still debated, and a number of hypotheses have been proposed. CFTR protein functions as an ATP-gated anion channel found in the apical membranes of epithelial

cells. CFTR transports chloride, bicarbonate and thiocyanate ions, but also has an important role in the regulation of other ion channels, such as the epithelial sodium channel (ENaC)[15]. In normal airways, chloride secretion by CFTR and sodium absorption by ENaC maintain airway surface liquid (ASL) at the depth required for cilia to beat efficiently, facilitating effective mucociliary clearance. The early 'high salt' hypotheses was that defects in CFTR resulted in a high concentration of sodium and chloride in ASL and subsequently inhibition of anti-microbial proteins, increasing the risk of infection[16]. However a later study showed that CF ASL was iso- or hypotonic, and also appeared to be depleted in CF epithelial cell cultures, which led to the 'low volume' hypothesis[17]. The theory was that defective CFTR chloride secretion and sodium absorption by ENaC was thought to lead to a reduced total mass of these two ions in ASL, a reduced osmotic gradient for water and a dehydrated, reduced volume ASL causing defective mucociliary clearance, and predisposing to airway infection. Viral infection also plays an important role in ASL homeostasis. Despite a rebalance of ion transport to maintain ASL volume under phasic simulation conditions, after a viral insult this motion-dependent ATP regulation of ASL height was disrupted *in vitro* in CF epithelial cells[18].

Animal models give further insight into pathophysiological mechanisms of CFTR dysfunction. CF pigs lacking CFTR display typical features of human disease. They are born with structural airway abnormalities including underdeveloped nasal sinuses, narrow proximal airways and hypoplastic submucosal glands[19] and show airway obstruction and air trapping as a likely consequence, even before inflammation can be detected[20]. Further studies in this animal model have shown impaired CFTR-related bicarbonate excretion, increasing the acidity of ASL and inhibiting anti-microbial defence peptides[21]. Mucociliary transport is also affected secondary to poor detachment of mucus from submucosal gland ducts[22].

Early human studies[1] and more recent animal studies show that there is little evidence of inflammation in CF lungs at birth[2]. It is clear however that infection and inflammation take hold even in the first few months of life[3]. Young children with CF commonly develop infection with bacterial pathogens, initially most frequently *Staphylococcus aureus* and *Haemophilus influenzae*. *Pseudomonas aeruginosa* becomes more dominant with age, strongly linked to lung function decline and a poorer prognosis[23]. Lung disease initially starts in the small airways with mucus plugging and obstruction[24]. Repeated bacterial infection drives predominantly neutrophilic inflammation. There is an exaggerated inflammatory response and necrotic neutrophils release DNA which

further increases viscosity of secretions. Necrotic neutrophils also release granule products, which are pro-inflammatory and tissue damaging. This eventually leads to bronchiectasis, irreversible lung damage and end-stage lung disease.

Given that in CF the cascade of infection and inflammation starts in early life and affects the developing lung, robust outcome measures to detect and define when lung disease occurs are needed so we can then target interventions and test treatment response. However, measurements in young children pose particular challenges, and the various options to describe and quantify lung disease in this population is addressed in the next section.

1.3 Detecting and monitoring lung disease in young children with CF

Research in adults and older children with CF describing the progression of lung disease and effectiveness of treatments report robust outcome and monitoring measures. Serial measurements of the forced expired volume in one second (FEV_1) can be used to define pulmonary exacerbations, response to treatment and be an outcome measure in clinical trials. Its rate of decline is understood by clinicians as a monitoring tool and to predict prognosis in their patients. Lung disease in older patients is also usually more advanced, and structural changes on computed tomography (CT) can be quantified by robust scoring systems[25].

Children below the age of six years present a particular challenge when conventional monitoring methods are considered. Infants are not able to perform active measures of lung function. Many centres do not undertake spirometry in children younger than six years of age, and in the laboratories that do, results are often normal in young children with CF. The effects of regular monitoring with tests that exposure the developing lung to ionising radiation, such as CT, mean that they must be implemented with care.

A number of alternative techniques to describe lung disease in young children have therefore been developed. The following section will summarise the available monitoring tools and adaptations required to measure lung physiology, structure, infection and inflammation in younger children, and their role as outcome measures in clinical trials. Their ability to detect disease and use as surveillance and monitoring

tools in clinically diagnosed and NBS cohorts of children will also be discussed in detail in section 1.4.

1.3.1 Measures of pulmonary function

1.3.1.1 Infant lung function techniques

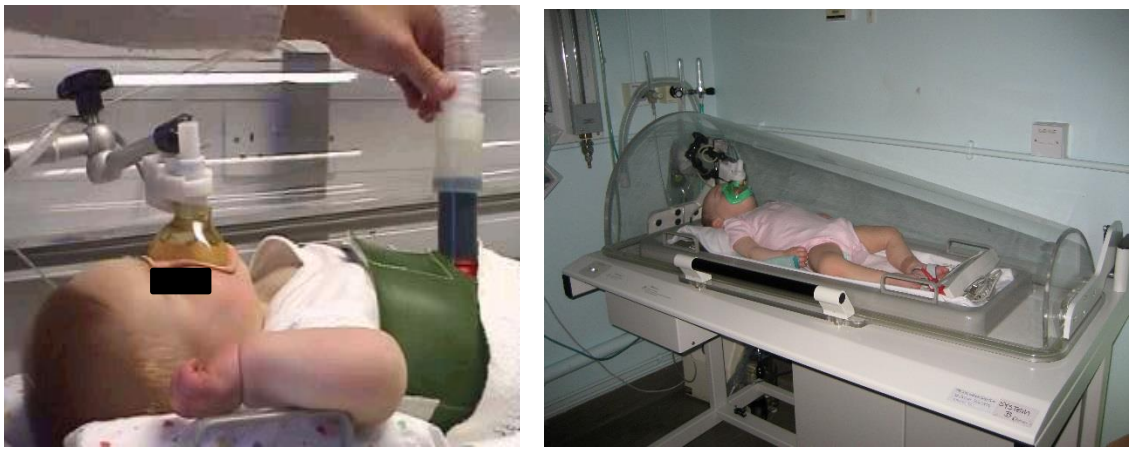
As infants less than two years are unable perform the forceful manoeuvres required in many lung function tests such as spirometry, a number of techniques have been developed that do not require active participation. Two giving the most useful and reproducible information in early CF lung disease are the raised volume rapid thoraco-abdominal compression (RVRTC) technique and whole-body plethysmography[26]. Both techniques are normally performed under sedation, or when the infant is asleep after feeding.

In the RVRTC technique[27], the lungs are inflated using a bag and mask towards total lung capacity with three to five breaths to produce an inspiratory pause. A vest previously wrapped around the infant's chest is quickly inflated and forces expiration to residual volume, reaching flow limitation and enabling measurement of forced expiratory flows and volumes[28]. 30cm H_20 inflation pressure is used when the infant is relaxed, and this allows flows from a reproducible lung volume to be assessed. The result is comparable with the expiratory flow volume curve in older children, but the forced expired volume in half a second ($FEV_{0.5}$) is normally reported, as most infants will have reached full expiration in one second due to the more rapid emptying of their lungs.

Lung volumes can be measured in an infant breathing against a closed shutter in a plethysmograph[29]. This device measures unknown lung volumes based on the principle of Boyle's law. This states that the product of pressure and volume is constant for a given mass of confined gas at a constant temperature. The infant breathes in the plethysmograph via a mask attached to a pneumotachometer which records air flow and volume. A shutter is then closed for less than ten seconds, retaining a fixed amount of gas in the lungs. As the infant breathes against the shutter, this gas expands and contracts in the closed system. If the changes in pressure and volume in the plethysmograph and the pressure at the mouth at the start of the

manoeuvre (assumed equal to atmospheric pressure, but this may not be correct if there is airflow obstruction) are known, the unknown volume of the lungs can be calculated. Plethysmographic functional residual capacity (FRC_{pleth}), the lung volume at the end of a tidal expiration can therefore be measured.

Figure 1.1: Infant raised volume rapid thoraco-abdominal compression and whole body plethysmography techniques



Legend: The figure on the left shows the raised volume rapid thoraco-abdominal compression technique. A sleeping infant wears a mask sealed to the face with putty to eliminate air leak, attached to a pneumotachometer. The green jacket around the infant's abdomen is rapidly inflated to a set pressure to produce a forced expiration. On the right, the closed whole body plethysmograph measures changes in pressure and volume to calculate plethysmographic functional residual capacity while an infant breathes against a closed shutter (carer consent given for inclusion of images)

There are published guidelines for testing both RVRTC[30] and plethysmography[29], and reference data for outcomes from both tests[31, 32]. Commercial devices for the measurement of infant lung function are available, and it must be noted that reference data should only be applied to measures collected with the same equipment using identical test protocols to avoid misinterpretation of results[33]. Within- and between-test reproducibility is good for both tests[34]. In a ten centre study of infants with CF, feasibility for RVRTC and plethysmography were relatively high (72% and 89% respectively)[35], but it was noted that despite meticulous training and quality control, feasibility varied significantly between sites depending on the experience of centres. Due to the need for sedation, adequate time for testing, highly trained staff and expensive equipment, infant lung function measures remain a research technique and are not a routine part of clinical care. It is also unknown whether abnormal infant

pulmonary function predicts later clinical outcomes in CF, and how to act on an abnormal result therefore is also unclear.

RVRTC and plethysmography both clearly discriminate infants with CF from controls in NBS and clinically diagnosed individuals[36-38], reflecting small airway obstruction, gas trapping and hyperinflation seen in early CF lung disease. Abnormal tests correlate with structural lung changes on CT[39] and inflammation on broncho-alveolar lavage (BAL)[40, 41]. In a group of 11 infants, lung function was shown to improve after antibiotic treatment of a pulmonary exacerbation[42], showing potential to detect change after treatment. RVRTC outcomes were used in the Infant Study of Inhaled Hypertonic Saline in cystic fibrosis trial, with $FEV_{0.5}$ showing a minor improvement with treatment[43]. The use of infant lung function outcome measures in interventional trials is limited due to the large numbers that would need to be enrolled; data from a multicentre observational study estimated that 150 infants would be needed in each treatment arm to detect a significant treatment effect and sufficiently power an interventional trial[35].

1.3.1.2 Lung function measures in preschool children

Spirometry

The preschool period between two and six years of age was previously referred to as 'silent' in that conventional lung function tests were not able to be performed, clinical symptoms are minimal, but lung disease is known to occur. Children less than five years of age are unable to meet quality control criteria for spirometry outcomes required by international guidelines in older children and adults[44, 45], but it is recognised that they can reproducibly perform a forced expiratory manoeuvre from as young as 2.5 years of age[45]. Quality control measures have therefore been adapted taking this into account, and standards for preschool spirometry were published ten years ago[46]. Feasibility of the technique improves with age, and by 5-6 years, most children are able to reproducibly perform this manoeuvre, but specialist laboratories with experienced staff are required to maximise success[47].

In an age-appropriate, relaxed and enthusiastic environment a maximal forced expiratory manoeuvre can be easily obtained in a preschool child. Training with games using bubbles or whistles and computerised incentives (such as blowing out candles) can help achieve maximal peak flow and vital capacity[47]. Quality control must include real-time inspection of flow-volume and volume-time traces. Criteria for preschool spirometry, adapted from international standards, are summarised in Table 1.1.

Table 1.1: Quality control for forced expiratory manoeuvres in preschool children

Start of test	Within-test	End of test	Repeatability
Rapid rise to peak expiratory flow	Smooth expiration, no glottic closure or cough	Plateau on volume time curve	2 manoeuvres within 0.1L or 10% of the highest value for FVC and FEV_t
Back extrapolated volume < 12.5% FVC or 80ml (whichever is greater)	No leak or obstruction of mouthpiece	Cessation of flow must not occur at 10% peak flow (may be able to report FEV_t)	1 manoeuvre may be reported if acceptable
	No early inspiration		

Legend: Preschool spirometry quality control criteria summarised from the American Thoracic Society (ATS) and European Respiratory Society (ATS/ERS) international guidelines[46]*. Abbreviations: FEV_t = FEV in 0.5, 0.75 or 1 second, FVC=forced vital capacity*

Because the lung empties more quickly than those of older patients, leading to shorter expiratory times, a full expiration is reached in many preschool children by one second. Therefore $FEV_{0.5}$ and $FEV_{0.75}$ are preferable outcome measures to FEV_1[45]. The largest FEV_t and FVC should be reported even if not taken from the same manoeuvre. Forced expiratory flows are taken from the expiratory loop with the largest sum of $FEV_{0.5}$ and FVC in a valid test[46].

When reporting results, it must be recognised that reference data from older children or adults cannot be extrapolated to those less than six years of age. Standard deviation ('z') scores are preferable to fixed cut offs (such as 80% of the predicted value often used in adults) as they also account for between subject variability[48] and the range of normality in children of different ages. Many software programs for commercial devices use a combination of reference equations to cover age ranges. The Global Lung Function Initiative 'all age' equations are based on measurements of over 4000 children, span an age range from 3 to 95 years and also consider ethnic group[49]. They include reference data for $FEV_{0.5}$ and $FEV_{0.75}$, use smooth reference curves in periods

of rapid growth and continuously cover the transition from child to adulthood[50]. They are therefore used in this thesis to report spirometric outcomes.
Mechanisms contributing to flow limitation are an important consideration when describing disease of the peripheral airways. Flow limitation is said to occur when flow does not increase despite increasing expiratory effort (or jacket pressure for RVRTC). The linear velocity of gas in the peripheral airways is very low, and resistance in the periphery of the lung has a lesser contribution to flow limitation during a forced expiration. Furthermore, if lung disease is regionally heterogeneous there may be increased flow through non-flow limited distal airways, masking reduced flow in other more obstructed airways. Therefore abnormal spirometry tends to represent pathology in the larger airways and is less sensitive to peripheral disease, such as that found in early CF.

Studies have shown that spirometric values are on average lower in preschool children with CF when compared to controls, but only a small proportion (9-36%) have an FEV_t below the normal range [7, 47, 51-54]. Spirometry is not sensitive to peripheral airway obstruction, the hallmark of early CF lung disease. Forced expiratory flows are reduced more than volumes in CF compared to controls[52, 53] and may be more sensitive to abnormality at this age. Although a familiar measure to clinicians, preschool spirometry in children with CF is so close to that in health that its use has been limited as an outcome measure in clinical trials as large numbers would be required to detect any significant intervention effect.

Plethysmography

The theoretical principles of plethysmography using a shutter to create a closed pressure and volume system were described in section 1.3.1.1. Whilst sleeping or sedated infants passively complete the manoeuvres necessary to measure FRC, it is extremely difficult for awake preschool children to tolerate breathing against a closed shutter. Specific airway resistance (sR_{aw}), a measure also derived from plethysmography, is a more acceptable test at this age. It is obtained during tidal breathing, assessing the relationship between airflow and change in plethysmographic pressure without manoeuvres during airway occlusion[55]. sR_{aw} is the product of airway resistance (R_{aw}) and FRC, and is derived by rearranging the equations used for plethysmographic ('box') measurement of these two outcomes[56].

R_{aw} is calculated using the principle of resistance being equal to pressure divided by flow, and is the rate of change in alveolar pressure to change in flow measured at the airway opening. An airway occlusion allows the relationship between change in mouth pressure (then equal to alveolar pressure) against box pressure to be derived.

$$R_{aw} = \frac{\Delta V_{box\ spont} / \Delta\ flow}{\Delta V_{box\ occ} / \Delta P_{mouth}}$$

where $\Delta V_{box\ spont}$ is the change in volume of the plethysmograph during spontaneous breathing, $\Delta V_{box\ occ}$ the change in box volume during efforts against an occlusion, and ΔP_{mouth} the change in mouth (alveolar) pressure against the airway occlusion[55]. As described in section 1.3.1.1 FRC is calculated by the change in box volume divided by the change in box pressure at the mouth (both when breathing against an occlusion), or:

$$FRC = \Delta V_{box\ occ} / \Delta P_{mouth}$$

These equations are combined to calculate sR_{aw} (R_{aw} x FRC):

$$sR_{aw} = \frac{\Delta V_{box\ spont} / \Delta\ flow}{\Delta V_{box\ occ} / \Delta P_{mouth}} \times \frac{\Delta V_{box\ occ}}{\Delta P_{mouth}}$$

They can subsequently be simplified, avoiding the need for airway occlusion to calibrate box pressure changes in terms of alveolar pressure changes:

$$sR_{aw} = \Delta V_{box\ spont} / \Delta\ flow$$

sR_{aw} has been measured successfully in children from 2 years of age[57]. Reference equations are available using measurements from over 2,872 healthy children as part of the Asthma UK initiative[55]. However, as part of this study, significant methodological differences between centres were noted, and a consensus for appropriate breathing frequency and other quality control factors are yet to be decided. Standard operating procedures are currently being developed.

As sR_{aw} is a product of airways resistance and FRC it is a useful measure in children with CF, as both are raised in obstructive lung disease or hyperinflation[34]. Two main studies have reported s*Raw* measures in children with CF[7, 54]. In both studies s*Raw* was significantly higher in children with CF than healthy controls, and was a feasible

measure in pre-schoolers (77-81%). In the latter study, s*Raw* was a useful serial measurement as it was persistently abnormal over a four-year period, and identified those with abnormal lung function earlier than spirometry[54]. A positive correlation has also been reported with lung clearance index[7]. s*Raw* has the potential to detect and monitor early CF lung disease in experienced laboratories, but its use as an outcome measure is presently limited by lack of specific methodological guidelines.

Forced oscillation and interrupter techniques

There are other techniques for measuring lung function in children but their role in detecting abnormalities in CF is less clear. Examples are the forced oscillation technique (FOT), also known as impulse oscillation, and the interrupter technique. They are both tidal breathing measures which are relatively easy to perform in pre-school children.

The forced oscillation technique involves applying a pressure wave of known frequency to the respiratory system and measuring the resulting flow. Respiratory impedance can be calculated as it is equal to pressure divided by flow. Oscillation waves generated by a loudspeaker are applied to the airway opening during tidal breathing. The pressure signal that is in phase with flow represents respiratory resistance (R_{rs}), and that not in phase the reactance (X_{rs}).

The interrupter technique measures the pressure at the mouth over a series of brief occlusions during tidal breathing. It assumes that alveolar pressure equilibrates with mouth pressure during the occlusions. Interrupter resistance (Rint), a measure of resistance of the respiratory system, is the ratio of pressure at the mouth measured after an occlusion and flow just before it[58].

There are conflicting results of FOT in young children with CF, with some studies showing abnormal R_{rs} and X_{rs} [59, 60] and others normal values[54, 61]. No relationship was found in these studies between FOT measures and respiratory infection. Most studies have shown that Rint does not distinguish young children with CF from healthy controls and that Rint remains stable over time despite other parameters indicating worsening disease[54, 62]. A multicentre study showed that spirometry in preschool children with CF was a more sensitive measure for detecting abnormality than forced oscillation[61]. Therefore, although feasible in preschool children, these techniques are rarely used to monitor disease in CF.

1.3.1.3 The inert gas washout technique

MBW is described here as it is a technique that can be used in both infants and preschool children. Inert gas washout was first described in the 1940s and examines gas mixing in the lung[63]. The bronchial tree divides into progressively smaller airways resulting in 23 airway generations. This division greatly increases the surface area of the alveolar membrane to enable efficient gas exchange. The conducting airways include generations 0-16, where linear velocity of gas is high but gas exchange does not take place. Spirometry reflects abnormalities mainly in the conducting airways where changes in linear gas flow affects forced expired flows and volumes. Generations 17-23 are the intra-acinar airways and alveoli where gas exchange starts to take place and flow velocity is low. At the entrance to the acinus the contributions to gas mixing of convection and diffusion become similar[64] and this is termed the 'convection-diffusion front'. Within the acinus, diffusion is the main gas transport mechanism. Resistance in the peripheral airways make little contribution therefore to flow limitation measured by spirometry, and abnormal function in these airways can be masked by normal flow in the distal airways. As gas mixing tests reflect the overall ventilation homogeneity of the lung, including the peripheral airways where early CF lung disease starts, they are more likely to reflect abnormality in young children with milder disease. Ventilation inhomogeneity theoretically can arise from pathology at three sites; proximally in the conducting airways (convection dependent), in the more distal airways and acini (diffusion dependent) and at the acinar entrance (diffusion-convection front)[65].

Multiple breath washout involves recording the concentration of an inert tracer gas and respiratory flow as the subject breathes through a sealed mouthpiece or mask. It is a tidal breathing measure and therefore can be measured in a sedated or sleeping infant, or in a passively cooperative preschool child. A mask sealed with putty is used in younger children and infants to ensure a tight seal and thus no leak during the washout. A number of inert gases can be used for this technique, but must have low or no solubility in blood and tissues to ensure they do not participate in gas exchange. Examples are sulphur hexafluoride (SF_6) and helium (He), which are 'washed in' to an equilibrium concentration before the gas supply is disconnected at end expiration, and the tracer gas 'washed out' by breathing air. Resident nitrogen (N_2) can also be used, which does not require a wash-in phase as it is washed out by breathing 100% oxygen (O_2).

In a normal lung, the distribution of gas between parallel units is relatively equal. During the washout phase, the tracer gas concentration progressively decays with each volume turnover of the lung. The lung clearance index (LCI) reflects the number of times the functional residual capacity must be 'turned over' to bring the tracer gas to reach a set concentration[28], commonly 1/40th of its starting value. This arbitrary cut off is used as historically it was the limit of the operating range of early N_2 gas analysers. It is a simple way to report overall ventilation inhomogeneity measured during the washout and is an index of the turnover volume divided by the measured FRC.

$$LCI = \frac{\text{Cumulative Expired Volume}}{FRC}$$

FRC can be calculated as the initial concentration of tracer gas is measured at the beginning (C_{init}) and end (C_{end}) of the washout, as is the total volume of gas expired.

$$FRC = \frac{\text{volume of gas expired}}{(C_{init}) - (C_{end})}$$

In diseased airways, inflammation, mucus plugging and airway wall damage alter gas mixing, and it becomes inhomogenous[66]. Gas is not distributed evenly between lung units, and washout of gas is faster in some areas and slower in those more poorly ventilated. Therefore a higher number of turnovers are required to washout the tracer gas, resulting in a higher LCI. FRC measured by MBW includes only the volume that equilibrates with tracer gas during tidal breathing, and may be a smaller volume than that calculated by plethysmography, which measures total thoracic gas[67]. LCI is insensitive to a completly occluded airway since gas wash-in and –out does not occur distal to the blockage.

Methodological considerations

SF_6 and N_2 are the most commonly used tracer gases recently employed in studies of young children with CF. Both have advantages and disadvantages. SF_6 has no taste, odour or colour and is non-toxic in concentrations used in MBW devices. However, it is not licenced for medical use and is a potent greenhouse gas, precluding its use in some countries. It is also costly and has limited availability, and therefore devices using lower concentrations of SF_6 may be preferable. 100% O_2 used to displace resident N_2 in the lungs (N_2 washout) is a much more readily available and affordable

gas. A wash-in phase is not needed by this method, but time is needed between washouts for gases in the lungs to re-equilibrate. However, this high concentration of O2 has been reported to alter tidal breathing in infants, and is therefore less used at this age[68]. Different LCI values are obtained during simultaneous N_2 and SF_6 washouts, thought to be secondary to back-diffusion of tissue N_2 into expired gas[69], so results are not directly comparable.

Gas concentrations are measured by a fast gas analyser. A commonly reported technique in studies of children with CF uses a mass spectrometer with 4% SF_6, but this device and associated equipment is custom made, and not available for widespread clinical use. A number of commercial devices have been developed for MBW analysis. The Innocor device (Innovision, Odense, Denmark) uses a photo-acoustic gas analyser with a gas reservoir bag and 0.1% or 0.2% SF_6, a lower concentration to that commonly used in mass spectrometry. It has been approved by the Food and Drug Administration for use in the US. It can be used in older children and a modified method *in vitro* for potential use in infants has been recently described[70]. Other commercial devices use ultrasonic flowmeters in mainstream (sensor at the sample site) or sidestream (a sample of expired gas is diverted to the analyser) positions. The Exhalyzer D (Eco Medics AG, Switzerland) uses 4% SF_6 in children under two years of age (avoiding 100% O_2 use in infants), and measures tracer gas concentration via a molar mass mainstream analyser. The lack of algorithms to account for the effect of temperature and humidity on mainstream molar mass measurement limits its use in older children in whom it has not yet been validated. It can be used for N_2 wash-out from preschool age, calculating N_2 concentration indirectly via O_2 (mainstream) and carbon dioxide (CO_2) (sidestream) measurement. The EasyOne Pro (ndd, Medical Technologies, Switzerland) is a further device for N_2 wash-out and measures sidestream molar mass (estimating N_2 from molar mass), but the large equipment deadspace means it is not suitable for use in infants, and it is yet to be validated in preschool children.

Guidelines for technique and quality control for MBW are available[71]; specific guidelines for preschool children have been published[46, 72] and are awaited in infants. In experienced centres, MBW is highly feasible, with success rates of over 80% in sedated infants and preschool children[7, 73]. There is greater within-test (30%)[74] variability of LCI in infants when compared to pre-school children (5.2%)[7] which may limit its clinical utility in the former age group. Reference data for LCI using 4% SF_6 and mass spectrometry have been published for children from two weeks to 19 years

of age[75]. As previously discussed, they are equipment and tracer gas specific, with higher LCI values reported for N_2 washout than when using SF_6[76]. To date, reference data have not been published for the aforementioned N_2 wash-out devices, and the Exhalyser D device measured a higher LCI, FRC and CEV than the EasyOne Pro in a comparison study[77]. Comparison to healthy control subjects are therefore required in studies using these methods of data collection.

Studies using LCI as an outcome measure

Although a technique described for many years, the rejuvenation of interest in using MBW to detect peripheral airway disease has stemmed from the development of faster gas analysers and commercial devices. In CF specifically, LCI has been shown to be a sensitive marker of abnormality in preschool[7, 78] and school-aged children[51] with mild disease, leading to a resurgence of this technique for use as a monitoring tool and outcome measure in clinical trials. Kraemer et al. described lung function in 142 children with CF from 6 to 20 years of age, and found that LCI was the earliest and strongest measure of progression than any other lung function measure in the study, including FEV_1 and plethysmography[79]. Two main groups, namely the LCFC, UK and the Australian Respiratory Early Surveillance Team for cystic fibrosis (AREST-CF, Australia) have described lung function, including LCI, in cohorts of clinically diagnosed and NBS children. As these reports are so pertinent to the work in this thesis, they are described in detail in section 1.4.

As LCI clearly distinguishes between children with CF and healthy controls, and is more sensitive than spirometry, it has great potential to identify children with early subtle lung abnormalities and therefore can be used as an outcome measure in clinical trials. To date, LCI has detected a treatment effect in trials of dornase alpha[80], inhaled hypertonic saline[81, 82], ivacaftor[83] and ivacaftor/lumacaftor[84]. Baseline LCI has also been shown to predict pulmonary exacerbations in children with CF, even in a subgroup with normal FEV_1[85]. Its use is currently limited to centres with facilities to perform the test, but validation of commercial N_2 washout equipment in pre-school children is currently underway, which should make the test more widely available. Studies have also shown that MBW washouts to 1/20th of the start concentration, as opposed to the arbitrary cut off of 1/40th, can significantly shorten the time of testing without compromising diagnostic information[86, 87], but may reduce sensitivity in younger children[86]. MBW can be used to inform clinical management in a population where other lung function tests are difficult to obtain, but it has still not been shown that regular monitoring of LCI results in an improved outcome[88].

1.3.2 Imaging techniques

Progressive structural changes, particularly bronchiectasis, are classical findings in adults with CF[89]. Abnormalities can also be found in young children; post-mortem histological specimens have shown bronchiectasis and mucus plugging from as early as four months of age[90]. Advances in CT imaging provide the opportunity to view lung abnormalities in increasing detail, and CT is still considered the gold standard for the detection of bronchiectasis[91]. It is much more sensitive than chest radiography in detecting CF lung disease[92]. As more sophisticated techniques and scoring systems are developed, there is opportunity to quantify architectural irregularities, even in children.

As infants and young children are unable to breath-hold cooperatively, scans are usually performed under general anaesthesia. Standardisation of lung inflation volume is particularly important, as the extent of structural change imaged varies with breathing phase. Images of inspiration and expiration have been compared in children with CF below five years of age; airway dilatation was detected in 30% of inspiratory scans but only 9% of expiratory scans[93]. Therefore recent studies using CT in infants have used a strict ventilation protocol during acquisition of images to minimise atelectasis, and administer a 'breath-hold' at a set volume (usually at 25cm H_20) during the inspiratory scan[3, 94]. A standardised agreed protocol between centres is crucial in research studies to enable comparison of outcomes.

Concerns over radiation exposure in such young children have led to the development of low-dose scanning techniques[95]. 'Limited slice' protocols are used taking three to six images at standard anatomical positions in inspiration and repeated in expiration to minimise radiation dose[96]. However due to the irregular distribution of lung disease in CF, limited slice protocols may neither capture all areas of structural abnormality[97] nor provide adequate resolution to quantify early disease[39].

A number of scoring systems can be used to quantify structural abnormalities seen on CT, and therefore may vary in reports from different cohorts. In infants, the LCFC and AREST-CF groups initially used a modified version of the Brody-II score[3, 94]. The score has 95% reproducibility in children between seven and 15 years[98] but it is important to note that it has not been validated in children under seven years. In more recent studies, AREST-CF use the PRAGMA-CF (Perth-Rotterdam Annotated Grid Morphometric Analysis for CF) system with an aim to quantify abnormality by annotating a grid overlaid on axial slices of the scan[99]. Automated quantitative scoring

systems also have potential to be more reproducible scoring measures, but again require validation in this population. Whilst different scoring systems describe the extent of similar parameters (such as bronchial dilatation and air trapping), it must be noted that they are not interchangeable when comparing reports.

CT scores have been shown to improve after treatment for a pulmonary exacerbation[100], dornase alpha[101] and inhaled tobramycin[102] in older children, demonstrating the potential to detect a treatment effect in interventional trials. Abnormalities in early childhood appear to predict lung disease progression, with abnormal CT score at a mean age of 7.5 years associated with significantly worse lung function and chest radiograph scores in later life[103]. However, CT data in infants and preschool children, and how imaging can predict future decline are scarce, likely due to concerns over frequent irradiation of developing lungs. The LCFC and AREST-CF groups have reported CT findings in NBS children with CF under 6 years of age, and these studies are discussed in detail in section 1.4.

In addition to the limitations of scoring systems and need for general anaesthesia, cumulative radiation doses in CF patients are a concern, particularly as life expectancy has increased significantly over the last decade. Magnetic resonance imaging (MRI) as a non-ionising radiation imaging technique could be an alternative to CT, but it is less often used in studies of the lungs due to air-tissue interfaces and low proton density that lead to weak magnetic resonance signals[104]. Acquisition time is often long and the environment disconcerting to young children, so many require sedation or general anaesthesia. However it is a rapidly developing field; techniques of improved structural protocols and contrast enhanced MRI can clearly show abnormal lung structure and perfusion even in infants and preschool children when compared to healthy controls, and can demonstrate response to treatment[105], supporting the development of MRI as an outcome measure. Hyperpolarized gases (typically He or Xenon) can be administered by inhalation before MRI in order to image ventilation defects in the lungs in fine detail. A study in children under 16 years of age with CF showed ventilation imaging was the most sensitive marker of abnormality when compared to CT, MBW and conventional MRI modalities[106]. The technique described requires a single breath hold, so is limited in very young children and infants, but a proof of concept study has recently been reported in non-sedated infants and preschool children[107]. Further development of this method shows promise as a monitoring technique that does not involve irradiation of young children.

1.3.3 Measures of lung infection and inflammation

A common management strategy in adults with CF is sputum culture-based pathogen surveillance and treatment with an appropriate antibiotic. Even when unwell, many infants and young children will be non-productive of, or unable to, expectorate sputum. Oropharyngeal swabs (OPS) are therefore often used in children, by asking the patient to cough onto a cotton swab placed at the back of the throat, or with a cough stimulated by placing the swab against the posterior pharynx. Sputum can also be induced by administration of nebulised hypertonic saline. These methods of collection have been compared to BAL, the 'gold standard' for sampling lower airway microbiology. As obtaining BAL involves sedation or general anaesthesia, alternative methods that accurately reflect lower airway infection are preferred. Although OPS have high specificity and negative predictive value, they have low sensitivity when compared to BAL, and poor predictive ability to reflect pathogens in the lower airways[108]. A within patient comparison of cough swab, induced sputum and BAL in children aged from six months (72 children under six years of age) showed a higher number of bacterial isolates in induced sputum when compared to OPS (92% versus 31%)[109], agreeing with earlier data showing poor diagnostic value of OPS for lower airway infection. They also compared induced sputum with single, two and six lobe BAL, and found more pathogens isolated as more lobes were sampled. Induced sputum found 69% of pathogens detected by BAL, but in some cases different organisms were isolated by the two techniques. In conclusion, a combination of induced sputum and six lobe BAL were proposed for lower airway microbial surveillance, and BAL could be reserved for those with negative induced sputum or persistent symptoms.

An ERS taskforce statement was published in 2000 giving guidelines for obtaining, storing and processing lavage fluid[110]. It is recommended that lavage is performed in the most affected lobe, or right middle lobe in the presence of diffuse disease, but there is no consensus on aliquot volume and number at present. As CF lung disease may not be uniformly distributed throughout the airway, lavaging a single lobe has the potential to miss areas of disease[111].

As well as pathogens, biomarkers of inflammation can be measured. The number of available assays is vast, but data on reproducibility of markers scarce. Techniques for assaying individual markers vary between laboratories, and can be complex and expensive. The European Cystic Fibrosis Society Clinical Trial Network Standardisation Committee published a review on BAL biomarkers as outcome

measures, and focus on neutrophil count or percentage, neutrophil elastase, interleukin-6 and interleukin-8 as most useful currently in CF[111]. Other difficulties with processing BAL are the varying volumes and dilution of fluid retrieved from each patient. Values of biomarkers can vary in sequential aliquots, and individual or pooled aliquots can be analysed[111]. A recent study reported a high level of concordance between pooled and non-pooled BAL samples for pathogen identification in young children with CF, and pooling of BAL did not negatively impact on the detection of inflammatory biomarkers[112]. Centres researching the role of BAL in young children with CF should therefore develop protocols in an attempt to standardise test procedures, but techniques still vary between reports.

Adverse events (such as fever) are common with BAL but usually minor[113]. Obtaining BAL samples to identify pathogens could therefore be a safe and useful surveillance and outcome measure in children less than six years of age. Inflammatory markers in BAL have been studied in trials of tobramycin inhalation solution[114] and rhDNase[115] but no clear change in any one marker was found. Furthermore, in a randomised controlled trial of children under five years of age of BAL-directed versus standard therapy in pseudomonas aeruginosa infection, no difference in the rate of pseudomonas eradication or CT score was found between the two approaches[116]. BAL remains useful in the early stages of trials to describe pathophysiological processes in CF[111], and free neutrophil elastase activity may be a potential marker for more severe disease, as shown in the AREST-CF studies described in detail in section 1.4. It is a useful clinical tool when infection is suspected in the face of otherwise negative microbiology, but it is still an invasive procedure requiring sedation or general anaesthesia, and without a clear benefit seen when treating infection detected only on BAL, routine surveillance using this method cannot yet be advocated.

1.4 Studies of early disease monitoring in young children with CF

1.4.1 Newborn screening

Newborn screening for CF in the UK is performed on a blood spot card obtained on around day five of life using an assay for immunoreactive trypsinogen (IRT). If the assay result is above a cut off level it is repeated and if persistently raised, DNA analysis is performed for the four most common CF mutations in the UK population. If two disease-causing CFTR mutations are found, a sweat test is performed to confirm

the diagnosis. In those with one mutation, IRT is repeated and extended CFTR analysis sent. If the second IRT is raised, again a sweat test is performed. The median age of diagnosis in the UK was two months in 2017[117], and newborn screening for CF has taken place nationwide since 2007.

Before NBS using the IRT assay became widely available, the diagnosis of CF was usually made by the detection of clinical signs. Children had often already developed respiratory symptoms and potentially irreversible lung damage by this time. NBS gives the opportunity to commence treatment shortly after birth, with an aim to prevent early pathology, rather than just treat the secondary effects of CF. There has only been one randomised controlled trial (RCT) of NBS for CF[118]. The Wisconsin study showed clear improvement in nutritional markers, but after ten years of follow up, NBS children had poorer chest radiograph scores, no difference in lung function and, of concern, a higher rate of *Pseudomonas aeruginosa* infection compared to those non-NBS[6]. Since this study there have been clear improvements in CF care, including segregation and regular surveillance of patients in specialist centres, the lack of which may have influenced the Wisconsin findings. This does underscore that screening without proper treatment facilities may actually be harmful.

Reports of observational studies comparing screened and unscreened cohorts give evidence for better pulmonary outcomes in NBS groups. The Wisconsin study reported chest radiograph scores at diagnosis; only 25% of NBS infants had evidence of established lung disease, compared to half of the clinically diagnosed group[6]. A recent Canadian registry study found lower incidence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in NBS patients compared to those diagnosed clinically within the same time period[119], and the Wisconsin study found later acquisition of *Pseudomonas aeruginosa* in NBS infants. In terms of long-term follow up, a study from the Netherlands showed a slower rate of lung function decline at 12 and 17 years compared to those clinically diagnosed[120, 121]. Australian NBS patients showed better lung function on transfer to adult care than those diagnosed clinically, and improved survival at 25 years of age[122]. Overall, in addition to clearly superior nutritional health in NBS patients, it is accepted that screening for CF can lead to improved outcomes in lung health, and has additional benefits of cost saving and reduced mortality and morbidity in early life[123].

Longitudinal cohort studies in both groups of children inform us of the evolution of CF lung disease. The two cohorts with the longest follow up to date of children with CF

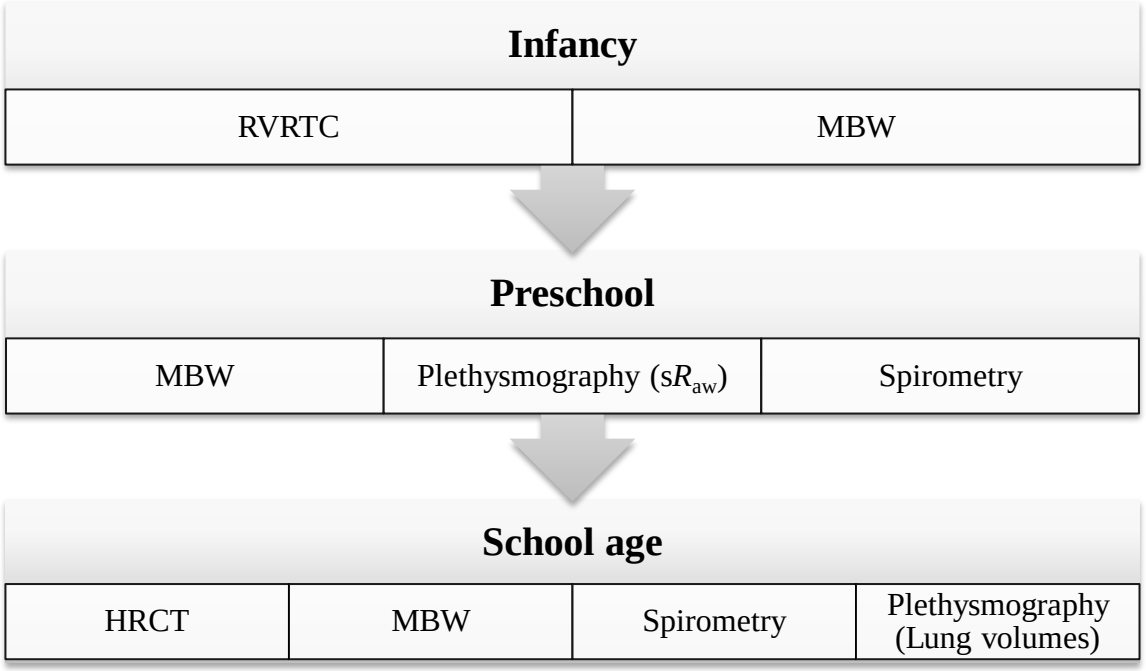
from diagnosis come from the aforementioned LCFC and AREST-CF groups. The LCFC report two cohorts; the first of clinically diagnosed children (cohort 1) with CF and a second cohort of NBS infants (cohort 2) diagnosed with CF around ten years after cohort 1. AREST-CF have studied children who were diagnosed by NBS. These studies are therefore discussed in detail in the following section as they form the main body of literature to inform the research in this thesis.

1.4.2 Studies in children clinically diagnosed with CF

1.4.2.1 The LCFC longitudinal study of clinically diagnosed children with CF

The first longitudinal study from the LCFC is an extensive report of the evolution of lung function in clinically diagnosed children with CF (cohort 1). The LCFC is a collaboration between six specialist CF centres in London with an aim to study and develop methods to monitor early lung disease from diagnosis. This group reported lung function and structure in children with CF from infancy to school age, and current follow up in adolescence is underway. Children with CF and contemporaneous healthy control children were recruited into the first phase of this study from 1999. Infants were tested shortly after diagnosis (median seven months), and in later infancy (up to 22 months) with follow up in the preschool years (2.5-6 years) and at school age (6-10 years). A summary of measures in these young children with CF at each study time point is presented in Figure 1.2

Figure 1.2: Measures at each time point of the London cystic fibrosis collaboration study of cohort 1 children with CF



Legend: Measures of the London cystic fibrosis collaboration study in clinically diagnosed children with CF in infancy (2-22 months), preschool (2.5-6 years) and school age (6-10 years).

In the first report from this group[124], infants (33 subjects with CF at diagnosis, 87 controls) underwent RVRTC at a median age of 30 weeks (range 7-93) when clinically stable. The median age of diagnosis of CF was nine weeks, but ranged from 0 to 55 weeks. Even at this early age, infants with CF had significantly poorer lung function than healthy controls, with mean $FEV_{0.4}$ [95% CI] 42ml [-54ml;-29ml] lower in those with CF. Importantly, the same deficit was found even in CF subjects without previously documented respiratory illness. Some infants were tested again at least six months later, with paired measurements in 34 infants with CF, after they had received treatment in specialist centres[38]. Despite treatment, no catch up in lung function was seen, and infants with CF had a mean [95% CI] $FEV_{0.5}$ z-score -1.6 [-2.7;-1.1] lower than controls at diagnosis and -1.9 [-2.5;-1.1] lower six months later. The mean deficit in $FEV_{0.5}$ was 20% in those with CF. Ominously, subsequent studies failed to show any evidence of catch-up growth.

The LCFC group went on to further explore pulmonary function in infants, and later recruited infants who underwent RVRTC and MBW (39 subjects with CF, 21 controls)

aged two to 22 months[73]. Again, infants with CF had significantly poorer lung function than controls. Mean LCI [95% CI] was 1.2 [0.7;1.7] units higher, and $FEV_{0.5}$ -1.7 [-2.3;-1.1] lower in those with CF. 22 (56%) of infants had an abnormally high LCI; 13 also had an abnormal RVRTC outcomes ($FEV_{0.5}$, FEF_{25-75} and FVC), and of those with abnormal RVRTC only six had an abnormal LCI. It was therefore proposed that these measures could be complementary in infancy, and when used together could detect disease in 72% of infants.

The next time point of this study was follow up of children in the preschool years. MBW, sR_{aw} and spirometry were measured in 40 children with CF (mean age [SD] 4.1 [0.9] years) and in 37 age matched controls again when children were clinically well[7]. LCI was shown to be the most sensitive marker of disease in preschool children with CF; 73% had an LCI above the upper limit of normal of the control group, and children with CF had a mean LCI [95% CI] 2.7 [1.9;3.6] higher. A lower proportion of children had abnormal sR_{aw} (47%) and $FEV_{0.5}$ (only 13%). Despite no association being found in infancy with bacterial isolates and lung function, preschool children infected with *Pseudomonas aeruginosa* had significantly higher LCI than those who did not. Kozlowska et al. then reported forced expiratory flows and volumes (FEFV) longitudinally from infancy to preschool age, with paired results in 48 children with CF and 33 controls[125]. Children with CF had a mean reduction of 7.5% in $FEV_{0.75}$ and 15.1% in FEF_{25-75} over the six year study when compared to controls. Those that had isolated *Pseudomonas aeruginosa* before any lung function test had a further reduction in FEFV outcomes (17% in $FEV_{0.75}$). Importantly, low lung function on infant testing was not associated with subsequent infection with *Pseudomonas* aeruginosa, and the lung function deficit was similar in children who no longer isolated this organism at preschool test and those who did. This suggests that infection precedes a fall in functional measures, and isolation of *Pseudomonas aeruginosa* does not just reflect poor respiratory status. Furthermore, no association was seen between total courses of intravenous antibiotics and FEFV parameters.

Finally, this clinically diagnosed cohort was tested between six and ten years of age, and the ability of preschool LCI and FEV_1 to determine the same lung function measures at school age were reported (48 subjects with CF, 45 controls)[51, 126]. LCI and FEV_1 were significantly poorer than controls at school age with mean difference [95% CI] of 3 [2.7;4.4] units and -1.28 [-0.8;-1.8] z-scores respectively. Preschool LCI was abnormal in 73% of children with CF, and had a positive predictive value of 95% for any abnormal school age lung function result and negative predictive value of 62%.

For FEV_1 (only 5% abnormal at preschool test) the positive predictive value of preschool FEV_1 was 100% but negative predictive value 25%. LCI remained normal at school age in 9 of 11 subjects with normal preschool LCI. LCI was considered a sensitive marker of early lung disease, as nearly all subjects with abnormal preschool LCI went on to have abnormal school age lung function, and two thirds had normal LCI at both preschool and school age test occasions. No significant differences were observed in any preschool or school age lung function outcome in children who had ever isolated *Pseudomonas aeruginosa* and those who had not. In addition to MBW and spirometry, high resolution CT (HRCT) was performed in 60 subjects with CF at school age on the same day as pulmonary function tests. 89% of children with abnormal LCI had abnormal HRCT. LCI was the most highly correlated measure with total CT score (Spearman's R 0.77) compared to other lung function measures (spirometry R=-0.43). Of the nine children with normal LCI, five had abnormal CT and of nine children with normal CT, five had abnormal LCI. It was therefore suggested that LCI could be an alternative measure to HRCT, and that CT could be limited to those with normal LCI, reducing cost and radiation exposure in young children.

In summary, the LCFC studies in clinically diagnosed children with CF show that lung function is abnormal at diagnosis and does not improve during infancy despite specialist treatment. In infancy, LCI and RVRTC are complimentary measures and early infection with *Pseudomonas aeruginosa* is an important determinant of later lung function. By the preschool years, LCI is the most sensitive marker of abnormality and can predict abnormal school age lung function. School age LCI also correlates highly with abnormalities of lung structure. LCI was therefore proposed to be an important marker for monitoring early disease and a suitable outcome measure for future clinical trials. Awaited reports of follow up in adolescence will show how these earlier measures can predict future decline.

1.4.3 Studies in children with CF diagnosed by newborn screening

1.4.3.1 The LCFC study of NBS infants with CF

After implementation of NBS in the UK, and the subsequent earlier identification and treatment of children with CF than those presenting with clinical symptoms, the LCFC recruited a second cohort of infants at diagnosis. In infancy, lung function (RVRTC,

plethysmography and MBW) under sedation were measured, with primary outcomes of $FEV_{0.5}$, FRC_{pleth} and LCI z-scores recorded for the three tests respectively. Infants underwent testing around three time points; three months, one year and two years of age. At one year, HRCT and BAL were performed under general anaesthesia in addition to lung function measures. At three months (mean age [SD] 11 [2] weeks), a similar deficit was found to those with a clinical diagnosis of CF, with all lung function outcomes significantly poorer than the age matched healthy control group[36]. In 71 infants with CF, mean [95% CI] $FEV_{0.5}$ z-score was -0.9 [-1.3;0.6] lower, FRC_{pleth} z-score 0.85 [0.4;1.3] higher and LCI z-score 0.5 [0.1;0.9] higher than controls, and an abnormal result found in 25%, 18% and 21% respectively. Overall, 44% of infants had at least one abnormal result.

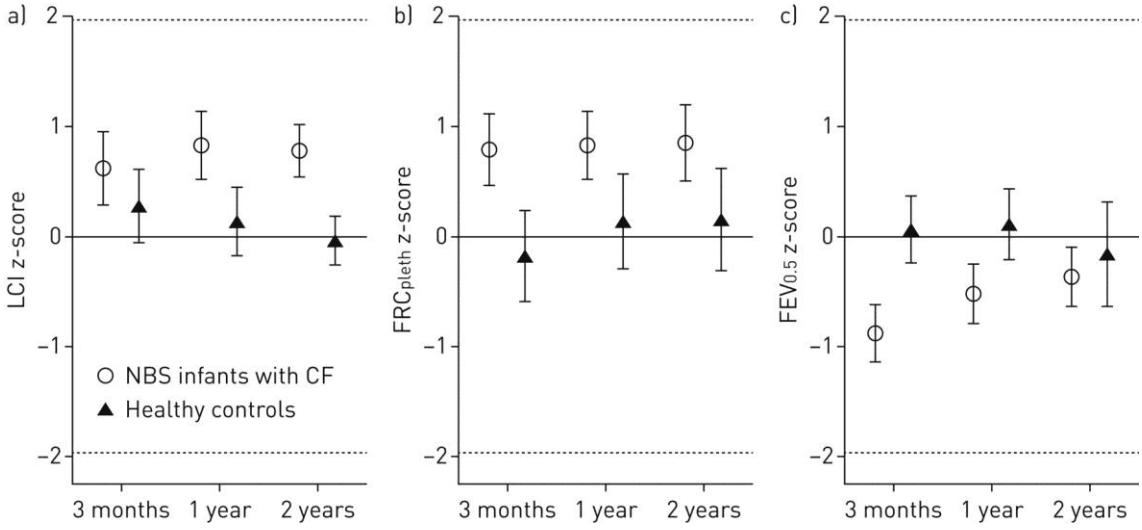
Infant lung function was repeated in these subjects at one year of age, with the addition of HRCT and BAL. Unlike those with a clinical diagnosis of CF, lung function showed an improvement over time in 72 NBS children with paired three month and one year measurements[127]. By one year, mean $FEV_{0.5}$ z-score [95% CI] was only 0.52 [0.89;0.15] lower than controls and increased by 0.59 [0.18;0.99] z-scores from the three month test. Furthermore, little change was seen in mean LCI (0.24 z-scores higher) and FRC_{pleth} (0.04 z-scores lower) when compared to controls at one year. The percentage of CF subjects with abnormal results decreased to 9% for $FEV_{0.5}$, was 16% for FRC_{pleth}, and 18% for LCI. Of 33% with any abnormal lung function test at three months, only 10% remained abnormal at one year. Somatic growth in CF subjects also improved; there was no difference to the control group at one year of age, and change in weight between tests was associated with lower (improved) FRC_{pleth}. On linear regression analysis, *Pseudomonas aeruginosa* was a significant determinant of one year FRC_{pleth} and clinician diagnosed wheeze was a predictor of LCI. No significant associations were found between other bacterial growths, nebulised mucolytic therapy, treatment for gastro-oesophageal reflux disease, or number of courses of antibiotics from birth with any of the one year lung function outcomes.

65 infants with CF also had chest HRCT at one year of age[94] using controlled ventilation under general anaesthesia. The Brody-II CT score was used by two experienced paediatric radiologists blinded to subject clinical and laboratory information, one of whom was Alan Brody who originally developed the score. The protocol used rendered more than 20 image 'slices' rather than the six used in the AREST-CF study[128]. The main finding of this study was that abnormalities were very mild in infants, so mild in fact that, apart from air trapping, inter- and intra-observer

repeatability was poor to fair for all other components of the score, including bronchiectasis and bronchial wall thickening. 90% of the differences in score were between a score of zero (normal) and 1 (mild disease), reflecting the subtle changes seen. Bronchial dilatation (defined as bronchial luminal diameter to arterial diameter ratio >1) was seen in 26% and air trapping in 42%. While change in any component of the Brody II score was seen in 52% of infants, only 7 (11%) and 2 (3%) were deemed to have an abnormal total CT score (>5% of the maximum possible score) by scorer A and B respectively. They concluded that further evaluation of CT scoring systems was needed, and without knowledge of the long-term implications of changes in infancy, CT with its associated risks could not be advocated as a regular monitoring tool or outcome measure in clinical trials.

Lung function at two years has recently been reported in these children[129]. In 62 infants with CF, $FEV_{0.5}$ z-score was now not statistically different to controls, with mean difference [95% CI] -0.21 [-0.7;0.29]. LCI was on average 0.81 [0.45;1.17] (p=0.001) z-scores higher and FRC_{pleth} 0.69 [0.11;1.26] z-scores higher (p=0.02) than controls. Abnormal results (>+/- 1.96 z-scores) for $FEV_{0.5}$, LCI and FRC_{pleth} were seen in only 7%, 15% and 19% of infants with CF for the three primary outcomes respectively. No child had abnormal LCI or $FEV_{0.5}$ at all test time points, and only two abnormal FRC_{pleth}. Figure 1.3 illustrates lung function across the first 2 years of life in these infants.

Figure 1.3: Lung function in newborn screened infants with CF from 3 months to 1 year from the London cystic fibrosis collaboration study



Legend: The evolution of lung function in 62 newborn screened infants followed up at two years in the London cystic fibrosis collaboration study, reproduced with permission from[129]

This study also reported evidence of 'tracking' in $FEV_{0.5}$ and FRC_{pleth}, with a highly significant relationship over the three test occasions for these individual outcomes. The same pattern was not seen in LCI, and despite this being the most sensitive marker of abnormality at two years, two year LCI was not predicted by earlier LCI measurements, either at three months or one year. When results were related to clinical status, a greater improvement in $FEV_{0.5}$ was seen in CF subjects who had not isolated *Pseudomonas aeruginosa* by the two year test. Apart from this, there were no other relationships seen between isolation of *Pseudomonas aeruginosa*, isolation of any other bacterial pathogen, or courses of intravenous antibiotics with magnitude or direction of change in all three lung function outcomes at two years.

This study did not demonstrate a progressive deterioration in lung function for NBS infants, and suggests that functional abnormality may be reversible, as infants with abnormal tests often reverted to normal at a subsequent test occasion. It did not therefore identify infants who could be targeted for novel therapies as no infants had persistently abnormal lung function throughout the study period.

In summary, the LCFC NBS infant studies show that abnormal lung function at three months recovers by one year, and is stable to two years in infants managed with standard UK CF care. Such mild changes were seen on one year chest CT that scoring was poorly reproducible. No subgroup of individuals was identified for which to target for enrolment in clinical trials of therapy, and neither CT nor infant lung function measures could be advocated for use in regular clinical monitoring or as trial end-points. The preserved lung function at two years suggested that new therapies may be deferred to a later time point in this cohort of children.

1.4.3.2 The AREST-CF longitudinal study of NBS children with CF

The AREST-CF group is a collaboration between specialist CF centres in Perth and Melbourne focussing on the assessment, treatment and prevention of CF lung disease in children under the age of seven years. Like the second LCFC study, they recruited and tested NBS infants with CF from diagnosis, but at the start of the work of this thesis, had a longer follow-up period to early school age (six years), whereas the then LCFC NBS reports were to two years. LCFC infants had CT and BAL only at one year of age, but the AREST-CF group performed these measures at diagnosis and then on a yearly basis, in addition to lung function. The differences in CT scoring systems

between the AREST-CF and LCFC reports are also essential to note. In the early studies their own scoring system was used (the 'AREST-CF CT score'), whereas the LCFC used the Brody II system. Bronchial dilatation was defined in the same way in both studies (bronchial luminal diameter to arterial diameter ratio >1), and is often referred to as 'bronchiectasis' in the AREST-CF reports. However, in longitudinal reports they note that some of these bronchiectatic changes regress. As bronchiectasis is historically thought of as an irreversible change, the term 'bronchial dilatation' is preferred when describing these observations in infants, and will be used in the description of the studies. Furthermore, in later reports, the scoring system changed and a quantitative CT scoring system used, the PRAGMA-CF score[99]. Details of the relevant scoring system used in each report are given below. Reports of lung function are discussed initially, followed by those describing structural changes, although there is overlap in some studies. Associations between lung function/CT score and BAL infection and inflammatory markers are summarised where reported.

Lung function

The first report of lung function and BAL in infants from the AREST-CF cohort was in 68 subjects with CF and 49 controls, from six weeks to 30 months[37]. In contrast to the LCFC study, normal lung function was seen in infants with CF less than six months of age, with mean $FEV_{0.5}$ [95% CI] at diagnosis only -0.05 [-0.73; 0.62] z-scores lower than controls. $FEV_{0.5}$ then declined to -1.15 [-1.57;-0.72] in those with a second lung function test over six months of age despite specialist care. There was no association between BAL markers and lung function. In this study it must be noted that a lower inflation pressure was used (20cm H_2O) for RVRTC than the recommended 30cm H_2O in ERS/ATS guidelines published after the study had taken place, and is likely to explain some of the differences seen between this and the LCFC study.

A further study of NBS infants described lung function (using 30cm H_2O RVRTC inflation pressure) and BAL at diagnosis, one year and two years[41]. Lung parameters were reported as standard deviation scores from published reference equations as no control group was studied. In agreement with LCFC data, lung function was abnormal at diagnosis, with mean $FEV_{0.5}$ z-score (SD) -1.4 (1.2) in AREST-CF infants (-1.2 (1.2) in LCFC infants). However, in contrast to improved $FEV_{0.5}$ at one year and stability at two years in LCFC patients, a progressive deterioration in $FEV_{0.5}$ mean z-score was seen to -2.4 (1.1) at one year and -4.3 (1.6) at two years. Decline was associated with infection with *Staphylococcus aureus, Pseudomonas aeruginosa* and BAL free

neutrophil elastase activity; a doubling of which was associated with mean $FEV_{0.5}$ z-score [95% CI] reduction of -0.46 [-0.77;-0.16]. Such a progressive deficit in lung function was concerning, with no apparent change in clinical status of well infants. However, custom made equipment for lung function measurement was used, and reference data to compute z-scores may not have been applicable unless identical test conditions (equipment and protocols) were in place. Without a control group for comparison, suspected measurement errors could not be detailed further.

MBW outcomes in infants and their relationship with CT changes were described in a separate report[130]. In 49 infants with CF aged 2-25 months, bronchial dilatation was seen in 27% and air trapping in 49%. However, LCI was not increased in the presence of CT abnormalities, and the study concluded that LCI could not replace chest CT to assess structural lung disease in infants.

A more recent publication describes the impact of BAL infection and inflammatory markers on LCI in 108 infants from diagnosis, at one year and two years of age[131]. Significant bacterial or fungal infection on BAL at the time of MBW measurement was associated with a mean increase in LCI [95% CI] of 0.4 [0.15;0.65] when compared to those with no infection, in particular with *Haemophilus influenzae and Aspergillus fumigatus* (but not *Staphylococcus aureus* or, surprisingly, *Pseudomonas aeruginosa*). In children with paired measurements, an accelerated decline in ventilation inhomogeneity was seen in those who isolated any 'pro-inflammatory' pathogen[132] (including *Staphylococcus aureus* and *Pseudomonas aeruginosa*) compared with those who remained infection free, indicating that those with early infection had subsequent lung function decline. BAL inflammatory markers did not show the same association.

The next follow up point was in early school aged children (four to eight years), describing the longitudinal effects of pulmonary infection, inflammation and structural abnormalities in infancy on later lung function[133]. Only minor differences in $FEV_{0.75}$ between the CF and control groups were found at school age; children with CF had mean [95% CI] $FEV_{0.75}$ 8.3% [-15.9;-6.6] lower. These children were the same who had a significant deficit in RVRTC outcomes in infancy to -4 z-scores in $FEV_{0.5}$ at two years, indicating that there was either substantial improvement by school age, or more likely, that there were errors of performance or interpretation in their infant lung function.

Nutritional outcomes in these school-age children showed a comparative deficit in the CF group, with mean weight and BMI z-scores 0.87 and 0.60 lower than controls respectively. Clinical predictors of poorer school age lung function in the first two years of life were pancreatic insufficiency, presence of free neutrophil elastase in BAL and infection with any pro-inflammatory pathogen. The greatest deficit in school age $FEV_{0.}75$ was seen in those with *Pseudomonas aeruginosa* (-16%) and *Haemophilus influenzae* (-15%). Free neutrophil elastase did not remain a significant predictor after adjustment for respiratory pathogens however, indicating infection with these pathogens are the main determinants of later lung function. Importantly, presence of air trapping and bronchiectasis were not associated with poorer school aged lung function. Children who received prophylactic antibiotics in the first two years had better lung function than those who did not. This study highlighted the importance of early pulmonary infection on later lung function.

The most recent report of lung function describes LCI using N_2 washout and BAL markers was in 58 preschool children aged 3-6 years[134]. 55% of preschool children had an LCI above the upper limit of normal (derived from a healthy control group), and mean LCI (SD) was 8.0 (1.45) in CF subjects compared to 6.67 (0.56) in controls. LCI was higher in those with pro-inflammatory pathogens (especially those with *Pseudomonas aeruginosa* on BAL), and total BAL cell counts, but not free neutrophil elastase activity. LCI was advocated as a useful marker of lower airway infection and inflammation at this age.

Lung structure

One of the initial studies from this group reported CT and BAL inflammatory markers in 57 infants shortly after a diagnosis of CF (median age 3.6 months)[3]. Evidence of lung disease was present on both inflammatory and structural markers, even at this early point. 81% had some abnormality on CT; 18% had evidence of bronchial dilatation and 67% air trapping. Bacterial infection was found in 21% and raised pro-inflammatory cytokines (77% detectable interleukin-8 and 30% detectable free neutrophil elastase activity) were present. When structural and inflammatory measures were compared, an association was seen between free neutrophil elastase activity and structural lung disease; the importance of which becomes more apparent in later studies. Of concern was that despite a high incidence of abnormality at such an early age, nearly all children in the study had no clinical signs of disease.

Lung structure and inflammatory markers were reported at diagnosis and then yearly to three years of age in a seminal paper from this group[135]. At three months of age, bronchial dilatation was seen in 29% of infants, and the point prevalence progressively increased at each study point (32% at 1 year, 44% at two years and 62% at three years). Air trapping was present in a high proportion (68% at diagnosis) and stayed stable throughout the study, seen in 69% at three years. A higher rate of bronchial dilatation was seen in those presenting with meconium ileus, respiratory symptoms at time of measurement, air trapping on CT and in subjects with detectable neutrophil elastase in BAL fluid. 'Persistent' bronchial dilatation (suggesting permanent change) was seen in 14% at 12 months of age and 32% at one year. The presence of neutrophil elastase at both time points was mostly strongly associated with this persistent structural change, and was highly significant (odds ratio 7 at one year, $p<0.001$ and 4 at three years, $p<0.01$). At three years, no other parameter, including earlier infection ('any' and individual organisms) showed a significantly increased odds ratio for structural change.

Follow up to six years of age has been reported describing longitudinal lung structure with paired CT scans in 49 children one year apart[136]. Using the AREST-CF CT score, bronchial dilatation was seen in all but 27 (19%) of children during the study. It was seen to persist in 75% of paired scans, but it must be noted that bronchial dilatation resolved in 26%, indicating that what is termed 'bronchiectasis' in the AREST-CF papers is not necessarily a permanent change that the term suggests. 63% showed progression of bronchial dilatation between scans. Air trapping was seen in 88% of children overall, which persisted in 81% of paired scans, progressed in 46% and resolved in 19%. Free neutrophil elastase and pulmonary infection were associated with progression of structural changes, and were therefore suggested as important early predictors of later disease.

Ramsey et al.[78] recently reported associations between structural disease and LCI in infants, preschool and school-aged children. LCI in infancy was insensitive to structural disease using the PRAGMA-CT score, but by 3-6 years it correlated with the total disease extent found on CT, and by 7-16 years with disease extent, bronchial dilatation (positive predictive value 83-86%) and air trapping. However, the negative predictive value in preschool and school age children was 50-55% so LCI could not replace chest HRCT to detect structural disease in these children.

In summary, the AREST-CF longitudinal studies report many important findings to improve understanding of the evolution of lung structure and function, and their association with infection and inflammation. CT abnormalities are present from an

early age in infants and can progress even in asymptomatic patients, and they propose that CT could be used as a trial end point. The development of a quantitative scoring system could standardise the interpretation of scans. Neutrophil elastase is an important predictor of later structural disease, but whether treating an early abnormal result offers any improvement in outcomes is unknown. LCI can predict bronchiectasis in preschool and school-aged children but not in infants, in line with earlier findings in clinically diagnosed children[126].

1.5 Hypotheses, aims and objectives

The overall aim of the thesis was presented in section 1.1.3 of this chapter as being "*to describe the evolution of lung function from diagnosis to six years of age in a NBS cohort of children with CF managed with standard UK care".* Specific hypotheses, aims and objectives were then formulated to generate answerable study questions.

1.5.1 Choice of outcome measures

Although the primary aim was to describe the evolution of lung function in NBS children to six years of age, the new data collected in this study were obtained at preschool follow-up of the LCFC cohort at three to six years of age. Appropriate lung function outcomes were therefore chosen for this age group to best reflect the pathophysiology of early CF lung disease. As discussed in section 1.3, a number of lung function measures are available for preschool children. In clinically diagnosed children with CF, LCI measured by the MBW technique is highly feasible and sensitive to abnormality[7], and can predict LCI and spirometry in school age children[51]. It has also longitudinally been shown to decline before other measures of lung function, and was the strongest marker to predict disease progression[79]. It was measured when the children participating in this study were infants, enabling longitudinal analysis of LCI from diagnosis to the preschool years. It was therefore chosen as the primary outcome measure. Although not as sensitive as LCI, plethysmographic total specific airway resistance (s*R*tot) was also shown to be abnormal in clinically diagnosed preschool children with CF, and was chosen as a secondary outcome measure. Finally, $FEV_{0.75}$ measured by spirometry was also included as a secondary outcome. This was chosen in preference to FEV_1 as some preschool children may complete the spirometric manoeuvre and reach forced vital capacity before one second[45]. Both plethysmography and spirometry are also highly feasible in an experienced laboratory[7].

These three outcomes were also measured in pre-schoolers in the first phase of the LCFC study, enabling direct comparison of the NBS and clinically diagnosed cohorts.

The AREST-CF surveillance programme includes yearly HRCT and bronchoscopic assisted BAL as well as lung function measures in NBS children. In the LCFC cohort, HRCT and BAL were only performed at one year of age. As discussed in section 1.4.3, there are clear differences between the cohorts. Lung function was much poorer in AREST-CF subjects by two years when compared to LCFC infants. CT abnormalities were also extremely mild and poorly reproducible in LCFC subjects, in contrast to findings of AREST-CF. CT could not therefore be ethically justified by the referring clinicians as an outcome measure in this study, given the relative preservation of lung function at two years. While the AREST-CF study has shown that free neutrophil elastase in BAL fluid is associated with structural lung disease in NBS children, its association with lung function is less clear, and no relationship was found with preschool lung function in the AREST-CF study. Furthermore, respiratory pathogens were stronger determinants of school-age lung function in the AREST-CF cohort, so the ability of neutrophil elastase to predict later pulmonary decline is still undetermined. Preschool LCFC subjects also had regular surveillance with cough swab or sputum cultures, and whilst some limitation in the detection of lower airway infection by these methods is recognised, they are more applicable to current practice. A randomised trial of BAL-directed therapy had also shown no difference when compared to standard therapy on the incidence of *Pseudomonas aeruginosa* and CT-score in children under six years[116]. Therefore bronchoscopy and BAL were not included in the LCFC preschool follow-up.

1.5.2 Primary hypotheses, aims and objectives

In view of the fact that the LCFC cohort of children had stable, if not improved, lung function in the first two years of life, the primary hypothesis of this study was:

1. NBS children with CF managed with standard UK care will have stable lung function at three to six years of age, which will be similar to contemporaneous healthy controls

The primary aims were:

1. To report lung function in NBS children with CF compared to healthy control children aged three to six years

2. To describe the evolution of lung function in NBS children with CF from diagnosis to six years of age

The specific primary objectives were therefore:

1. To measure LCI, s*R*tot and $FEV_{0.75}$ using MBW, plethysmography and spirometry in preschool children with CF and healthy controls (contemporaneous, and also studied longitudinally), and compare results between the two groups

2. To report lung function longitudinally from diagnosis (~three months) to six years of age in NBS children with CF

1.5.3 Secondary hypotheses, aims and objectives

The secondary hypotheses were:

1. NBS children will have better lung function than preschool children with a clinical diagnosis of CF born a decade earlier

2. Infant lung function measures and nutritional markers cannot predict preschool lung function in NBS children with CF

3. Clinical markers of infection (isolation of bacteria on respiratory samples), and markers of disease severity (for example courses of intravenous antibiotics) in infancy will not be significantly associated with preschool lung function primary outcomes

4. Inflammatory markers measured in bronchoalveolar lavage in infancy cannot predict lung function at three to six years of age

5. Abnormalities on CT (air trapping, bronchial dilatation and total CT score) measured in infancy cannot predict preschool lung function

The secondary aims were:

1. To compare results of preschool LCI, s*R*tot and $FEV_{0.75}$ in NBS children with CF with the same measures in a previous (non-contemporaneous cohort) of clinically diagnosed children with CF at the same age

2. To investigate whether measures of lung function, structure, infection, inflammation and disease severity in infancy can preschool predict lung function

The secondary objectives were:

a) To measure LCI, s*R*tot and $FEV_{0.75}$ using MBW, plethysmography and spirometry in preschool children with CF and healthy controls, and compare results to those measured in a preschool cohort of children diagnosed with CF a decade earlier

b) To describe the anthropometric characteristics (height, weight and body mass index) from birth to preschool age in subjects with CF and their association with preschool lung function (primary outcomes LCI, s*R*tot and $FEV_{0.75}$ z-score)

c) To examine whether there is an association between infant lung function measures ($FEV_{0.5}$, LCI and FRC_{pleth}) with the preschool lung function primary outcomes

d) To investigate the association between clinical markers in infancy, such as bacterial isolates, and markers of disease severity, such as courses of intravenous antibiotics, known to be associated with lung function decline in older children with preschool lung function

e) To determine the relationship between CT and BAL parameters measured at one year in the LCFC cohort of NBS children and preschool lung function primary outcome measures measured at three to six years in the same children

1.5.4 Structure of thesis

This thesis is presented in seven chapters. The methodology is presented in Chapter 2, followed by four results chapters. Chapter 3 reports lung function in NBS preschool children with CF compared to healthy controls. Chapter 4 compares preschool lung function results in NBS children to those with a clinical diagnosis of CF diagnosed a decade earlier. Chapters 5 and 6 investigate whether measures of clinical status, lung function, infection, structure and inflammation can predict preschool lung function in NBS children with CF. Each results chapter is accompanied by a discussion relative to the principal findings of each study, and Chapter 7 presents a general discussion of the implications of results as a whole, with final conclusions and suggestions for future research.

2 Methods

2.1 The LCFC longitudinal studies of children with CF

This thesis compares tests in infancy performed as part of earlier studies of children with CF by the LCFC. The LCFC was established in the UK in 1999. Five centres (Great Ormond Street Hospital for Children, The Royal Brompton Hospital, The Royal London Hospital, University Hospital Lewisham, St. Helier Hospital and King's College Hospital) prospectively recruited infants with CF born in South-East England. The aim was to develop methods to monitor early lung disease in children with CF.

The first phase of the LCFC cohort followed children clinically diagnosed with CF from diagnosis to adolescence born between 1999 and 2002. Children first had infant lung function measurements (RVRTC) at diagnosis (~seven months) and repeated at least six months later. The MBW technique then became available in the laboratory and further infants were recruited, undergoing MBW and RVRTC age two to 22 months. Preschool follow up of children at three to six years measured MBW, plethysmographic specific airway resistance (sR_{aw}) *and* spirometry. At school age (6-10 years) children underwent MBW, plethysmography (sR_{aw} and lung volumes) and in addition to lung function tests HRCT was performed. Reports of longitudinal lung structure and function from these studies are summarised in section 1.4.2.1.

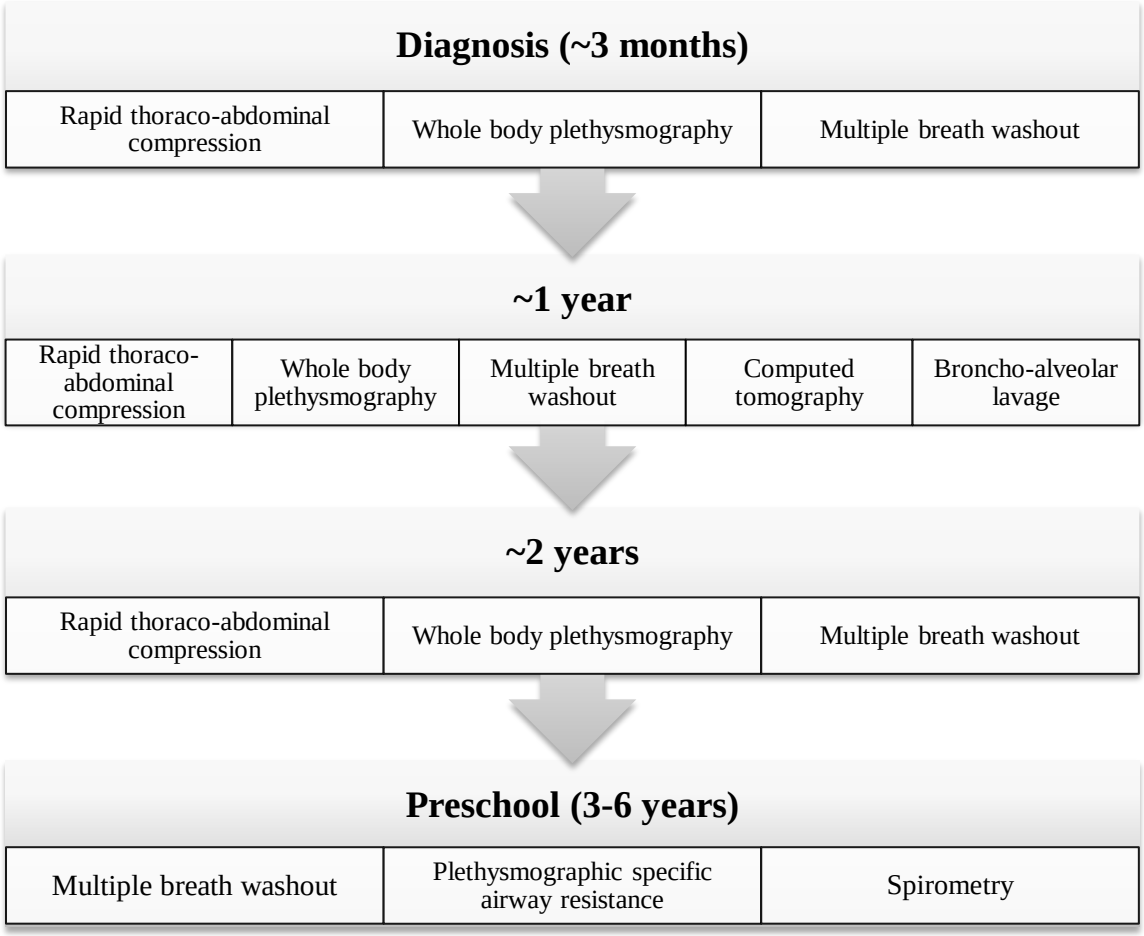
The introduction of new-born screening in the UK meant that most children with CF were identified shortly after birth, often before any clinical signs of lung disease were apparent. The opportunity emerged to study this additional group of children considering the differences associated with an earlier diagnosis. This second phase of the LCFC study recruited infants with CF diagnosed by NBS born between 2009 and 2011. The main results in this thesis report lung function of the same NBS infants during the preschool years (Chapter 3), and include longitudinal data collected from diagnosis as part of the infant study (Chapters 5 and 6). As the initial recruitment, measures and protocols in infancy are relevant to the current preschool study they are summarised below. Chapter 4 compares preschool lung function measurements of NBS children with the clinically diagnosed cohort, the methodology of this first phase of the LCFC study is summarised there.

2.2 Overview of study design

Data presented in this thesis are measurements from subjects participating in a prospective longitudinal observational cohort study of lung function, structure and inflammation in NBS children with CF from diagnosis to preschool age (three to six years). Lung structure and function up to two years of age (the 'LCFC NBS infant study') have been previously published[36, 94, 127, 129] and are summarised in section 1.4.3.1. Infants were recruited and tested as part of an earlier study (not by myself), Principal Investigator Professor Janet Stocks.

This thesis reports lung function at preschool follow up of LCFC NBS infants. Preschool children were re-recruited and tested by myself and assistants (detailed on page 2). Pulmonary function measurements (multiple breath washout, plethysmographic specific airway resistance and spirometry) were performed in children with CF and healthy controls at Great Ormond Street Hospital, London UK. Measures in infancy and at preschool test are summarised in Figure 2.1.

Figure 2.1: Study protocol at each time point of the London cystic fibrosis collaboration newborn screened infants longitudinal study



Legend: Study protocol at each time point. In infancy, lung function measurements were repeated at each study visit, with the addition of high resolution computed tomography and bronchoalveolar lavage at one year. Preschool follow-up (current study) tested multiple breath washout, plethysmographic specific airway resistance and spirometry

2.3 Recruitment and measures in the LCFC NBS infant study

2.3.1 Recruitment of infants

Children with CF

Children with CF were recruited from the six participating specialist paediatric CF centres of the LCFC between 2009 and 2011. At each centre, children identified by

newborn screening underwent diagnostic sweat testing and if necessary, extended genotyping for confirmation of the diagnosis. Children diagnosed with CF presenting with meconium ileus were also included. Children were recruited at a follow up approximately two weeks after diagnosis by their consultant at the participating centres. If consent was obtained, infants were enrolled into the LCFC NBS infant study with an aim to attend the three tests occasions at three months, one year and two years of age.

Healthy infant control subjects

Control subjects were recruited from the Homerton Hospital, London and University College Hospital, London by research assistants to the LCFC infant study between 2009 and 2011. Eligible subjects were identified from birth lists sent from these participating centres to the research team, and families contacted after approval from their General Practitioner. All subjects were term (>36 weeks gestation) and had no recorded congenital abnormalities, severe illness or known lung disease. If consent was obtained these families were invited to attend for infant lung function testing and underwent the same protocol as the CF infants, with the exception of bronchoscopy and CT at 1 year of age.

2.3.2 Inclusion and exclusion criteria for the LCFC NBS infant study

Healthy control infants

The following inclusion criteria applied when subjects were recruited as infants:

- Healthy term infants (born at ≥36 weeks gestation) whose parents consented to infant lung function measurements

Exclusion criteria:

- Diagnosis of respiratory condition (e.g. upper airway abnormality or history of apnoeic episodes)
- Previous hospital admission for respiratory condition (e.g. lower respiratory tract infection)
- Chronic medical condition likely to affect lung function (neonatal lung disease or neuromuscular, bone, renal or cardiac disease)
- History of chronic diarrhoea or failure to thrive
- Inability of parents or carers to give informed consent

- Recruitment contra-indicated because of psycho-social factors

Children with CF

Inclusion criteria:

- Infant diagnosed with CF by NBS (positive NBS test, diagnosis confirmed by sweat test and/or genetic analysis) or presentation with meconium ileus with diagnostic confirmation of CF referred by one of the six tertiary respiratory centres in the greater London area
- Born at full term (≥36 weeks gestation)

Exclusion criteria:

- Additional respiratory or other medical condition likely to affect lung function testing results
- Parents unable to give informed consent
- Recruitment contra-indicated because of psycho-social factors
- History of upper airway pathology or apnoeic episodes

2.3.3 Measures in infancy

Infant lung function (RVRTC, plethysmography and MBW) was performed shortly after diagnosis (around three months of age) and repeated in the same children at one and two years of age. In addition to lung function, HRCT and bronchoscopic assisted BAL were performed at one year.

On all three test occasions lung function was measured comprising:

- RVRTC measuring a forced expiratory flow volume curve, primary outcome $FEV_{0.5}$, and secondary outcomes of forced expiratory flows between 25% and 75% of lung volume (FEF_{25-75}) and forced vital capacity (FVC)
- Body plethysmography, primary outcome FRC_{pleth}
- Multiple breath washout, primary outcome LCI and secondary outcome FRC_{MBW}

Detailed methodology of infant lung function techniques used in the LCFC NBS infant study can be found in a previous publication[36]. At 1 year of age children also

underwent volumetric thoracic CT scan performed under general anaesthetic with adherence to a strict pre- and intra-scan ventilation protocol, scored using the Brody-II scoring system[137]. Flexible bronchoscopy and BAL were performed immediately after CT again with a strict protocol for collection of lavage samples and analysed for bacterial, fungal and mycobacterial growth, viral immunofluorescence and inflammatory mediators. Further details of CT and BAL methodology is described in Chapter 6.

2.4 Methodology of the current NBS preschool follow-up study

Original data presented in this thesis was collected at preschool follow-up of subjects participating in the NBS LCFC infant study between three to six years of age. A more detailed description of recruitment and measures in this study is therefore presented in the following sections of this chapter.

2.4.1 Ethical approval

This study was approved by the National Research Ethics Service (NRES) Committee London- Bloomsbury REC (Ref No: 12/LO1668) and the Local Research Ethics Committees of each of the six collaborating centres.

2.4.2 Recruitment of preschool children

We identified and compiled data sheets of infants with CF and healthy controls who took part in the infant LCFC study and had successful measurements (i.e. completed the full test protocol) on at least one infant test occasion. Parents of children who had consented to be contacted for future research were contacted to participate in the preschool follow-up study. Families were contacted by post initially with a written information sheet detailing the tests involved in the study (Appendix 1), and the consent form for the study so that they had the opportunity to read this before the test occasion. Parents were given at least two weeks to do this and were then contacted by the author or a research assistant (Emma Raywood [ER] and Sarah Legg [SL]) to discuss participation and to answer any questions regarding testing.

2.4.3 Inclusion and exclusion criteria

Healthy control children:

Inclusion criteria at preschool follow-up were as follows:

- Age >2 years and < 6 years at time of recruitment
- A valid result on at least one infant lung function test occasion

Exclusion criteria:

- A new diagnosis (after infant test occasion) of any respiratory condition or upper airway pathology (including recurrent wheeze, asthma and chronic cough)
- Use of medication for reactive airways disease (e.g. bronchodilators) in the twelve months preceding test occasion
- Frequent use of antibiotics for respiratory symptoms (arbitrarily, <5 courses in the twelve months preceding test occasion)
- A new diagnosis of a chronic medical condition likely to affect respiration (i.e. neuromuscular, bone, renal or cardiac disease)
- Symptoms of respiratory illness or the use of medication for respiratory illness in the three weeks preceding test occasion
- Parents unable to give informed consent
- Re-recruitment contra-indicated because of psycho-social factors

Children with CF:

- Age >2 years and <6 years at time of recruitment
- A valid result on at least one infant lung function test occasion

Exclusion criteria:

- A new diagnosis of a chronic medical condition likely to affect respiration (i.e. upper airway pathology, neuromuscular, bone or cardiac disease)
- Symptoms of respiratory exacerbation or the use of medication for respiratory illness in the three weeks preceding test occasion
- Parents unable to give informed consent
- Re-recruitment contra-indicated because of psycho-social factors

Children with CF were managed on a standardised treatment protocol agreed by members of the participating centres in the LCFC NBS infant study (Appendix 2).

2.5 Pre-test procedure

2.5.1 Preparation for lung function tests

All measurements were performed at the Great Ormond Street Hospital (GOSH) lung function laboratory. Two research associates (the author and ER or SL) were required for testing on every occasion. Before the family arrived, two roles were allocated; one associate would have responsibility for interacting with the child during testing, the other's role was to operate equipment, assist testing and complete the test questionnaire with parents. Study appointments were arranged to coincide with a time when the child was most cooperative (for most around 11am) and not when the child would normally be tired or asleep. For those whose routine clinical care was at GOSH, an appointment was arranged on the day of a clinic visit, but again testing for most commenced between 12 and 1pm.

When the child and their carer(s) arrived at the lung function laboratory, they were shown to a waiting area. The mask used for the first test in the lung function protocol (MBW) was then introduced to the child using a game, enlisting the carers' help if needed. Once the child was familiar with the mask, they were also shown how the therapy putty was used to create a seal between the mask and their face. When the child was comfortable wearing the mask (Figure 2.2) they were then shown to the clinical area for testing. This pre-test procedure was aimed to increase the likelihood of the child wearing the mask, and was completed away from the test area as some children became nervous at initial sight of the test equipment. This was particularly true for children with CF, as they may have had blood sampling in similar environments, and required reassurance that this would not take place at this visit. Children were given a chart (Appendix 3) with steps and sticker rewards for each part of the study protocol, and indication of a prize at the finish as an incentive to complete testing. The visit was described to the child as a series of games and not referred to as a 'test'.

Before proceeding with testing, one of the associates would check carer's understanding of the test procedure, and ask them to complete the written consent form.

Figure 2.2: Pre-test procedure

Legend: A research associate undergoing the pre-test procedure with a preschool child. The child is comfortable wearing the mask used for multiple breath washout, whilst being shown the sticker chart. The aim was to gain rapport to maximise the feasibility of lung function testing (carer consent given for inclusion of images)

2.5.2 Test day questionnaire

The parental questionnaire is available in Appendix 4. It differed for subjects with CF and healthy controls in order to collect appropriate information, as well as identifying if any exclusion criteria were met. For children with CF, the questionnaire included medical history (including any surgery), current treatments (nutritional and respiratory), hospital admissions and courses of oral and intravenous antibiotics. Clinical status at time of test (frequency of coughing) and parental smoking history were also recorded. For healthy controls, similar questions were asked regarding medical history with the omission of treatments only relevant to subjects with CF. A retrospective clinician questionnaire was also completed after the test date (section 2.7.2).

2.5.3 Anthropometric measurements

Measurement of height (without shoes) was taken using a calibrated wall mounted stadiometer (Seca 264 wireless stadiometer, Hammer Steindamm 9-25 22089

Hamburg, Germany) to two decimal places with the feet, body and back of head against the stadiometer with the child looking directly ahead. This was repeated two to three times until two measurements were within 0.5cm of each other and the mean value of the two recorded. Sitting height was measured in a similar way with the child sitting up straight on a stool (height 45.50cm) and calculated by subtracting the stool height from standing height. Weight was measured without shoes and with minimal clothing using daily calibrated electronic scales (Marsden MC-250, Marsden W/M group limited, Henley-on-Thames, Oxfordshire, UK) recorded to two decimal places. Standard deviation scores for height, weight and body mass index were calculated to adjust for age and gender.

2.5.4 Clinical examination

Examination was performed by a clinician (the author or Paul Aurora, Ema Kavalinute and Anjay Pillai). Respiratory rate, signs of respiratory distress, location of crackles or wheeze (if present) and any other abnormality on examination were recorded. In addition, pre- and post-test transcutaneous heart rate and O_2 saturations were recorded using a pulse oximeter (Rad-5v, Masimo Corporation, 40 Parker Irvine, CA 92618 USA).

2.6 Preschool lung function protocol

2.6.1 Choice of outcome measures

The following lung function tests were performed, the rationale for each is discussed in section 1.5.1.

- MBW, primary outcome LCI
- Whole body plethysmography, primary outcome s*R*tot
- Spirometry, primary outcome $FEV_{0.75}$

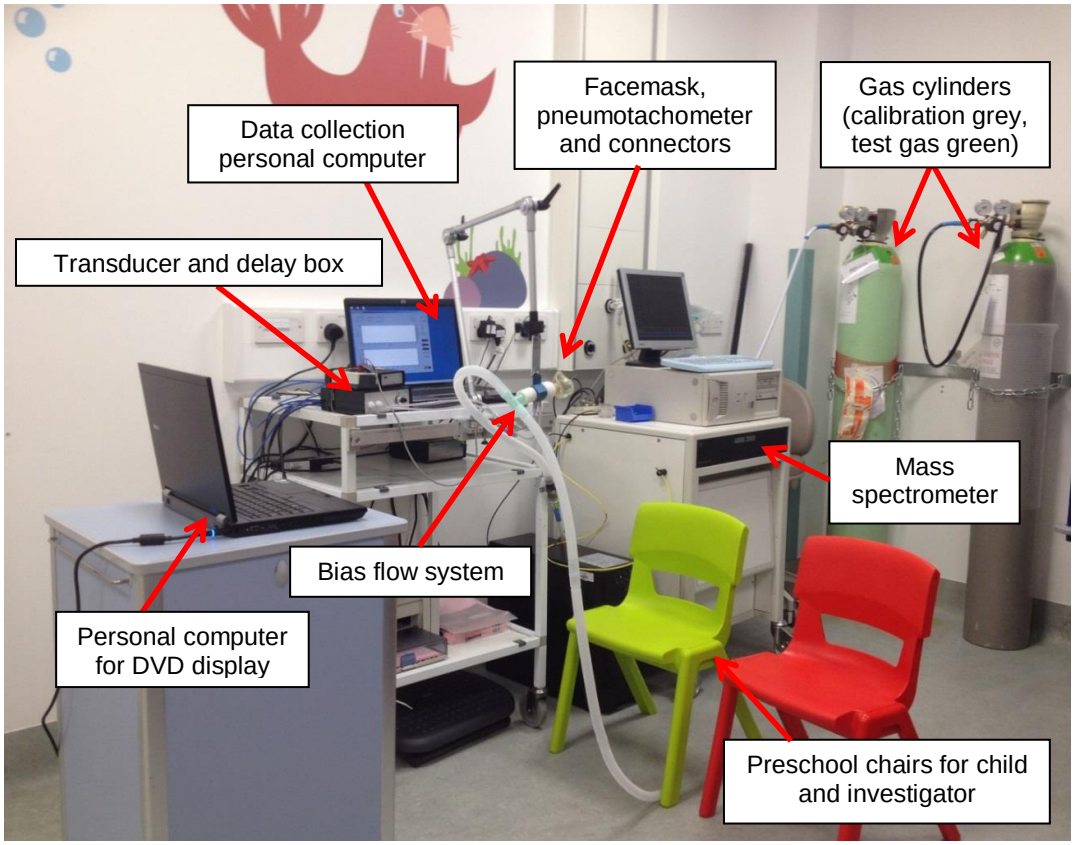
They were performed in the above order as bronchoconstriction or alteration of airway mechanics can occur with the deep inspiration in forced spirometry, and affect the tidal breathing that is required for MBW and plethysmography.

2.6.2 Multiple breath washout

2.6.2.1 Equipment and calibration

Figure 2.3 shows the laboratory room used for MBW testing. The equipment for gas analysis, interface with child (mask, pneumotachometer and connectors), pressure transducer and equipment calibration will be described in following sections.

Figure 2.3: Equipment for multiple breath washout measurement



Legend: Equipment for multiple breath washout measurement in the lung function laboratory. The child sits on the green chair and investigator on the red chair. The pneumotachometer, mask and connectors are released from the supporting arm and held by the investigator during testing

Pneumotachometer, transducer and demodulator

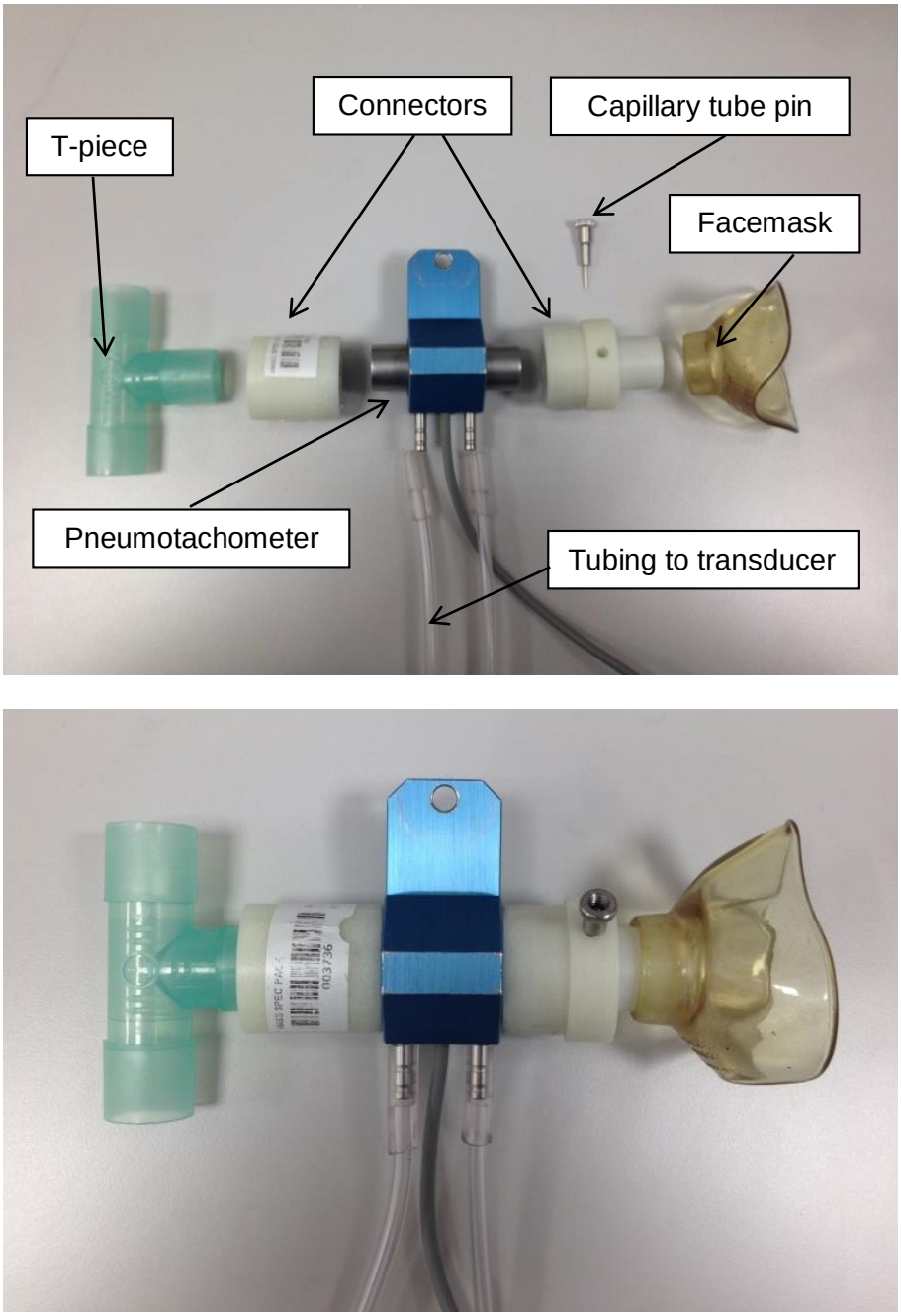
To measure and display flow, a pneumotachometer-transducer-demodulator system was used. Gas flow was measured by a Fleisch number one pneumotachometer

(Fleisch, Lausanne, Switzerland). The inspiratory port of the pneumotachometer was clearly labelled so calibration and data collection was performed in the correct orientation. The pneumotachometer was connected to a pressure transducer box (Model MP45-14-871, Validyne Corp, Northridge, USA) using equal lengths of polyethylene tubing (Tricoflex SA, Hozelock Tricoflex, Vitry-le-François, France). A high gain demodulator (Model CD19, Validyne Corp, Northridge, USA) as part of a multi-channel modular transducer system (Model MC1, Validyne Corp, Northridge, USA) then converted the pressure signal to an analogue flow trace that could be displayed in real time during testing.

Facemask and connectors

Figure 2.4 illustrates the assembly of the facemask, pneumotachometer and connectors. A size two Rendell-Baker facemask (Ambu International, Bath, Avon, UK) was chosen as an appropriately sized interface for preschool children and was sealed to the face using therapeutic putty (equal parts mixture of medium and hard putty, Mobilis Rolyan, Patterson Medical Ltd, Nottinghamshire, UK) to eliminate any gas leak from the mask. The amount applied served to minimise dead space of the mask (20ml without putty) without obstructing the child's mouth and therefore leading to nose breathing. The mask was fitted to a custom made acetyl/nitrile connector (manufactured by Mr R Taylor, Department of Biomedical Engineering, GOSH, London, UK) connecting it to the inspiratory port of the pneumotachometer. This connector had a small opening in which the pin from the capillary tube of the mass spectrometer was inserted for gas sampling, shaped to prevent leakage of gas.

Figure 2.4: Facemask, pneumotachometer and connectors for multiple breath washout



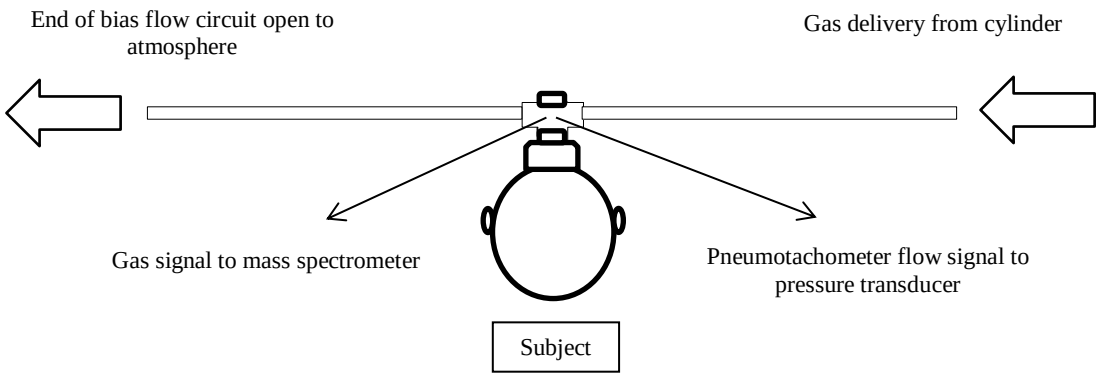
Legend: Facemask, pneumotachometer and connectors used for multiple breath washout; disassembled (above) and assembled (below)

Gas bias flow system

A second custom-made connector fitted the expiratory port of the pneumotachometer to the SF_6 gas supply via a T-piece and elephant tubing. SF_6 was delivered via a bias flow system (Figure 2.5) from a cylinder of medical grade gas containing 4% SF_6, 4%

He, 21% O_2, and balance N_2 (British Oxygen Company, Guildford, UK). The gas cylinder was connected to a first piece of elephant tubing and one port of the T-piece, the middle port of the T-piece attached to the expiratory port of the pneumotachometer, then a second length of elephant tubing was connected to the remaining port of the T-piece with its end open to the atmosphere.

Figure 2.5: Gas bias flow system for multiple breath washout



Legend: In the bias flow circuit, gas is delivered from a cylinder to a t-piece via elephant tubing. Gas flow is set higher than the subject's tidal flow rate so the subject can breathe in the gas to equilibrium concentration whilst excess gas flows into the atmosphere

Gas analysis

A quadrupole mass spectrometer was used for gas analysis (AMIS 2000, Innovision, Odense, Denmark), which identifies gases based on their mass-to-charge ratio and measures the abundance of gas phase ions. The quadrupole refers to four metal electrode rods arranged so that two positive and two negative rods lie in parallel. Gas samples are drawn down a capillary tube into a vacuum chamber at a rate of 15ml/min. Ions are then created by contact with an electrical filament, and then accelerated towards a detector. A direct current applied to the electrodes determines the overall positive or negative charge, and an alternating current is also applied, creating an oscillating electrical field. The voltage is varied so at any one time, only ions of a certain mass charge ratio (*i.e.* only one gas of a particular molecular mass) can travel between the rods to the detector. Others have unstable trajectories, collide with the

electrodes, and are therefore filtered out. With this system, rapid cycling of the electrical field allows up to twelve gases to be measured sequentially, displayed online and saved for later analysis. The mass spectrometer was set to measure SF_6, He, N_2, O_2) and CO_2. The analogue output from the mass spectrometer was linked to a personal computer that also displayed input from the demodulator (see below) so that gas concentration and flow could be displayed in real time during testing.

Calibration and delay switch

The pneumotachometer was calibrated (unheated) before each test with separate calibration constants for inspiratory and expiratory flows using a custom made 241mL syringe (Department of Biomedical Engineering, GOSH, London, UK) by the author, ER or SL. Once calibrated the pneumotachometer was heated to 37 degrees for testing (Heater model FWS4D, Hugo Sachs Elektronik, March, Germany).

According to manufacturer's instructions the AMIS 2000 was two point calibrated once daily using certified concentration calibration gas (alpha-gravimetric standard, British Oxygen Company, Guildford, UK) containing 3.97% He, 3.98% SF_6 , 7.04% CO_2, 21% O_2 and 64% N_2. Signal to noise ratio of greater than 100 was accepted. A short calibration was performed immediately before each measurement. To allow for the delay between the gas bolus measured at the capillary inlet of the mass spectrometer and processing to the recording software programme, a custom delay switch was used (Mr E Bergsten, Swedish Defense Research Agency, Department of Defense Medicine, Linköping, Sweden). The delay was measured daily using a software programme that recorded a median of ten gas bolus delay measurements and rise times for SF6, He and CO_2 signals. The median delay was then incorporated into the online recording programme to align flow and gas concentration signals.

Analogue gas flow and volume signals from the demodulator, and gas concentration from the mass spectrometer were recorded by a personal computer (Compaq 6820s, Hewlett Packard, Palo Alto, California, USA) in a custom made software programme (TestPoint, Capital Equipment Corp., Billerica, MA, USA). During the test the software displayed online flow, volume and SF_6 concentration signals. The software corrected the flow and volume signal for body temperature, pressure, water vapour saturated (BTPS) conditions and also for changes in gas viscosity of each gas sample, as the gas composition varied during the test.

2.6.2.2 Data collection

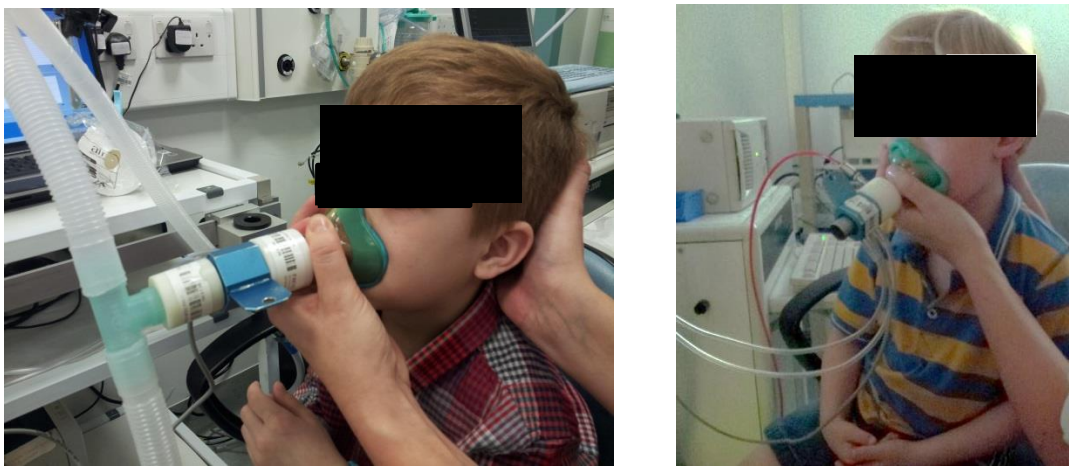
When the child entered the laboratory for MBW measurement, overhead lights were dimmed (described to them as a 'cinema') as some children became nervous at first sight of the equipment. A digital video disk (DVD) of a popular children's show (Peppa Pig © Astley Baker Davies Ltd / Entertainment One UK Ltd 2003) was displayed for the child to watch during testing on a personal computer or DVD player set on a trolley so the screen was at the child's eye level with the neck slightly extended. This particular DVD had a calm storyline so the child was unlikely to talk or laugh during data collection and it therefore encouraged regular tidal breathing. The child would sit on a preschool chair closest to the data collection PC and one investigator (the author, ER or SL) seated in the adjacent chair, holding the facemask to the child's face and supporting the back of the child's head for the test duration. The other investigator stood behind the child and managed the gas supply, examined online gas flow and concentration traces and disconnected the bias flow system at the start of recording (see below). The child's carer(s) also sat in the room to reassure the child.

Once the mask was in place the medical grade gas supply (4% SF_6, 4% He, 21% O_2, balance N_2) was turned on for the wash-in phase. If the child was breathing faster than their resting respiratory rate (measured before testing) they were encouraged to breathe more slowly, but this was rarely necessary. Flow of gas in the circuit was set to a level higher than the maximum inspiratory flow of the subject (250-400mL/second). SF_6 gas 'wash-in' was complete when SF_6 concentration was greater than 3.8% and inspiratory to expiratory end tidal SF_6 concentration variability was less than 1% for at least ten seconds, assessed on the online numerical display of SF_6 concentration. Recording of the MBW trace was then commenced and this stable wash-in phase was captured for at least five breaths. A stable tidal volume for five breaths or the preceding 30 seconds was required before the start of the wash-out.

In the wash-out phase, the gas supply was disconnected by removing the second connector from the expiratory port of the pneumotachometer at the start of expiration by the second investigator. This was done with as little disturbance to the child as possible to ensure breathing pattern was unchanged. SF_6 concentration not reaching zero in the first inspiration of the washout phase was an indication that disconnection did not occur at the start of expiration, and the test was re-attempted. Figure 2.6

shows the set-up for preschool children during the wash-in and wash-out phase of MBW.

Figure 2.6: Preschool children during wash-in and wash-out phases of multiple breath washout



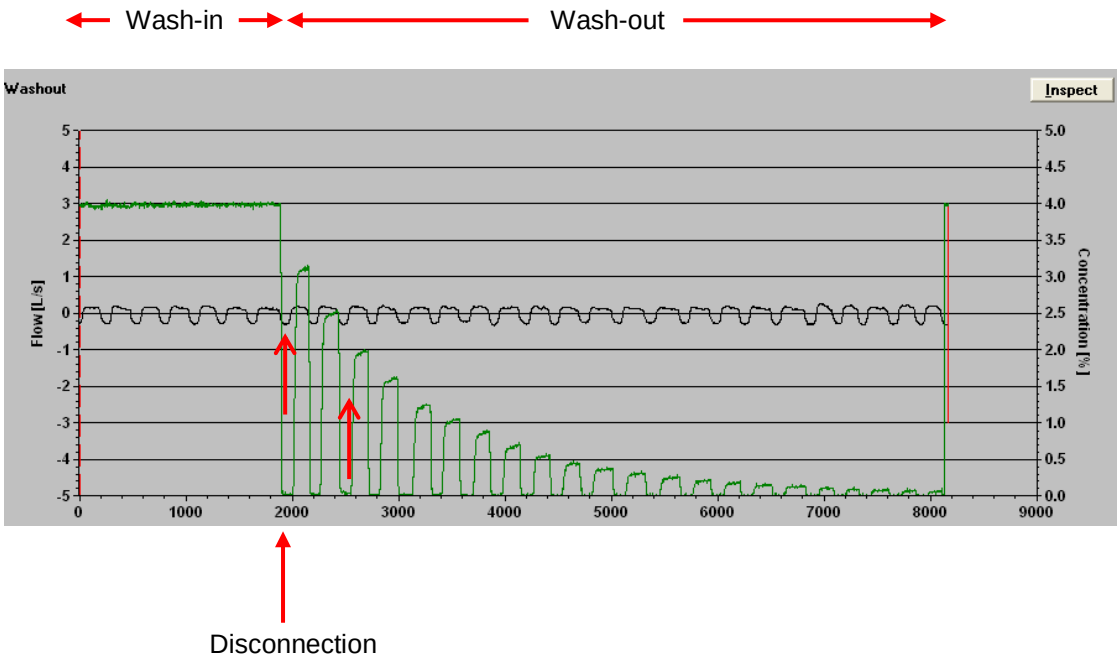
Legend: Left: the investigator supports the back of the child's head whilst holding the facemask, connectors, pneumotachometer and bias flow system on the child's face. Right: the distal connector and bias flow system has been disconnected for the wash-out phase (carer consent given for inclusion of images)

Acceptability criteria were used according to the European Respiratory Society / American Thoracic Society consensus statement for inert gas washout measurement, published in 2013[71]. Breathing pattern was visualised and examined in real time from the washout display on a personal computer. At the start and throughout the washout, stable lung volumes were required during relaxed tidal breathing. If any large breaths were seen (which can mobilise trapped gas), a further run was attempted. If a large breath was taken during the first five breaths of the washout, then the run was abandoned and wash-in phase restarted. Coughing, laughing or talking during the wash-out were also criteria for run exclusion.

If the seal was felt to be broken (for example by the child moving their face) with a resulting gas leak after recording was commenced, even if not seen on visual inspection of the gas concentration signal, the test was abandoned and re-attempted. During washout recording the online trace was inspected for signs of air leak; a sudden offset or drift in the volume trace, or sharp drop of SF_6 concentration suggested this.

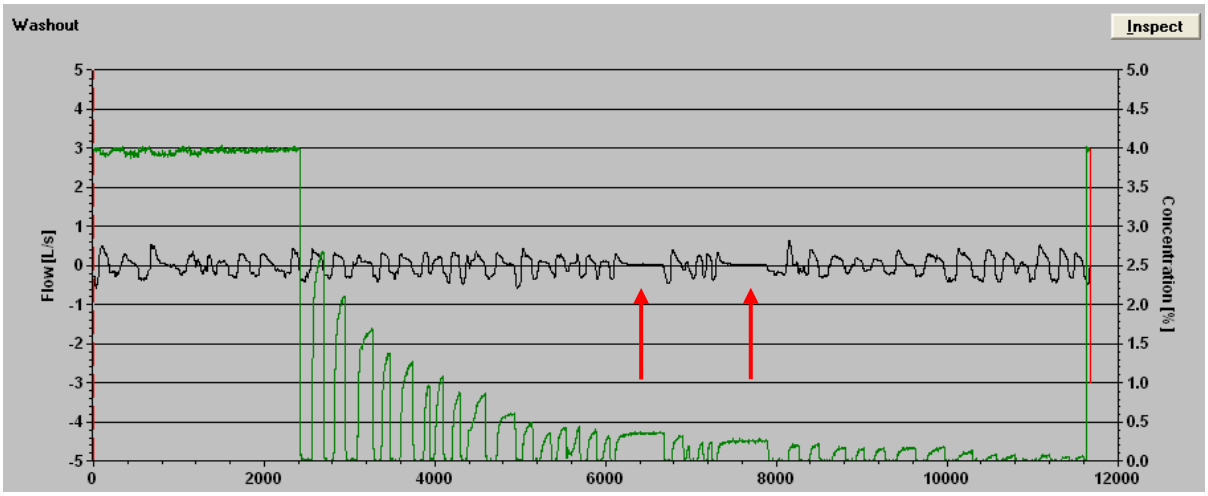
Washout was complete when SF_6 concentration was <0.1%, *i.e.* <1/40th of the starting gas concentration, for at least 5 consecutive breaths. At test completion, if the child was comfortable, the gas delivery system was reconnected and the above steps repeated until three acceptable MBW traces were obtained. Further details of acceptability criteria can be found in Appendix 6. Figure 2.7and Figure 2.8 are examples of high and poor quality MBW curves.

Figure 2.7: Multiple breath washout trace showing a high quality curve



Legend: An example of a high quality multiple breath washout curve. The green trace represents SF_6 concentration shown on the right vertical axis (horizontal axis is time). There is a stable wash-in concentration at 4%, disconnection of bias flow gas supply at the start of expiration and regular tidal breathing throughout the wash-out phase. During inspiration the SF_6 concentration is zero when the child breathes air. There is decay of SF_6 resident in the lungs over the course of the wash-out as it is progressively diluted by air

Figure 2.8: Poor quality multiple breath washout trace



Legend: There is irregular breathing and pauses (arrows) during the wash-out phase, giving a poor quality multiple breath washout trace

2.6.2.3 Data analysis

Each acceptable MBW recording was analysed breath-by-breath in custom made software (TestPoint, Capital Equipment Corp., Billerica, MA, USA, written by Mr E Bergsten, Swedish Defense Research Agency, Department of Defense Medicine, Linköping, Sweden). Details of this analysis procedure can be found in Appendix 6.

The FRC was determined by dividing the cumulative expired volume (CEV) of SF_6 by the change in end tidal SF_6 concentration at the start and end of the washout. The LCI was then calculated by dividing the CEV by the FRC and represents the number of FRC volumes, or turnovers (TO), required to reduce the SF6 concentration to 2.5% of starting concentration. Final LCI and FRC were reported as a mean of the three best acceptable MBW tests. Variability of FRC was required to be <25% between acceptable runs. Results were exported from the software and entered into the database (Rebase software; Rebase, London, UK), with data checked on transfer by two associates (the author, ER and SL).

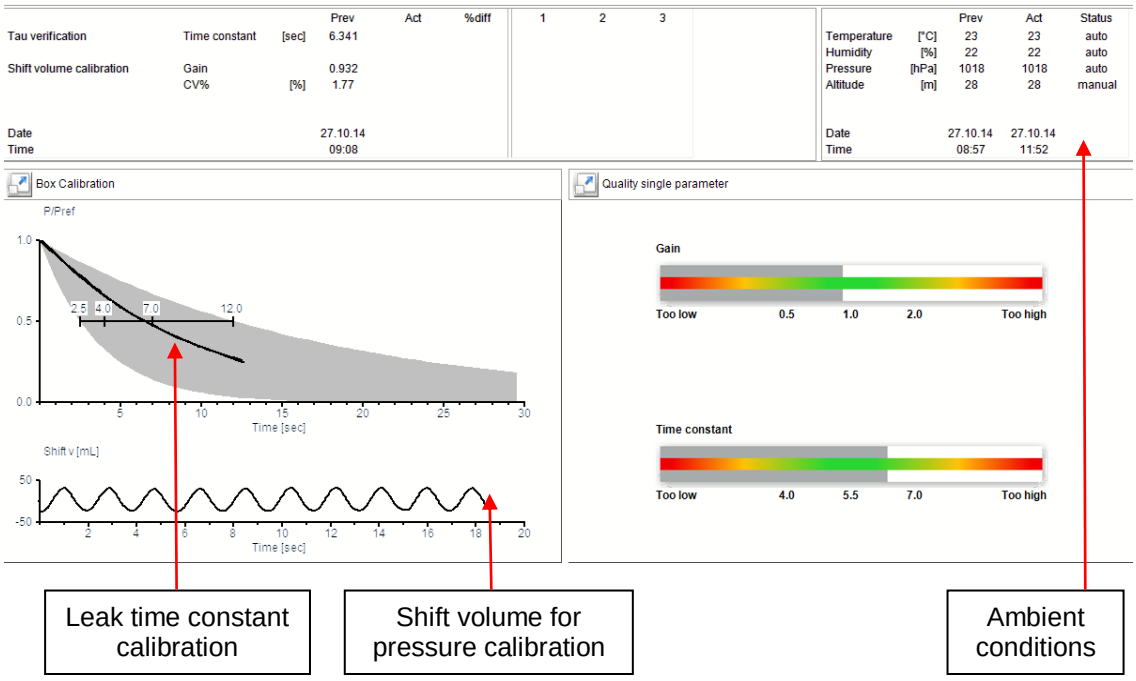
2.6.3 Plethysmographic specific airway resistance

2.6.3.1 Equipment and calibration

Specific airway resistance (sR_{aw}) was measured using a whole body constant volume variable pressure plethysmograph (Jaeger Master Screen Version 5.02, VIASYS Healthcare, Hochberg, Germany) using software incorporated by the manufacturer (SentrySuite Software V2.11.1, Carefusion Corporation, San Diego, California, USA). Ambient temperature, barometric pressure and humidity were recorded with each flow and volume calibration, and calibration performed according to manufacturer's instructions.

Pressure calibration was performed once a day with the door of the plethysmograph closed (Figure 2.9). The plethysmograph is open to the atmosphere by a small leak tube which minimises slow pressure changes not attributable to respiratory manoeuvres, such as small temperature fluctuations when a subject breathes inside the device. After a two minute pressure stabilisation period the time constant of this mechanical leak is checked by the software and three trials are required to be between four and seven seconds, with differences between tests of <1.5 seconds. The median result is selected and saved by the calibration programme. If this did not occur the calibration was repeated after checking the door seal. The pressure transducers were then calibrated by a 50mL motor-driven syringe which rapidly introduces and withdraws air into the plethysmograph. Changes in gas pressure measured then reflect changes in gas volume due to compression and decompression of gas. As this is performed without a subject inside the plethysmograph, an accurate weight must be entered for testing to compensate for the decrease in gas volume. BTPS correction factors for the volume signal were calculated by the computer software, using the ambient conditions.

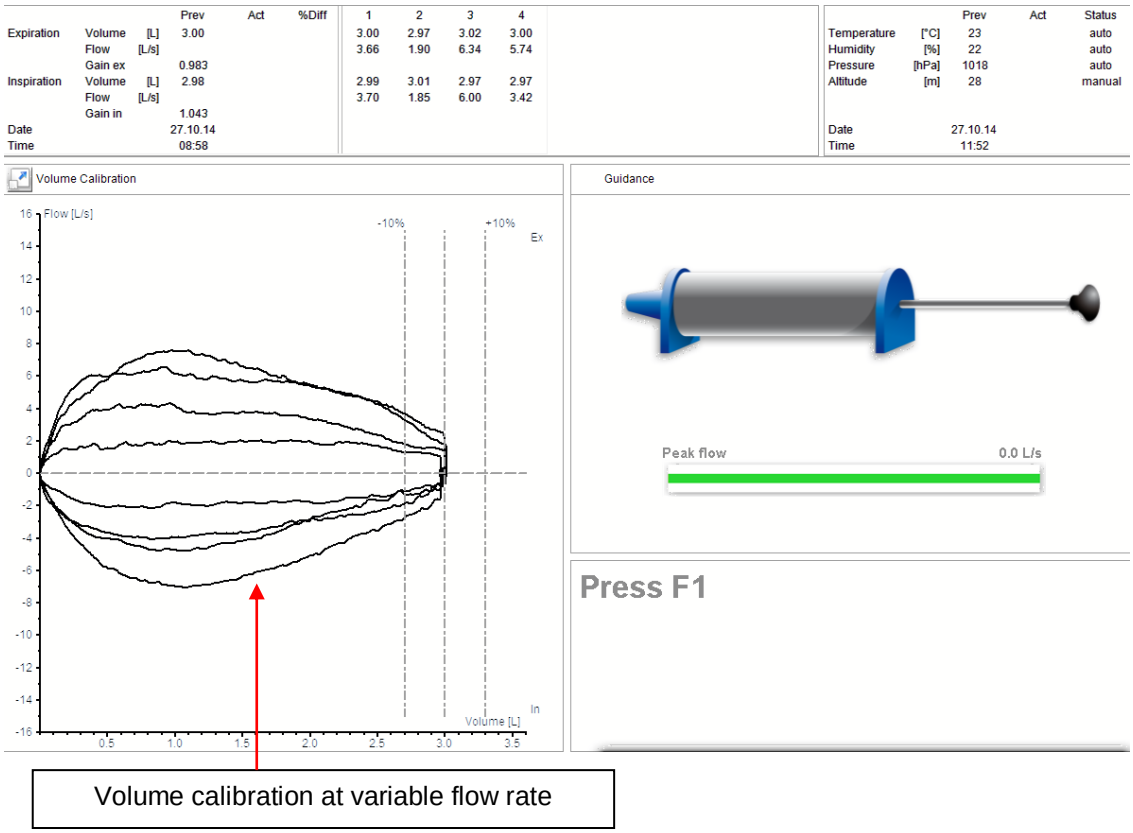
Figure 2.9: Pressure and time constant calibration of the plethysmograph



Legend: Display of Sentrysuite software during plethysmograph calibration: the leak time constant is calculated with three trials required to be between four and seven seconds. Pressure calibration according to shift volume is calculated using an in-built 50mL syringe, and volume adjusted for body temperature, pressure and water vapour saturated (BTPS) conditions

Volume calibration (Figure 2.10) of the pneumotachometer was performed on a daily basis using a 3L syringe over a range of flow rates (at least two measurements at each of high, medium and low flows). Calibrated inspiratory and expiratory volumes were required to be within 1% or 100ml, whichever was the greater.

Figure 2.10: Volume calibration of the plethysmograph pneumotachometer



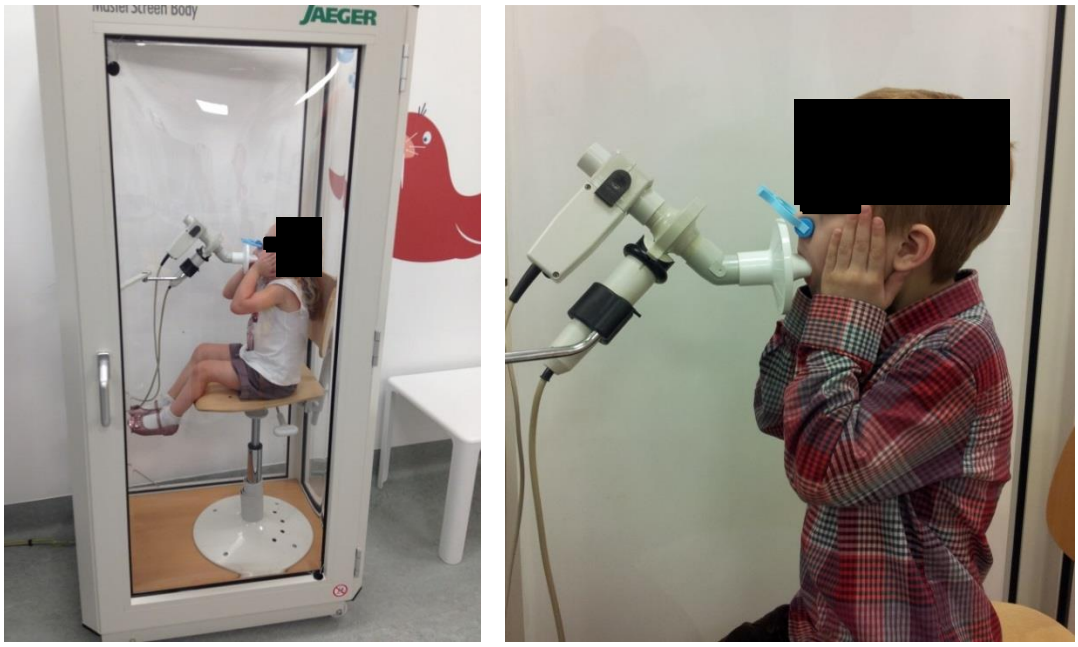
Legend: Volume calibration (horizontal axis) of the pneumotachometer at varying flow rates (vertical axis)

2.6.3.2 Data Collection

Before entering the plethysmograph the children were taught the necessary steps to complete sR_{aw} manoeuvres. Children would sit at a small table with one of the investigators (the author, ER or SL) and were asked to learn three sequential steps. The first was to fit a nose-clip and keep in in place for at least ten seconds. With the nose-clip in place, the second step was to make a good seal with their lips around the mouthpiece of a plastic bacterial filter (GVS filter technology Ltd, Morecambe, Lancashire, UK) this was subsequently attached to the pneumotachometer in the plethysmograph. The third step was to tidally inspire and expire with hands placed on the cheeks. When the child could competently complete all three steps they entered the plethysmograph for testing. This pre-test procedure was necessary as it was difficult for children of this age to learn these steps while inside the plethysmograph.

Once seated for testing the height of the chair and pneumotachometer (mounted on a stand) were adjusted for each child with the neck slightly extended (Figure 2.11) as flexion of the neck increases upper airway resistance. The door was closed for 30 seconds before beginning the test so that the pressure inside the plethysmograph could equilibrate. Then the child was asked to undertake the previously learnt manoeuvres and could hear the investigator's instruction via a microphone and speaker inside the plethysmograph. The child was encouraged to breathe at a rate of 30-45 breaths per minute (standard procedure) to minimise the effect of breathing frequency on the measurement. Some children were able to do this easily with just verbal cues from the investigators to inspire and expire; others found it easier to follow an incentive built into the software.

Figure 2.11: Preschool children performing plethysmography



Legend: Preschool children within the plethysmograph wearing a nose-clip breathing tidally on a bacterial filter attached to a pneumotachometer; left: view of whole plethysmograph, right: close up of apparatus (carer consent given for inclusion of images)

The software allowed for 10 pressure-flow loops to be viewed online and saved at any one time. When a tidal breathing pattern was established and ten acceptable loops were visualised in real-time, the series was saved. Three sets of acceptable flow-

pressure loops were attempted and recorded. No manual adjustment to the loops were made.

2.6.3.3 Data analysis

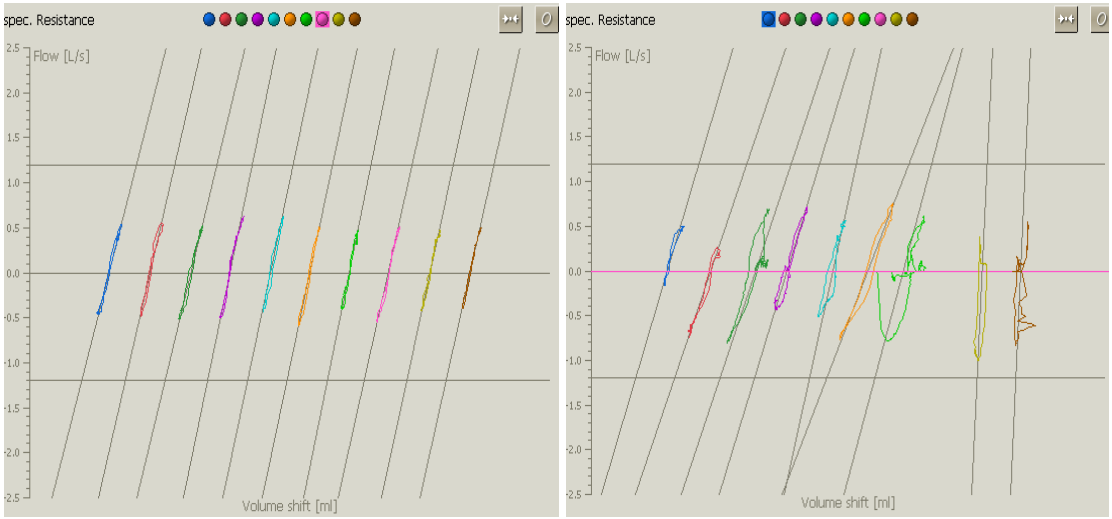
After testing, each saved recording was printed and visually inspected (by the author and ER), and over-read by a senior physiologist (Jane Kirkby [JK]). Total specific airway resistance (s*R*tot) was the primary outcome in this study, calculated by the Sentrysuite plethysmograph software from the slope of a line connecting flow points at maximal change of plethysmographic volume. Acceptable and unacceptable recordings are shown in Figure 2.12. Results were exported from the software and entered into a database (Rebase software; Rebase, London, UK), with data checked on transfer by two associates (the author, ER and SL).

A quality control score was applied as detailed in Kirkby *et al*[55], which consisted of the following:

1. Respiratory rate is between 30-45 bpm
2. Breaths are super-imposable (i.e. parallel slopes)
3. Breaths are of similar size and shape
4. Breaths are reasonably closed at zero flow
5. There are no obvious distortions to the breath (e.g. glottic closure, cough, talking)
6. There is more than one acceptable trial available

More than three of the above criteria had to be met, or the traces were rejected. More details of the test procedure and quality control for specific airway resistance can be found in Appendix 7.

Figure 2.12: Technically acceptable and unacceptable specific airway resistance recordings



Legend: Left: acceptable specific airway resistance recording; traces are superimposable, of similar size and shape and closed at zero flow (left vertical axis). Right: unacceptable recording with some loops open at zero flow, and loops of varying size and shape

2.6.4 Spirometry

2.6.4.1 Equipment and calibration

Spirometry data were collected using a Jaeger MasterScope spirometer (VIASYS Healthcare GmbH, Höchberg, Germany). A heated screen pneumotachometer is connected to a PC based system with incorporated Jaeger software (SentrySuite Software V2.11.1, Carefusion Corporation, San Diego, California, USA). Ambient temperature, relative humidity and barometric pressure were recorded prior to testing. Volume calibration of the pneumotachometer was performed once daily according to manufacturer's instructions with a 3L syringe at multiple flow rates to an accuracy of 3mL (1%). The pneumotachometer was connected to a bacterial filter (GVS filter technology Ltd, Morecambe, Lancashire, UK).

2.6.4.2 Data collection

Before testing, children were taught to take a maximal inspiration and forced expiration by means of a game. On some occasions a 'party whistle' was used to facilitate this. Testing was performed in a seated upright position with the neck slightly extended. Online incentive games built into the software were used. First the 'candles' incentive to produce a maximal peak flow (fast expiration) and then the 'bowling' incentive to produce a maximal forced vital capacity. Other games were available in the software and selected depending on which measure (peak flow or expiration to vital capacity) needed to be improved, and to keep the child's interest during the procedure. The difficulty of each incentive game could be increased or decreased to ensure maximal manoeuvres were obtained and the child motivated to complete the task.

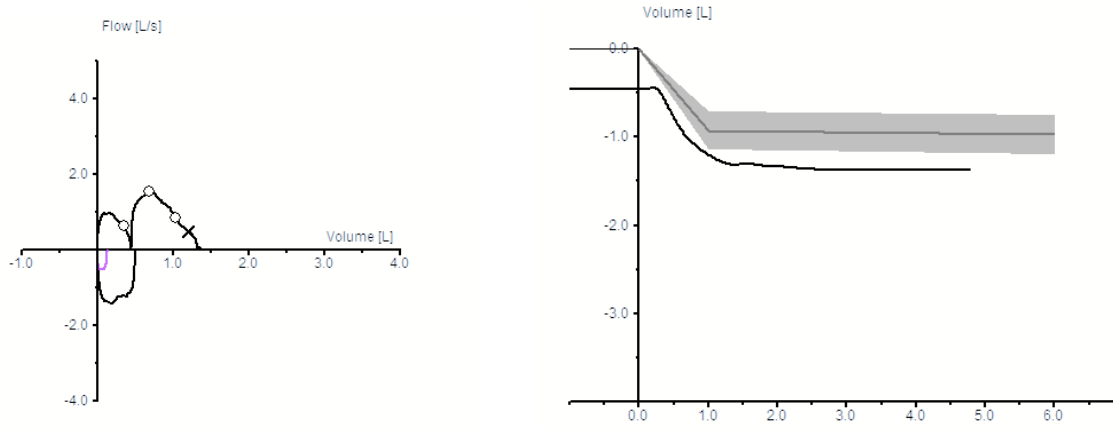
Figure 2.13: A preschool child performing spirometry and examples of incentive games

Legend: Left: A child performing spirometry with an incentive game, the aim of which was to blow the ball out of the tube (carer consent given for inclusion of images). The investigator can visualise the flow-volume loop for each attempt on the screen. Right: other examples of incentive games (bowling and candles)

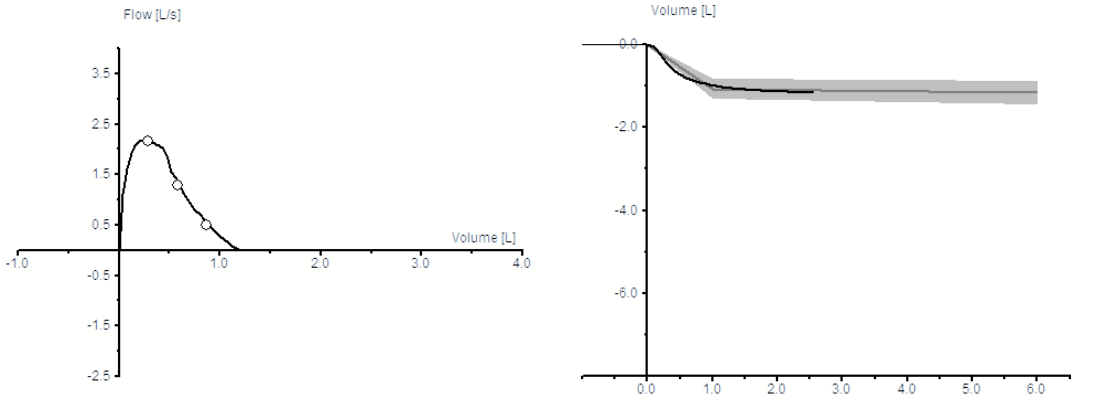
Both Jaeger and SentrySuite software enabled visual inspection of flow-volume and volume-time traces by the investigator so the effort of each attempt could be judged. A maximum of 10 attempts could be recorded by both software versions; once this was reached the attempts were saved to file and a new test file created if needed. The session was terminated once three or more good quality repeatable manoeuvres were obtained, or if the child became unsettled or showed signs of fatigue. Acceptable and unacceptable recordings are shown in Figure 2.14.

Figure 2.14: Examples of acceptable and unacceptable spirometry traces

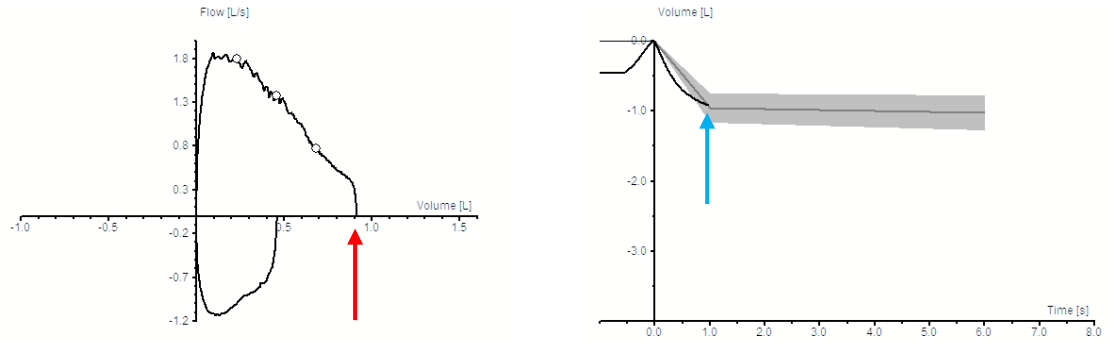
a)



b)



c)



Legend: Flow-volume traces are shown on the left and volume-time on the right; a) The flow-volume trace shows an unacceptable attempt as there is a slow rise to peak flow and two blows are attempted; b) shows a later acceptable attempt by the same child with a fast rise to peak flow, smooth descending limb of the flow-volume curve, and a clear plateau on the volume-time trace; c) an unacceptable attempt with early termination, sharp 'drop off' on the flow volume loop (red arrow), and volume-time trace does not reach a clear plateau (blue arrow)

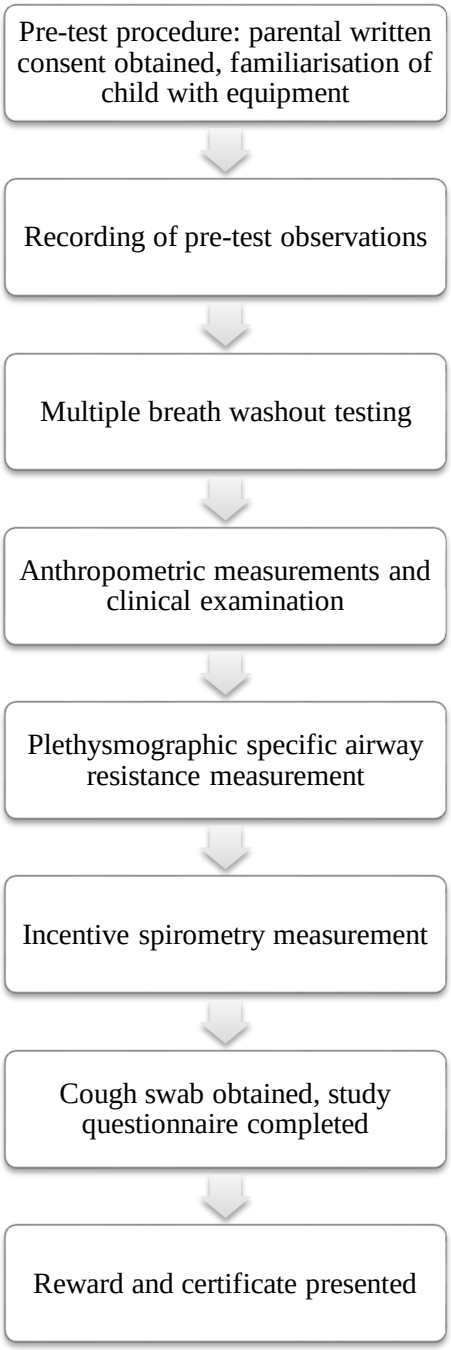
2.6.4.3 Data analysis

Offline quality control was performed by two investigators (the author and ER or SL) and over-read by a senior physiologist (JK). Acceptable tests were reported in accordance with ATS/ERS guidelines for preschool spirometry[46], with quality control factors as summarised in section 1.3.1.2. SentrySuite Software allows selection of acceptable manoeuvres and de-selection of unacceptable manoeuvres. $FEV_{0.75}$ was the primary outcome measure reported in this study. Once acceptable traces had been selected, the software reported the largest $FEV_{0.75}$ and FVC even if not from the same manoeuvre. Results were exported from the software and entered into a database (Rebase software; Rebase, London, UK), with data checked on transfer by two associates (the author, ER and SL).

2.6.5 Summary of test procedure

A summary of the test procedure is shown in Figure 2.15.

Figure 2.15: Summary of test protocol



Legend: First the pre-test procedure was performed to obtain written consent and to ensure the child was comfortable with research team members and equipment before testing. The lung function protocol was followed with cough swab (section 2.7.1), study questionnaire and reward for the child at the end of the study visit

2.7 Post-test procedure

2.7.1 Microbiology

In children with CF an oropharyngeal swab (OPS) was taken using a sterile swab and container (Transswab, Medical Wire and Equipment Co. Limited, Wiltshire, England). The child was asked to open the mouth as wide as possible, the swab placed in the mouth and directed towards the back of the oropharynx, ensuring the cotton bud at the end of the swab did not touch the oropharynx, and the child asked to cough. The swab was then placed in culture medium and sent to the microbiology laboratory at Great Ormond Street Hospital for culture and antibiotic sensitivity of any bacterial or fungal growth, in accordance with CF Trust guidelines: (https://www.cysticfibrosis.org.uk/~/media/documents/the-work-we-do/care/consensus-docs-with-new-address/laboratory-standards.ashx?la=en).

2.7.2 Clinician questionnaire

For children with CF, a clinician questionnaire (Appendix 5) was sent out retrospectively to the child's clinician at the tertiary study site to be completed from the child's medical records. This was designed to obtain accurate information of medical history and treatment relevant for subjects with CF. The author visited each participating centre and completed a record form for each study participant with medical and/or specialist nursing staff. Treatment was sub-divided into nutritional and pulmonary therapies completed on a yearly basis. Data could therefore be transferred from annual review summaries kept by each centre as part of the child's clinical record. Complications of CF and other significant medical history was also detailed. Dates and length of oral and intravenous antibiotic courses, and dates of any positive bacterial or fungal isolates recorded. This study was not designed or powered to assess which treatments in early childhood were associated with better lung function outcomes, but collection of this data allowed exploratory analysis of these factors.

2.7.3 Data integrity and storage

Each subject had been assigned a unique study number in the LCFC NBS infant study. The same numbers were used to anonymise study questionnaires and lung function

data. Paper copies of complete questionnaires and lung function results were kept in a file for each subject in a locked filing cabinet. Electronic copies were also scanned and kept in portable document format, saved on a secure hard drive. Subject contact details and study identification key were kept in a password protected database. Lung function results and questionnaire data were summarised in a spreadsheet which was subsequently cross-checked for errors. To facilitate analysis and longer-term storage, this data was uploaded to a customised database (Re-Base Ltd, London, UK) and re-checked for errors after transfer.

2.8 Summary

The protocol for the cohort 2 preschool follow up study is summarised above in Figure 2.15. The results of this study will be presented in the following first results chapter. Methodology for data analysis (and power calculations where indicated) for each results study are presented in the relevant chapter.

3 Lung function in newborn screened preschool children with CF

3.1 Introduction

It is clear that lung disease is detectable from an early stage, even shortly after birth in children with CF. However, it is difficult to measure lung function in children under six years of age. Preschool children pose a particular problem as, unlike infants, they are too old to sedate adequately and tests requiring active cooperation, such as spirometry, are often normal in children with CF at this age. The period between two and six years of age were often termed the 'silent years' of lung function. However, work from the LCFC in clinically diagnosed children with CF (cohort 1) showed that LCI, measured by the MBW technique, is a sensitive, non-invasive functional marker of early lung disease in the preschool years, and becomes abnormal before other tests of lung function[7].

These earlier studies were conducted in children whose diagnosis of CF was made when they had developed clinical signs and symptoms. With the introduction of newborn screening, children are diagnosed, and therefore treated, much earlier, and usually before respiratory symptoms arise. In view of this and other improvements in CF care since clinically diagnosed children were studied, the LCFC recruited a new cohort of NBS infants who underwent infant pulmonary function tests at three months, one year and two years of age[36, 127, 129]. Although lung function was poorer than controls at three months, an improvement was seen in $FEV_{0.5}$ at one year of age, and all primary outcome measures ($FEV_{0.5}$, FRC_{pleth} and LCI) remained stable throughout the study with standard UK CF care. Very few infants (7-19% depending on which measurement is considered) had abnormal lung function at two years of age.

This first results chapter reports follow up of the same LCFC infants in cohort 2, diagnosed with CF by NBS, between three and six years of age, and investigates whether lung function stability is maintained to the preschool years.

3.2 Hypothesis, aims and objectives

3.2.1 Hypothesis

In view of stable lung function reported at one and two year follow up in cohort 2 of the LCFC NBS study, the hypothesis was that in children with CF, lung function remains within the normal range at follow up in the preschool years, when compared to contemporaneous controls.

3.2.2 Aim

The aim of this study was to report lung function in children with CF and managed with standard UK CF care, and contemporaneous healthy control children, between three and six years of age.

3.2.3 Objectives

The specific objective was to measure and compare lung function in preschool children with CF diagnosed by NBS with an age and gender matched contemporaneous recruited healthy control group for the following primary outcome measures:

- LCI measured using the MBW technique
- Specific total airway resistance (s*R*tot) measured by plethysmography
- $FEV_{0.75}$ measured by spirometry

3.3 Methods

3.3.1 Subjects and measurements

Children with CF were originally recruited as infants born between January 2009 and July 2011 from the six participating specialist CF centres, and controls from a central London hospital as part of the LCFC longitudinal study of newborn screened children with CF. Recruitment in infancy is described in detail in section 2.3.

Families who had consented to be contacted for future research and had lung function data at one or more infant test occasions (three months, one year and two years) were invited to return for testing at preschool age (three to six years) between June 2013 and June 2015. Exclusion criteria listed in section 2.4.3 were re-visited on initial telephone contact and in detail using the test day questionnaire (Appendix 4) at the study visit, to ensure children were still eligible for the study. Children were invited to attend when well, with no symptoms of, or treatment for, respiratory infection in the three weeks before their preschool test occasion.

After completing the test day questionnaire and pre-test procedures, lung function measurements were made in the same laboratory for all subjects in the following order:

- MBW using a mass spectrometer-SF_6 tracer gas system with mask interface while the subject was sitting upright, primary outcome LCI z-score, with LCI reported as the average of three acceptable MBW traces
- Plethysmographic specific airway resistance (sR_{aw}), primary outcome s*R*tot
- Incentive spirometry, primary outcome $FEV_{0.75}$ z-score

If the full protocol was not completed on the first visit, subjects were invited to attend a second test occasion around six months later. A third test occasion was offered (at any time after the second test occasion) if the second visit was not complete. Data were collected retrospectively from each CF centre to describe the clinical course of the children, and details of current and past medications, courses of intravenous antibiotics and isolates on cough swab, sputum or BAL were noted. Lung function and clinical data were exported to a research database, linked by test occasion (Rebase software; Rebase, London, UK). A more detailed description of the recruitment process, exclusion criteria, clinical data collection and lung function methodology for the preschool NBS follow up study is described in Chapter 2.

3.3.2 Statistical analysis

Summary data are presented as mean (SD), median (IQR) or median (range). Standard deviation scores (z-scores) were calculated using published reference equations from healthy control data to express growth (height, weight, BMI[138]) and lung function parameters (LCI[75], s*R*tot[55] and $FEV_{0.75}$[49]). Lung function measurements were made using identical equipment and protocols as reference data. For $FEV_{0.75}$, ethnicity specific data were not available at the time of testing, so equations for white individuals

were used for all subjects (7/67 children with CF were of non-white ethnicity and 4/41 controls).

For lung function primary outcomes, an abnormal result was defined ≥ 1.96 z-scores for LCI and s*R*tot and ≤ -1.96 z-scores for $FEV_{0.75}$. For group comparisons between subjects with CF and healthy controls, Chi-squared tests were used for categorical variables and Mann-Whitney U or student t-tests for numeric data, depending on whether the data were normally distributed. Differences are presented with 95% confidence intervals where appropriate. Linear regression analysis was used to investigate the relationship of lung function parameters with age.

Statistical analyses were conducted using SPSS software (IBM SPSS Statistics for Windows, Version 24.0). Figures were produced using SPSS and GraphPad Prism software (version 7.00 for Windows, GraphPad Software, La Jolla, California, USA). Statistical significance was taken as $p<0.05$.

3.3.3 Power of study

96 children with CF and 62 controls tested as infants were available for preschool follow-up (a ratio of 1.5:1). The power calculation accounted for multiple comparisons (as there were three primary outcomes) calculated as z-scores (expected standard deviation of 1) and unequal groups. It was estimated that 60 children with CF and 40 controls would detect a difference in SD score of 0.7 (or greater) in the primary lung function outcomes (LCI, s*R*tot and $FEV_{0.75}$) with 80% power at the 5% significance level. Having said this, the actual numbers recruited were opportunistic and driven by the size of the original cohort and retention in the study.

3.4 Results

3.4.1 Recruitment accrual

A total of 154 children (92 with CF and 62 healthy controls) had been enrolled as infants to the NBS LCFC longitudinal study, in whom at least one valid infant lung function test result had been obtained, and whose families had consented at that time to be contacted for follow up studies. Attempts to contact all families were made in the

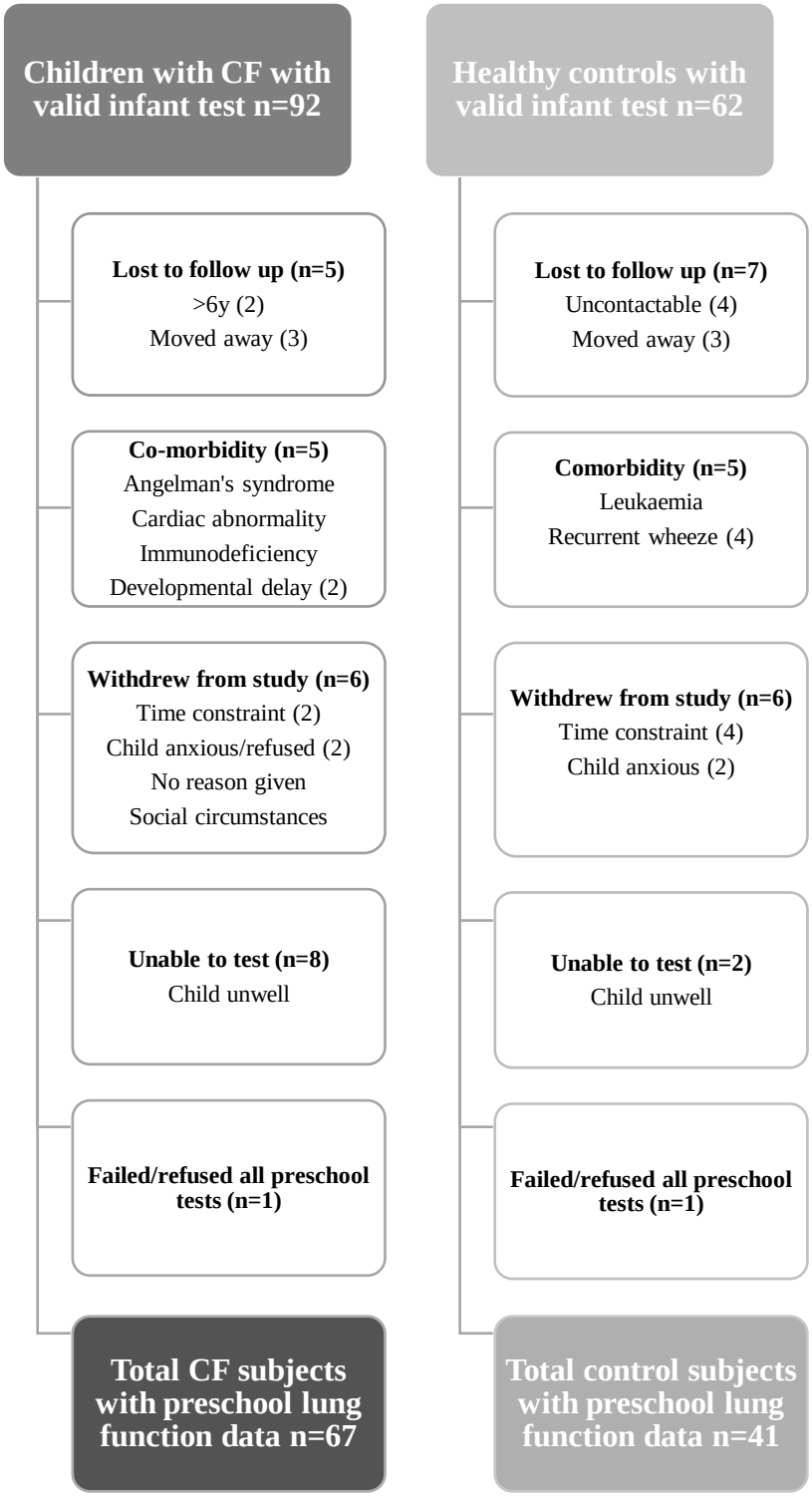
first two months of the study period (between June and August 2013), but was on-going throughout the study duration. If contact details had changed, the child's CF centre (or general practitioner for controls) were telephoned in an attempt to obtain updated contact information.

Of the 92 children with CF, 25 (27%) were unable or ineligible to attend for testing. Five children were lost to follow up: two children were over six years of age when the study started, and three families had moved out of area or overseas and were unable to attend. Five were excluded due to medical co-morbidity: one child had Angelman's syndrome, two had severe developmental delay and their parents felt they would not be able to understand test procedures, one had an immunodeficiency and one had a cardiac condition (both diagnosed after two years of age). The parents of six children withdrew consent: two wanted to avoid additional time visiting hospital, two because they felt their child would be too anxious, or the child declined to participate, one gave no reason and one family had complex social circumstances (parental illness). A further eight children were unwell at time of study visit and/or during the study period and met clinical exclusion criteria. This was most commonly due to subjects receiving a course of oral or intravenous antibiotics within three weeks of the test date.

Of the 62 healthy controls enrolled as infants, 21 (34%) were unable to be tested. Seven were lost to follow up: we could not contact four families and three had moved out of the area or overseas. Five children had co-morbidities and met exclusion criteria: one had been diagnosed with leukaemia and four had developed recurrent wheeze and/or had taken treatment for wheezing episodes in the year preceding the study. Six families withdrew from the study; four because of time constraints and two felt their child would be too anxious to complete the test procedure. One child was too unwell with upper respiratory tract infections each time they were contacted to attend.

Of the children that were able to attend, a further two children (one from each group) either refused or failed all preschool lung function tests. Subsequently 67 in the CF group and 41 healthy controls attempted testing on at least one occasion and had at least one successful lung function measure. Recruitment and accrual is summarised in Figure 3.1.

Figure 3.1: Recruitment and accrual in cohort 2 children with CF and healthy controls at preschool age



Legend: Numbers in brackets represent the number of subjects (n) for each category

3.4.2 Background characteristics of children with CF and healthy controls

Background characteristics for children with CF and healthy controls are summarised in Table 3.1. The mean age at testing for all children was 4.2 years (range 3.1-6.0 years). More children with CF were approaching six years of age at the start of the study, and there were a higher number of subjects in this group, so initial recruitment efforts were concentrated on CF subjects to ensure inclusion in the study. As a result, children with CF were statistically significantly older (4.8 years) on average than controls (4.4 years). Those with CF had significantly lower birthweight, and were shorter and lighter at the time of test occasion than controls (as has been previously reported), but differences were clinically minor and BMI z-scores were not significantly different between the groups. CF and control groups were otherwise well matched for gender and ethnicity.

Table 3.1: Comparison of background characteristics in children with CF and healthy controls

	CF	Controls	Δ [95%CI] CF-HC
Number of subjects	67	41	
Male (%)	31 (46%)	19 (46%)	0% [-19%; 18%]
White ethnicity (%)	58 (87%)	34 (83%)	4% [-10%; 19%]
Age at test (years)	4.76 (0.75)	4.41 (0.62)	0.35 [0.08; 0.63]*
Birthweight z-score#	-0.38 (0.96)	-0.02 (0.74)	-0.36 [-0.71; -0.01]*
Weight z-score#	-0.11 (0.84)	0.43 (1.15)	-0.54 [-0.92; -0.16]**
Height z-score#	-0.23 (0.87)	0.23 (1.21)	-0.46 [-0.90; -0.03]*
BMI z-score#	0.09 (0.67)	0.25 (0.96)	-0.16 [-0.47; 0.15]

*Legend: Data presented as mean (SD) or mean difference (Δ) [95% CI] in children with CF minus healthy controls (CF-HC). #=calculated using British 1990 reference[138] for height, weight and body mass index (BMI). *represents statistical significance p<0.05 and **p<0.01. Children with CF had significantly lower height and weight z-scores than control subjects at preschool test occasion, but BMI was not significantly different*

3.4.3 Clinical characteristics of subjects with CF

Details of clinical characteristics and relevant pathogens are presented in Table 3.2, and treatments at preschool test in Table 3.3. Clinical characteristics (age at diagnosis, genotype, presentation with meconium ileus and pancreatic status) were available for all 67 children with CF. Other clinical data (pathogen isolation and treatment at preschool test) were not available for three children as their care had been transferred to a CF centre outside of London, and ethical approval for collection of data was only applicable to London sites. All children with CF apart from three were diagnosed before ten weeks of age. These three children with a later diagnosis had equivocal screening results, and all were pancreatic sufficient. Seven children (10%) presented with meconium ileus. Children with a later diagnosis or meconium ileus presentation were not outliers in any growth or lung function measurement, and inclusion or exclusion of these subjects did not change the study results, so they remained in the analysis. 41 (61%) of children were homozygous for the p.Phe508del mutation, 20 heterozygous (30%) and seven (10%) had two other mutations.

Most children (78%) had 'ever' isolated *Pseudomonas aeruginosa*, and 19% had a positive isolate in the 12 months preceding preschool test (only one child had chronic infection with *Pseudomonas aeruginosa* as defined by the Leeds criteria[139]). Over half (60%) had previous *Staphylococcus aureus* infection, 57% *Haemophilus influenzae* and 24% *Stenotrophomonas maltophilia* 'ever' but positive isolates were less common in the year before preschool test (27%, 9% and 6% respectively). One child isolated methicillin-resistant *Staphylococcus aureus* (MRSA) in the 12 months preceding preschool test and three children 'ever'. Only four children (6%) had *Aspergillus fumigatus* infection 'ever', all isolated in the year before preschool testing. No other organisms were found in children with CF which are known to cause significant infection in this disease; no children isolated non-tuberculous *Mycobacteria*, *Achromobacter, Serratia* or *Burkholderia* species between diagnosis and preschool testing.

55% of children remained on prophylactic antibiotics at preschool testing, 38% were receiving mucolytic therapy and 23% were on long term nebulised antibiotic therapy. The total number of intravenous (IV) antibiotic courses from birth varied widely (zero to nine), with 40 (66%) of children having at least one course, but most children (78%) had not received a course in the year preceding preschool testing. One child received nine courses of IV antibiotics from birth, three children 5-6, eight children 3-4 and 30

children 1-2. No children included in the analysis received CFTR modulators at any time point of the study.

Table 3.2: Clinical characteristics and bacterial pathogens in children with CF

Clinical characteristics:	
Age at diagnosis, median weeks (range)	3.4 (1.1 – 30.5)
p.Phe508del homozygous	41 (61%)
p.Phe508del heterozygous	20 (30%)
Presented with meconium ileus	7 (10%)
Pancreatic insufficiency	58 (87%)
Bacterial pathogens isolated prior to preschool testing:	
Pseudomonas aeruginosa	50 (78%)
Staphylococcus aureus	40 (63%)
Haemophilus influenzae	38 (59%)
Stenotrophomonas maltophilia	16 (24%)
Bacterial pathogens in the 12 months preceding testing:	
Pseudomonas aeruginosa	12 (19%)
Staphylococcus aureus	17 (27%)
Haemophilus influenzae	6 (9%)
Stenotrophomonas maltophilia	4 (6%)
Age at first isolate of bacterial pathogen:	
Pseudomonas aeruginosa	1.21 (0.12 – 4.34)
Staphylococcus aureus	1.38 (0.04 – 5.76)
Haemophilus influenzae	1.05 (0.02 – 4.67)
Stenotrophomonas maltophilia	2.26 (0.51 – 5.92)

Legend: Data presented as median (range) or n (%) as indicated, based on 64 subjects, apart from 'clinical characteristics' where data for all children were available

Table 3.3: Details of treatment in children with CF

Treatment at preschool test:	
Treatment for gastro-oesophageal reflux disease	26 (41%)
Oral antibiotic prophylaxis	35 (55%)
Long term nebulised antibiotic	15 (23%)
Ursodeoxycholic acid	12 (19%)
Nebulised rhDNase	20 (31%)
Nebulised hypertonic saline	11 (17%)
Any mucolytic therapy	24 (38%)
CFTR modulator therapy	0 (0%)
Long term azithromycin	5 (8%)
Treatment for ABPA 'ever'	1 (2%)
IV antibiotic courses from birth, median (range)	1 (0-9)
IV antibiotic courses 12 months before test, median (range)	0 (0-2)

Legend: Data presented as median (range) or n (%) as indicated, based on 64 subjects. Abbreviations: rhDNase = recombinant human deoxyribonuclease, ABPA = allergic bronchopulmonary aspergillosis, IV = intravenous

3.4.4 Feasibility of study protocol

117 subjects (73 children with CF and 44 healthy controls) attended for lung function measurements. Of children with just one test occasion, one child with CF refused all tests and one healthy control attempted but failed all tests. A further seven children (five with CF and two controls) attempted testing but were then excluded from the final dataset when the test day questionnaire revealed either co-morbidity or illness (despite pre-test telephone screening).

Of the 116 children attempting any lung function test, a valid result was obtained in 79 (68%) for all three lung function tests (the fully study protocol) on their first visit; 48 (67%) of children with CF and 31 (70%) of controls. MBW had the highest success rate in both groups. The average number of MBW runs required to obtain three acceptable traces was 5 (including washouts that were terminated early due to within test quality control fail). Up to 10 runs were attempted if required and tolerated, and MBW testing did not exceed one hour. The coefficient of variation for LCI of the three

repeat MBW runs was 5.6% (CF 5.7%, controls 5.6%) and 6.9% for FRC (CF 6.7%, controls 7.3%), similar to those in other studies of preschool children[140, 141].

Apart from the children who refused testing altogether (not included in the feasibility analysis), the most common reason for test failure was not meeting quality control criteria, as discussed in section 2.6. For MBW, this was often distraction; talking or breaking of the mask seal before the wash-out was completed. For specific airway resistance, children who failed to produce acceptable measurements found the sequence of manoeuvres difficult to learn and reproduce in the plethysmograph, despite the pre-test procedure. For spirometry, quality control fail was most often due to poor technique. Some children were unable to produce rapid rise to peak expiratory flow and others a plateau on the volume time curve. A further reason for failing spirometry was lack of repeatability (two manoeuvres within 0.1 liters or 10% of the highest value for forced vital capacity and forced expired volume in 0.75 seconds).

Feasibility for the full protocol and for each lung function test is shown in Table 3.4. Older children were more likely to obtain valid results for each lung function test, and for the full study protocol, as shown in Table 3.5.

Table 3.4: Feasibility for each lung function test and the full study protocol for all children and by diagnosis on first test occasion

	MBW	sR_{aw}	Spirometry	All tests
All children (n=116)	106 (91%)	95 (82%)	89 (77%)	79 (68%)
CF (n=72)	67 (93%)	56 (77%)	56 (78%)	48 (67%)
HC (n=44)	39 (89%)	39 (89%)	33 (75%)	31 (70%)

Legend: Data presented as number of subjects, n (%). Success was highest in multiple breath washout. Feasibility for each test were similar in children with CF and healthy controls (HC). Abbreviations: MBW = multiple breath washout, sR_{aw} = specific airway resistance

Table 3.5: Feasibility of each lung function test and the full study protocol by age at first test (all children)

Age	MBW	sR_{aw}	Spirometry	All tests
3.0-3.9y (n=36)	32 (89%)	25 (69%)	21 (58%)	20 (56%)
4.0-4.9y (n=58)	52 (90%)	48 (83%)	46 (79%)	37 (64%)
5.0-6y (n=21)	21 (100%)	21 (100%)	21 (100%)	21 (100%)

Legend: Data presented as number of subjects, n (%). Success rates increased with age over three to six years. Abbreviations: y=years, MBW = multiple breath washout, sR_{aw} = specific airway resistance

Of the 37 children who could not complete the full study protocol on first test occasion, 23 children (20 with CF and 3 controls) were able to attend a second test occasion. 17 children (74%), 15 with CF and two controls, then completed the full protocol at second visit. Two children, both with CF, attended a third test occasion and successfully performed all three lung function tests on their third visit.

The median age (range) at first preschool test occasion was 4.21 (3.10-6.01) years, at second test 4.95 (3.85-6.05) years and at third test 5.72 (5.48-5.95) years. The median interval (range) between first and second test occasion was 0.8 (0.19-1.68) years and 0.63 (0.26-0.99) between second and third. If children attended for more than one visit, results for summary data were chosen from the most complete test occasion.

3.4.5 Comparison of preschool lung function in children with CF and healthy controls

Table 3.6 presents lung function results for MBW, sR_{aw} and spirometry in children with CF and healthy controls at preschool follow-up. Overall, there were significant differences between CF subjects and controls for all primary lung function outcome measures (LCI, s*R*tot and $FEV_{0.75}$), contrary to the hypothesis which had been based on the stability of lung function to two years of age previously reported[129]. The greatest

difference was seen in LCI, with mean z-score 1.47 higher in the CF population ($p<0.001$), indicating significant ventilation inhomogeneity in this group when compared to controls. LCI z-score ranged from -1.0 to 7.1 in those with CF, and from -0.8 to 2.1 in controls. Importantly, LCI was the only lung function parameter outside the normal range (≥1.96 z-scores) at a mean of 2.14 z-scores in children with CF. Specific airway resistance was also increased; with s*R*tot 0.72 z-scores higher in the CF group ($p<0.01$) and spirometry poorer with $FEV_{0.75}$ 0.54 z-scores lower ($p<0.05$) in those with CF. 26 (39%) children with CF had abnormal LCI; but only 5 (7%) abnormal s*R*tot and 7 (11%) abnormal $FEV_{0.75}$. Lung function z-score data are presented graphically in Figure 3.2.

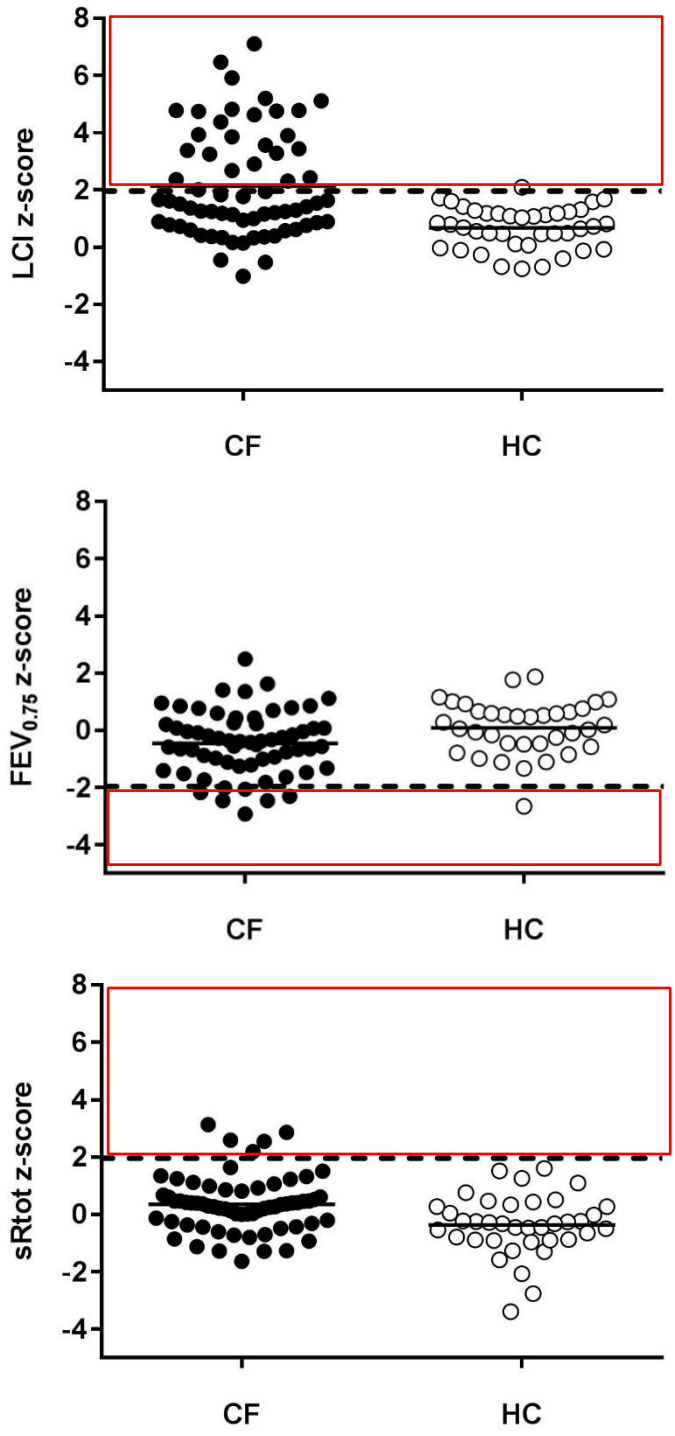
Table 3.6: Comparison of lung function results between children with CF and controls

	Cystic Fibrosis	Healthy Controls	Δ [95%CI] CF-HC
LCI	8.28 (1.67)[a]	7.09 (0.45)[b]	1.19 [0.76; 1.63]***
LCI z-score[#]	2.14 (1.84) [a]	0.67 (0.72)[b]	1.47 [0.96; 1.97]***
FRC_{MBW}[#]	0.41 (0.82)[a]	0.39 (0.73)	0.03 [-0.29;0.35]
s*R*tot z-score[#]	0.35 (0.99)[c]	-0.37 (1.04)[d]	0.72 [0.32; 1.13]**
$FEV_{0.75}$ z-score[#]	-0.45 (1.12)[e]	0.09 (0.93)[f]	-0.54 [-0.98; -0.10]*
FVC z-score	0.03 (1.03)[i]	0.25 (0.90)[f]	-0.22 [-0.63; 0.19]
$FEV_{0.75}$/FVC z-score	-0.45 (1.11)[e]	0.09 (0.93)[f]	-0.32 [-0.64; -0.00]

Legend: Data presented as mean (SD), and mean difference (Δ) [95% CI], primary outcome measures in blue. Number of subjects: a=66; b=39; c=65; d=38; e=62; f=35; g=60; h=34; i=64. [#]=z-scores calculated from published reference equations[49, 55, 75]. Abbreviations: LCI = lung clearance index, sRtot = specific total airway resistance, $FEV_{0.75}$ = forced expired volume in 0.75 seconds.

Children with CF had significantly poorer lung function than control subjects for all primary outcome measures at preschool test, the most significant difference seen in LCI ($p<0.001$) which was outside the normal range at 2.14 z-scores.

Figure 3.2: Lung clearance index, specific airway resistance and forced expired volume z-scores in preschool children with CF and controls

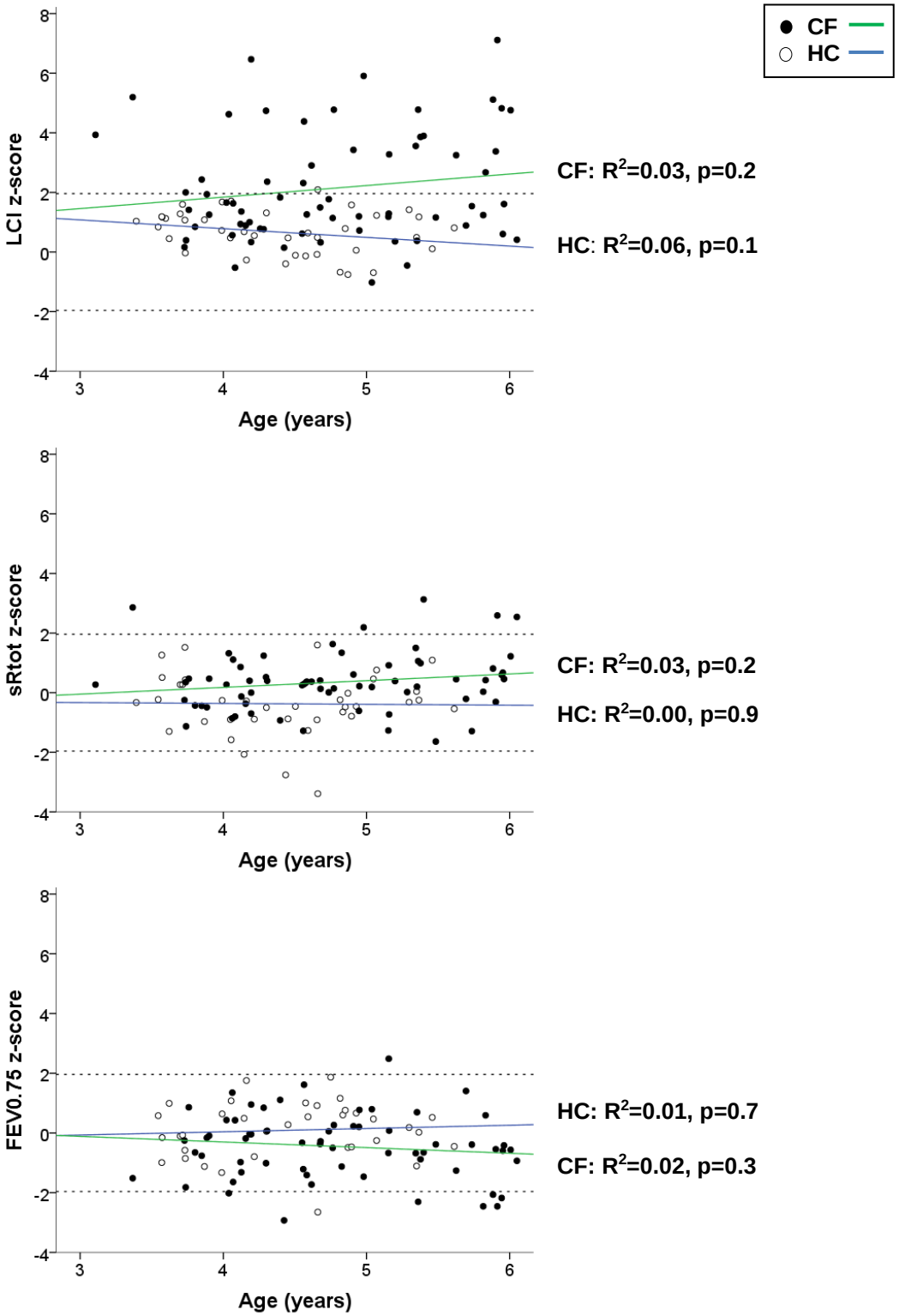


Legend: Children with CF presented as filled black circles, control children open circles. Solid horizontal lines represent the mean of each group, and broken lines +1.96 z-scores for lung clearance index (LCI) and specific airway resistance (sR_{tot}), a result above this line being abnormal, and -1.96 z-scores for forced expired volume in 0.75 seconds ($FEV_{0.75}$), a result below this line is abnormal; subjects with abnormal results shown in the red boxes. By preschool test occasion, children with CF had significantly poorer lung function than healthy controls for all three lung function outcome measures

3.4.6 The effect of age on lung function parameters

As the age range at preschool test was relatively wide (3.1 to 6.0 years), linear regression was used to investigate whether there was any decline in lung function parameters with age, or if mean results were representative of all children within the preschool age bracket. An increase in LCI z-score was seen in subjects with CF from three to six years, but the confidence intervals were wide (mean regression coefficient [95% CI] 0.39 [-0.21;0.98]) and the linear relationship not significant (R^2 0.03, p=0.20). LCI is predicted to rise by 0.4 z-scores with every year of age in subjects with CF. s*R*tot also increased with age in CF subjects (0.23 [-0.09;0.54]) and $FEV_{0.75}$ decreased (-0.19 [-0.57;0.20]) but again linear associations were not significant, nor were changes with age in controls and any of the lung function primary outcomes. Mean group values were therefore considered representative of children between three and six years of age. R^2 and p-values for each measure in CF subjects and controls are shown in Figure 3.3.

Figure 3.3: The relationship between preschool lung function primary outcome measures and age



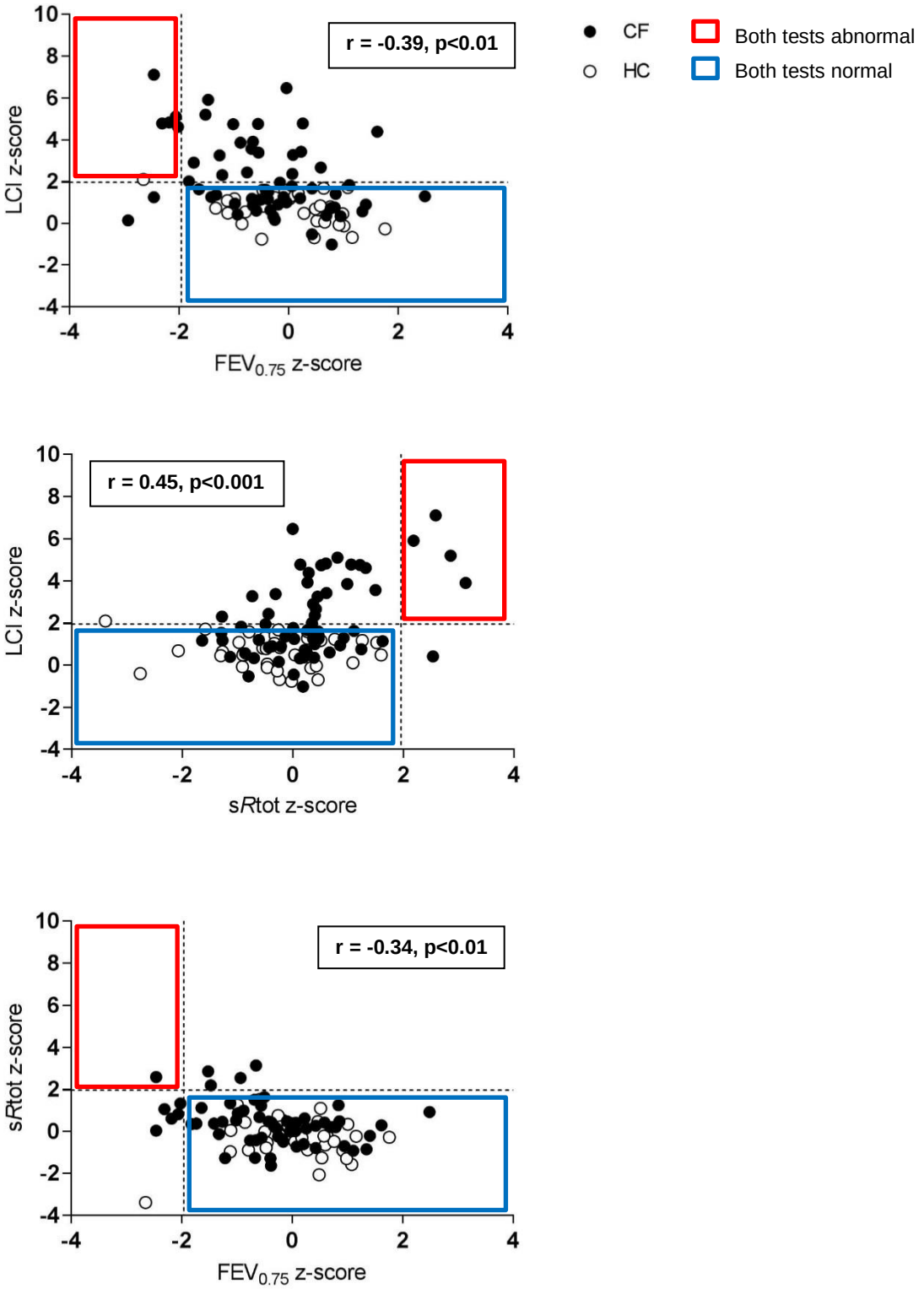
Legend: Subjects with CF shown as closed black circles, controls open circles. Broken lines represent limits of normality for each lung function measure (+/- 1.96 z-scores). Regression lines illustrate change with age for children with CF (green) and controls (blue) lines. No significant linear relationships were found between lung function measures and age.

3.4.7 Relationship between lung function measures

Results were further examined to investigate what proportion of CF subjects had abnormal results for more than one measure to identify a subgroup with a significant deficit across all parameters. Furthermore, if all subjects with abnormal s*R*tot and $FEV_{0.75}$ also had abnormal LCI, there would be little additional information gained from undertaking the former two tests in preschool children and LCI could be used as the preferred monitoring tool or outcome measure. Figure 3.4 illustrates correlations between the three lung function primary outcomes. In subjects with CF, there was a significant moderate negative correlation between LCI and $FEV_{0.75}$ z-scores ($r= -0.38$, $p<0.01$), and between s*R*tot and $FEV_{0.75}$ z-scores ($r= -0.34$, $p<0.01$) and a moderate positive correlation between LCI and s*R*tot z-scores ($r=0.45$, $p<0.001$).

Of seven children with CF and abnormal $FEV_{0.75}$, all but two children had abnormal LCI. Five children had abnormal s*R*tot and of these, all but one had an abnormal LCI. For those with a normal LCI, only two children had abnormal $FEV_{0.75}$ and one child abnormal s*R*tot.

Figure 3.4: The relationship between lung function measures in children with CF and healthy controls



Legend: Children with CF presented as black circles, and control children open circles. Broken lines represent +1.96 z-scores for lung clearance index (LCI) and specific airway resistance (sRtot), and -1.96 z-scores for $FEV_{0.75}$ (limits of normality). LCI identifies nearly all children with CF who also have abnormal results for sRtot and $FEV_{0.75}$

3.5 Discussion

3.5.1 Summary of principal findings

The principal findings of this study were:

1. NBS preschool children with CF have significantly poorer lung function than healthy controls for all primary lung function outcomes

2. LCI z-score was the most sensitive marker of abnormality in NBS preschool children with CF, with 39% having an abnormal result, and only 7% having an abnormal s*R*tot z-score and 11% an abnormal $FEV_{0.75}$ z-score. Nearly all children with an abnormal s*R*tot or $FEV_{0.75}$ z-score also had an abnormal LCI z-score (4/5 and 5/7 respectively)

3. LCI z-score was the only lung function primary outcome outside the normal range at 3-6 years in children with CF, with mean (SD) z-score of 2.14 (1.84)

4. Height and weight z-score were significantly poorer than controls in NBS children with CF, but BMI z-score was not different to controls

3.5.2 Review of hypothesis

The hypothesis of this study was:

'In view of stable lung function reported at one and two year follow up in cohort 2 of the LCFC NBS study, the hypothesis was that in children with CF, lung function remains within the normal range at follow up in the preschool years, when compared to contemporaneous controls

The principal finding of this study disproves this hypothesis, as LCI z-score was outside the normal range by three to six years in children with CF, and all lung function primary outcome measures were significantly worse when compared to controls.

3.5.3 Comparison of this study to other reports

The objective of this study was to report lung function in preschool children with CF and compare it to healthy control subjects. Children with CF had significantly poorer lung function in all primary outcome measures when compared to controls. Other recent cross-sectional reports of LCI in NBS children with CF come from the AREST-CF cohort of children[134] at preschool follow up. These are the same children who had significantly abnormal infant pulmonary function tests (-4.3 z-scores in $FEV_{0.5}$ from RVRTC at two years). In their study of 58 children aged three to six years, children with CF had a higher LCI (mean [SD] 8.00 [1.45] compared to control subjects (6.67 [0.56]). The upper limit of normal was defined from the study control population as 7.71. However, the raw LCI values are not directly comparable to those reported in this thesis as different tracer gases were used. The AREST-CF group used N_2 washout whereas the LCFC study used SF_6 washout, and N_2 is known to give higher LCI results than SF_6[142].

The proportion of children with abnormal results however can be compared; 55% children with CF in the AREST-CF study had abnormal LCI compared to 26/67 (39%) in this thesis. There is a suggestion therefore of a difference between cohorts, in that the AREST-CF subjects have poorer lung function than those of the LCFC. However, in the AREST study, a significant proportion (39%) of children had respiratory symptoms at the time of testing, whereas the LCFC preschool children were asymptomatic. As pulmonary exacerbations are associated with higher LCI values[143], this alone could have led to the higher proportion of children with abnormal LCI in the AREST-CF study. A separate report from the AREST-CF group, designed primarily to correlate CT abnormalities with LCI, reported LCI in a subgroup of 39 preschool children (some with repeated MBW measurements). A similar proportion of children (58%) had abnormal LCI, but it is likely that there is overlap between the two studies and some of the same subjects were reported twice. Clinical symptoms were not reported in this latter study.

A recent report from Canada is also important to include with respect to preschool LCI in children with CF[144]. This study looked at repeated measures of LCI between 2.5 and six years of age (in 78 children with CF and age matched control subjects); with N_2 MBW measurements at enrolment, and one, three, six and 12 months after. Spirometry was also measured. As found in this thesis $FEV_{0.75}$ was within the normal range, but unlike this study there was not a significant difference to control children.

LCI was, however, a sensitive marker of abnormality and significantly different to controls; 67% of children with CF had an abnormal LCI at the first study visit, and LCI significantly deteriorated on average [95% CI] by 0.40 [0.14;0.66] LCI units per year (the same rate of decline as shown in this study). This remained true when the analyses were limited to children without symptoms, with a deterioration of 0.34 [0.08;0.60] LCI units per year. Although the children reported in this thesis did not have repeated measures, there was a tendency for LCI to increase with age as seen in Figure 3.3. This is further evidence that LCI is abnormal in the preschool population and suggests it can deteriorate with age.

Although not the primary focus of this chapter, this study also reported nutritional markers which are known to be closely associated with lung function outcomes in children with CF[145]. Preschool children were found to have significantly poorer height and weight z-scores than control subjects. These differences were minor, and not reflected in BMI z-score which showed no difference to controls. Despite the Wisconsin RCT showing improvement of nutritional outcomes in NBS children compared to non-NBS children with CF, it seems there is still a difference between healthy children and those with CF despite current early management of pancreatic insufficiency. The aforementioned Canadian study investigating the change in preschool LCI also reported poorer nutrition in CF patients compared to controls[144], as has the AREST-CF group at early school age (4-6 years)[133]. It may be that minor nutritional deficits start to become apparent at this age, which was not seen in LCFC infants[129], with a subsequent detrimental effect on lung function.

3.5.4 Strengths and limitations of the study

In agreement with other studies of preschool children[7], there was excellent feasibility for the three lung function tests employed. LCI was the most sensitive marker of abnormality in this study, and highly feasible in this age group, so there is potential for its use as a monitoring tool in clinical practice or outcome measure in trials. This is discussed in more detail in chapter 7.

A mass spectrometer was used for SF_6 gas analysis in this study, not available in many centres, and is mostly therefore a research tool. With the recent development of commercial N_2 wash-out devices using 100% O_2 which is freely available, although not directly comparable to SF_6 washout studies, there is potential for MBW to be used in clinical monitoring using similar methodology to that employed in this study.

A further strength of this study is as well as measurements being made in children with CF, a contemporaneous healthy control group was also studied for comparison. Although lung function results are reported as z-scores calculated from published reference equations from healthy subjects using the same methodology and equipment, the potential misinterpretation of lung function data using reference data is well documented[33]. In fact in this study, the control population had a slightly higher mean (SD) LCI z-score of 0.67 (0.72) than expected. Reasons for this are explored in Chapter 4, but could be secondary to change in the control population over time. Raw LCI data were however also still significantly higher (mean [95% CI] 1.19 [0.76; 1.63]) than controls in preschool children with CF in this study.

Lung function tests were performed in a set order for all subjects (MBW first, then plethysmography and finally spirometry), as deep inspiration required for spirometry could in theory affect airway calibre and therefore tidal volumes, which would have a secondary effect on MBW and sR_{aw} measures. Therefore the individual feasibility of these tests cannot be derived from this study, apart from MBW which was measured first. Feasibility was lower for plethysmography than MBW, and lower still for spirometry which may reflect children's fatigue as the test protocol progressed.

Children were tested when in a stable clinical condition. A minority (five children) initially tested as infants and available for recruitment at preschool age were too unwell during the study period to be tested. They either did not meet eligibility criteria (no cough, course of antibiotics that was not regular treatment or positive bacterial isolation in the three weeks preceding test), or had spent time in hospital making it difficult for them to attend the study visit. The implication of loss of subjects to follow up is discussed in the final chapter of this thesis.

Most children in the CF group perform airway clearance twice daily as part of their routine care. Techniques for this differ greatly between individuals, and some children may not have performed airway clearance before lung function testing. A further limitation of this study was that was not possible to standardise this treatment which could potentially impact on lung function results; children who had performed effective airway clearance prior to testing could have had better results because of sputum clearance, or worse because airways which were completely occluded, and thus silent, were partially unblocked, giving a new abnormal signal. However, neither in research nor clinical practice can adherence to treatment such as airway clearance be objectively determined.

3.5.5 Meaning of the study, conclusions and future research

The overall aim of this thesis is to describe the evolution of lung function in children with CF less than six years of age managed with standard UK CF care. This chapter reports lung function in preschool children; and shows that despite spirometry and sR_{aw} being within the normal range, LCI measured by MBW is abnormal by this time point. In clinically diagnosed preschool children with CF, LCI has been shown to predict lung function at school age[51], with a positive predictive value for preschool LCI 94% to predict an abnormal school-age result. School age LCI also correlates with abnormalities on CT at this age[126]. Longitudinal studies of children with CF to school-age are needed to investigate whether this relationship also exists in NBS individuals.

LCI is also a useful outcome measure for clinical trials as a non-invasive and sensitive marker in NBS pre-schoolers, and this will be discussed in detail in the final chapter of this thesis. To use LCI as a trial end point, power calculations are often based on historical studies carried out more than ten years ago, but there are many differences in CF care today compared to this time. A comparison of lung function results between NBS and clinically diagnosed children with CF would also give insight into any differences between these two groups, and is explored in the next chapter.

4 An era comparison of lung function in preschool children with CF

4.1 Introduction

Chapter three reported cross-sectional lung function in the LCFC cohort 2 of children with CF who were diagnosed by NBS. Data were collected from 2013-2015 and represent the children with CF that we see and treat today. An earlier longitudinal study was conducted by the LCFC between 2001 and 2005 in children diagnosed clinically with CF (cohort 1), before NBS was introduced. These children were enrolled at preschool age to undertake the same lung function tests as the current study, namely multiple breath washout, plethysmographic sR_{aw} and spirometry. Previous work has shown that at two years of age, for most infants in cohort 2, lung function was within the normal range[129], and reviewed in chapter 1. However, the work of the previous chapter has shown that preschool lung function in cohort 2 is abnormal. In addition to their age and symptoms (many not properly treated) at diagnosis, there are of course many differences between these cohorts, including new therapies and more widespread application of standard treatments. An era comparison can give information as to whether pre-school lung function in children with a NBS and more recent diagnosis of CF remains better than those diagnosed a decade earlier, or if in fact the better functional status of cohort 2 was merely transient (i.e. present at age two years and disappeared by the preschool testing, in which case it would be difficult to argue the benefits of NBS).

This chapter will compare lung function between the two cohorts to see if the advantage of the NBS cohort is maintained. The analysis will determine whether power calculations for interventional studies using lung function outcome measures can be made with data from clinically diagnosed children with CF, or need to be based on studies in NBS children (of whom by definition there are rather fewer). It is accepted from the outset that if any difference is found, the cause of that difference cannot be determined from this data.

4.2 Hypothesis, aims and objectives

4.2.1 Hypothesis

Despite the deterioration in lung function from age two to preschool, MBW, sR_{aw} and spirometry outcomes remain significantly better in newborn screened preschool children with CF when compared to children clinically diagnosed a decade earlier.

4.2.2 Aim

The aim was to compare lung function (multiple breath washout, sR_{aw} and spirometry) in NBS children with CF aged three to six years with the same measurements made in preschool children in the study performed 10 years earlier.

4.2.3 Objectives

The objectives were:

- To measure lung function primary outcomes (LCI, s*R*tot and $FEV_{0.75}$) in NBS children and a contemporaneous healthy control group at three to six years of age (as reported in chapter 3)

- To directly compare these primary outcomes between NBS preschool children with CF and those with a clinical diagnosis tested a decade earlier at the same age

4.3 Methods

4.3.1 Subjects

As detailed in chapter three, children with a diagnosis of CF made by NBS and contemporaneous healthy controls were recruited for lung function testing between three and six years of age. Data were collected between 2013 and 2015. The

recruitment process, study protocol, methodology and eligibility criteria are described in Chapter 2, section 2.4.

For the historical comparison, data were available for preschool children clinically diagnosed with CF and healthy controls enrolled in a study performed a decade earlier. Children had been tested at preschool age between 2001 and 2005 and underwent the same three lung function tests (MBW, plethysmographic specific airway resistance and spirometry) at the same centre using identical protocols. For clarity, children participating in the study between 2001 and 2005 are referred to as cohort 1 and those tested between 2013 and 2015 cohort 2. Data from cohort 1 were collected as part of an earlier study (Principal Investigator Professor Janet Stocks), and not by myself.

A detailed description of the methodology employed in the cohort 1 study can be found in an earlier publication[7]. This cohort was chosen for comparison as the study was conducted in a near identical method to the more recent cohort 2 study. There were minor differences in recruitment between studies as follows. In the cohort 2 study, children with CF had been recruited from six London tertiary CF centres as infants and were invited back for testing at preschool age. In cohort 1, initial testing was performed in preschool children recruited from GOSH, and then extended to other LCFC centres once feasibility had been established. In addition, not all these children had previous infant lung function performed. The participating centres were the same in both cohorts as described in Chapter 2. For healthy control subjects, preschool children in cohort 1 were recruited from local schools and playgroups, as opposed to a postnatal ward in cohort 2.

The inclusion and exclusion criteria for subjects with CF and healthy controls were identical in both cohorts. Subjects performed the three lung function tests (MBW, plethysmographic specific airway resistance and spirometry) in that order in the same laboratory at GOSH in both cohorts.

4.3.2 Study design

The study was a cross-sectional comparison of lung function measurements between preschool children with CF in cohort 1 and cohort 2. Lung function primary outcomes were compared between children with CF and healthy controls in both cohorts, and then the CF populations were compared (cohort 1 versus cohort 2).

4.3.3 Statistical analysis

Presentation of summary data and group comparison analyses were performed as described in section 3.3.2. To investigate any effect of different control samples between cohorts, the mean differences between CF and healthy control children in cohorts 1 and 2 were compared for LCI z-score (as this was the most sensitive measure of abnormality in both cohorts, and noted to be higher in cohort 2 controls than expected in chapter 3). Multiple linear regression was used to describe differences in the control populations for LCI z-score between cohorts. The regression model included all subjects (children with CF and healthy controls) with LCI z-score as the dependent variable. Binary predictors of cohort (coded as 0 for cohort one and 1 for cohort two) and CF (0 for healthy controls and 1 for children with CF) were added to the model, allowing for an overall shift in average between cohorts, and a difference attributable to CF (the same difference for each cohort). An interaction term was then added between CF and cohort to investigate for a significant difference between the CF minus healthy control mean difference between cohorts. Statistical analyses were conducted using SPSS software (IBM SPSS Statistics for Windows, Version 24.0). Figures were produced using SPSS and GraphPad Prism software (version 7.00 for Windows, GraphPad Software, La Jolla California USA). Statistical significance was taken as $p<0.05$.

4.3.4 Power of the study

Lung function data were available for 89 children with CF in cohort 1 and 69 children in cohort 2 (a ratio of 1.25:1). The power calculation accounted for multiple comparisons (as there were three primary outcomes) calculated as z-scores (expected standard deviation of 1) and unequal groups. 43 children in each cohort would detect a difference in SD score of 0.7 (or greater) in the primary lung function outcomes (LCI, sRtot and $FEV_{0.75}$) with 80% power at the 5% significance level. When adjusting for unequal groups with the known recruitment ratio, 49 children in cohort 1 and 40 children in cohort 2 would detect the same difference in the three outcome measures of 0.7 at the same power and significance level. Therefore the study was adequately powered to detect a difference between cohorts 1 and 2.

4.4 Results

4.4.1 Subject background characteristics

In cohort 1 there were 89 subjects with CF, aged from 3.0-5.9 years and 56 healthy controls, aged from 2.7 to 5.8 years. There were no significant differences in gender, ethnicity, age at test or birthweight z-score between children with CF and controls.

Background characteristics limited to children with CF in cohorts 1 and 2 were compared and are summarised in Table 4.1. Children were well matched for gender and ethnicity and there was no significant difference in birthweight z-score. There was, however, a significant difference in age at test between cohorts, with children in cohort 2 older than cohort 1 by six months on average. In cohort 1, apart from five children with antenatal bowel pathology suggestive of CF and one with a family history, all children had a clinical diagnosis of CF, with 23 (26%) presenting with meconium ileus, a significantly larger proportion of children than in cohort 2. As expected, age at diagnosis was later in cohort 1 who were diagnosed with CF clinically, compared to those in cohort 2 diagnosed by NBS. There were no significant differences in the proportion of children with pancreatic insufficient or CF genotypes (p.Phe508del homozygous and heterozygote) between cohorts.

Table 4.1: Comparison of background characteristics between cohorts 1 and 2 limited to children with CF

	Cohort 1	Cohort 2	Δ [95%CI] (C1-C2)
Number of subjects	89	67	22
Male	37 (42%)	31 (46%)	4% [-12.42;20.34]
White	81 (91%)	58 (87%)	4% [-6.58;15.60]
Age at test (years)	4.25 (0.78)	4.76 (0.75)	**-0.51 [-0.75;-0.26]*****
Birthweight z-score$^{\#}$	-0.34 (1.05)	-0.38 (0.96)	0.04 [-0.29;0.37]
Age at diagnosis (weeks)	26.8 (37.4)	4.56 (4.53)	**22.23 [13.13;31.32]***
p.Phe508del homozygous	58 (65%)	41 (61%)	4% [-11.9;20.0]
p.Phe508del heterozygous	23 (26%)	20 (30%)	4% [-10.8;19.2]
Other/unknown mutation	8 (9%)	6 (9%)	0% [-9.74;10.78]
Meconium ileus	23 (26%)	7 (10%)	**16% [2.88;27.98]***
Pancreatic insufficiency	85 (96%)	58 (87%)	9% [-0.48;19.88]

Legend: Data are presented as number of subjects (%) or mean (SD). The mean difference [95% CI] is calculated as cohort 1 minus cohort 2. $^{\#}$ *= calculated from British 1990*[138]*.* **represents statistical significance p<0.05 and* * *p<0.001.*
Subjects in cohort 2 were older at preschool test occasion than cohort 1, and diagnosed significantly earlier than those in cohort 2

Table 4.2 summarises the results of anthropometric and lung function measurements in children with CF and healthy controls in both cohorts, and a comparison of these measures between cohorts. Height and weight z-scores were significantly poorer in clinically diagnosed children with CF when compared to controls in cohort 1. As reported in Chapter 3, the same was also (unexpectedly) found in NBS children. However, BMI z-score was not different in children with CF compared to controls in either cohorts. When the cohorts were compared, there were no significant differences in height, weight or BMI z-score; those diagnosed by NBS did not have better nutritional outcomes when compared to those diagnosed clinically.

4.4.2 Comparison of lung function primary outcome measures between cohorts 1 and 2

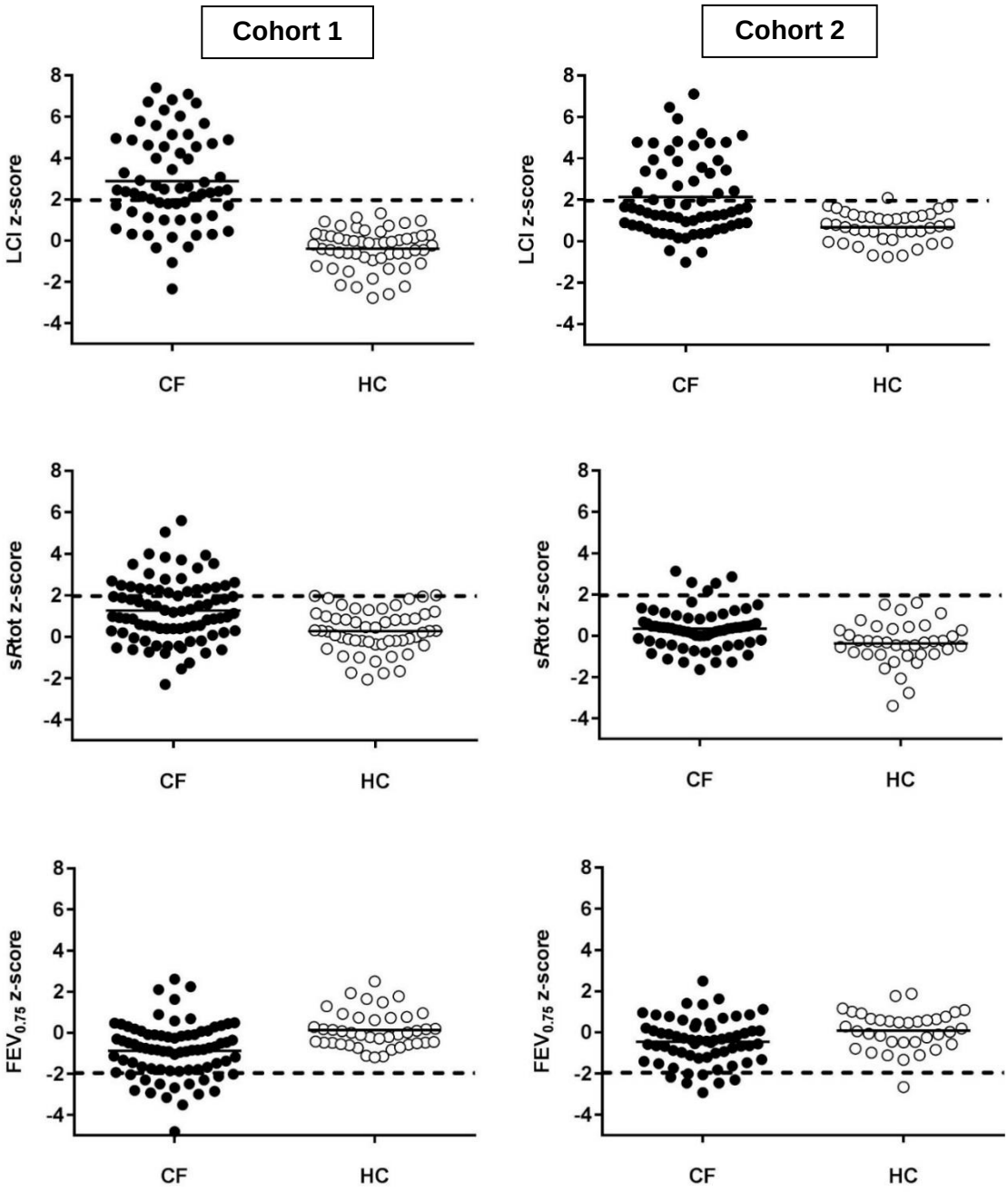
As seen in cohort 2, children with CF in cohort 1 had significantly poorer lung function than healthy controls for all of the primary outcomes. When children with CF in the two cohorts were compared, lung function measurements were significantly better in cohort 2, with mean z-score [95% CI] in LCI 0.74 [0.36;1.44] lower, s*R*tot 0.92 [0.49;1.35] lower and $FEV_{0.75}$ 0.43 [-0.84;-0.01] higher than children in cohort 1 (Figure 4.1). In healthy control subjects, there were no significant differences in height, weight or BMI z-score in the two cohorts. However, a significant difference was seen in LCI and s*R*tot z-score; control subjects in cohort 1 had a mean [95% CI] LCI z-score -1.06 [-1.43;-0.70] lower and s*R*tot z-score 0.64 [0.20;1.07] higher than healthy controls in cohort 2. There was no significant difference in $FEV_{0.75}$ z-score between controls in both cohorts. The reasons for differences between control groups is investigated further in section 4.4.3. The table also shows the discrepancy between cohort 1 and cohort 2 in the mean difference (children with CF minus healthy controls). For LCI this was 1.8 z-scores lower, s*R*tot 0.28 z-scores lower and $FEV_{0.75}$ 0.47 z-scores higher in cohort 2 when compared to cohort 1.

Table 4.2: A comparison of anthropometric and lung function results in cohorts 1 and 2

	Cohort 1			Cohort 2			Cohort 1-Cohort 2		[C1 (CF-HC)] – [C2 (CF-HC)]
	CF	**HC**	**Δ CF-HC [95% CI]**	**CF**	**HC**	**Δ CF-HC [95% CI]**	**Δ CF-CF [95% CI]**	**Δ HC-HC [95% CI]**	**Δ CF-HC C1-C2**
Weight z-score	0.03 (0.89)	0.31 (0.64)	-0.28 [-0.55;-0.00]*	-0.11 (0.84)	0.43 (1.15)	-0.54 [-0.92; -0.16]**	0.14 [-0.14;0.42]	-0.12 [-0.49;0.24]	0.26
Height z-score	-0.08 (0.98)	0.32 (0.71)	-0.41 [-0.71;-0.11]**	-0.23 (0.87)	0.23 (1.21)	-0.46 [-0.90; -0.03]*	0.15 [-0.15;0.45]	0.10 [-0.29;0.84]	0.05
BMI z-score	0.14 (0.80)	0.14 (0.74)	0.00 [-0.26; 0.27]	0.09 (0.67)	0.25 (0.96)	-0.16 [-0.47; 0.15]	0.51 [-0.19;0.29]	-0.11 [-0.46;0.23]	-0.16
LCI z-score	2.88 (2.20)[a]	-0.39 (0.96)[b]	3.27 [2.62;3.92]***	2.14 (1.84)[g]	0.67 (0.72)[h]	1.47 [0.96; 1.97]***	0.74 [0.36;1.44]*	-1.06 [-1.43;-0.70]***	1.8
s*R*tot z-score	1.27 (1.51)[c]	0.27 (1.02)[d]	1.00 [0.54;1.47]***	0.35 (0.99)[i]	-0.37 (1.04)[j]	0.72 [0.32; 1.13]**	0.92 [0.49;1.35]***	0.64 [0.20;1.07]**	0.28
$FEV_{0.75}$ z-score	-0.88 (1.33)[e]	0.13 (0.88)[f]	-1.01 [-1.41;-0.60]***	-0.45 (1.12)[k]	0.09 (0.93)[l]	-0.54 [-0.98; -0.10]*	-0.43 [-0.84;-0.01]*	0.04 [-0.38;0.46]	0.47

*Legend: Data presented as mean (SD) and mean difference (Δ) [95% CI]. Abbreviations: C1=cohort 1, C2=cohort 2. *represents statistical significance of p<0.05, **p<0.01 and ***p<0.01. Number of subjects for each test as follows: a=64, b=53, c=82, d=53, e=77, f=41, g=66, h=39, i=65, j=38, k=62, l=35. Children with CF in cohort 2 had significantly better lung function than cohort 1 for all primary outcome measures*

Figure 4.1: Lung function primary outcome measures in children with CF and healthy controls in both cohorts



Legend: Solid lines represent the mean of each group; broken lines +1.96 z-scores for lung clearance index (LCI), specific airway resistance (sRtot), and -1.96 z-scores for $FEV_{0.75}$ (limits of normality). Lung function was improved for all measures in cohort 2 compared to cohort 1

Table 4.3 summarises the number of children with abnormal lung function results for each of the primary outcome measures. A result was classified abnormal if it was greater than 1.96 z-scores for LCI and s*R*tot, and less than -1.96 z-scores for $FEV_{0.75}$. Although the most significant difference in z-scores between cohorts was seen in s*R*tot, a much smaller number of children had abnormal results than LCI. Only five children had an abnormal s*R*tot in cohort 2, whereas 26 had an abnormal LCI. A similar pattern was seen in $FEV_{0.75}$ z-score, with only seven children in cohort 2 with an abnormal measurement. When the cohorts were compared, a significantly lower proportion of subjects in cohort 2 had abnormal results in LCI (25% less) and sRtot z-scores (23% less) than cohort 1, but there was no difference in the proportion of children with an abnormal $FEV_{0.75}$ between cohorts.

Table 4.3: A comparison of the proportions of children with an abnormal lung function result in cohorts 1 and 2

	Cohort 1	Cohort 2	Relative Difference (Cohort 1-2)	Absolute Difference (Cohort 1-2)
LCI z-score	41 (64%)[a]	26 (39%)[d]	61%	**25%****
s*R*tot z-score	26 (32%)[b]	5 (8%)[e]	25%	**24%*****
$FEV_{0.75}$ z-score	15 (19%)[c]	7 (11%)[f]	58%	8%

*Legend: Data presented as number of subjects (%) with an abnormal result in each cohort. Absolute group differences were compared by the Chi^2 test (or Fisher's exact if numbers were <20 in either group). Total number of subjects for each test as follows: a=64, b=82, c=77, d=66, e=65, f=62, ** indicates significance p<0.01, ***p<0.001. There were significantly more children with an abnormal lung clearance index (LCI) and specific airway resistance (sRtot) z-score in cohort 1 when compared to cohort 2, but no significant difference in the proportion with abnormal $FEV_{0.75}$ z-score.*

4.4.3 Adjustment for control samples between cohorts

In Chapter 3, LCI z-score in healthy controls was higher than expected at 0.67 (standard deviation 0.72). Table 4.2 shows a significant difference in LCI and sR_{tot} between the cohort 1 and cohort 2 control population. To investigate potential differences in the control sample populations, and considering the cohort 1 control group was a larger sample size than cohort 2, differences between CF and controls in the two cohorts were also investigated.

So far lung function measurements have been expressed as z-scores using published reference equations in this study; abnormal results being +/-1.96 z-scores using the reference population. It is also possible to define the limits of normality from the contemporaneous healthy control data as +/-1.96 times the standard deviations (SD) from the control mean; with the upper limit of normal (ULN) ≥ +1.96 SD for LCI and s*R*tot, and lower limit of normal (LLN) for $FEV_{0.75}$. These values were calculated from the study control population are shown in Table 4.4.

Table 4.4: Upper and lower limits of normal for lung function primary outcome measures calculated from the study control population

	Cohort 1	**Cohort 2**
LCI	7.45	7.97
s*R*tot	1.81	1.61
$FEV_{0.75}$	0.63	0.60

Legend: Upper (for LCI and sRtot) and lower (for $FEV_{0.75}$) limits of normal calculated from the study control population (mean +/- [1.96 x standard deviation]), expressed as raw values (LCI units, centimeters of water per second and litres respectively)

The percentage of children with CF identified with abnormal results from the z-score conversion and using +/-1.96 SD from the measured mean in the control study population could then be compared, and is shown in Table 4.5. Although different numbers of children would be identified as abnormal using the two methods, the

proportions in each sample were not significantly different when compared by Chi^2 analysis.

Table 4.5: Children identified as abnormal using reference equation z-scores and the study control population to define normality

	Cohort 1			Cohort 2		
	z-s	**U/LLN**	**Δ [95%CI]**	**z-s**	**Control**	**Δ [95%CI]**
LCI[a]	41 (64%)	48 (75%)	11% [-5;26]	26 (39%)	26 (39%)	0% [-16;16]
s*R*tot[b]	26 (32%)	30 (37%)	5% [-9;19]	5 (7%)	8 (12%)	5%[-5;16]
$FEV_{0.75}$[c]	15 (19%)	19 (25%)	6% [-7;19]	7 (11%)	2 (3%)	8%[-1;18]

Legend: Data presented as number (percentage) of children. Abbreviations: z-s=abnormality defined by z-score ≥ 1.96 (LCI and sRtot) or ≤1.96 ($FEV_{0.75}$); U/LLN=upper (LCI and sRtot) or lower ($FEV_{0.75}$) limit of normal defined as mean +/- (1.96 x standard deviation) of the control population; Δ=difference in percentage. Number of subjects in cohort 1/cohort 2: a=64/66, b=82/65, c=77/62.
The same proportion of subjects were identified as abnormal using both methods in cohort 2 for LCI, and there were no significant differences in proportions identified as abnormal in the two methods to define abnormality

Multiple linear regression was used to further investigate the differences in the control populations for LCI z-score between cohorts. Table 4.6(a) shows the regression model which includes all subjects (children with CF and healthy controls in both cohorts) with LCI z-score as the dependent variable. Table 4.6(b) shows the model with the interaction term added between CF and cohort to investigate for a significant difference between the CF minus healthy control mean difference between cohorts. LCI in the CF group was on average [95% CI] 2.44 [1.98;2.90] z-scores higher than the healthy controls, and there was no significant difference between cohorts. However there is a significant interaction, with the CF minus heathy control difference being 1.81 [0.93, 2.69] z-scores less on average in cohort 2 after adjusting for an average increase of

1.07 [0.38, 1.76] z-scores in cohort 2 and an overall average increase of 3.27 [2.66, 3.88] z-scores amongst children with CF in cohort 1. In summary, even allowing for differences in the control group values, the NBS children had a better LCI than the symptom diagnosed group at pre-school testing.

Table 4.6: Multiple linear regression model to account for differences between cohort 1 and 2 control populations

(a)

	Coefficient	95% CI	p-value
Constant	0.59		
Cohort 2	0.00	-0.45;0.45	0.99
CF	2.44	1.98;2.90	<0.001

(b)

	Coefficient	95% CI	p-value
Constant	0.39		
Cohort 2	1.07	0.38;1.76	0.99
CF	3.27	2.66;3.88	<0.001
CF*cohort	-1.81	-2.69;-0.93	<0.001

*Legend: (a) A regression model of all subjects (children with CF and healthy controls) with LCI z-score as the dependent variable, and (b) with the interaction term (CF*cohort) added. After adjusting for the differences in control populations, the mean difference of LCI z-score (CF-HC) was on average 1.81 z-scores less in cohort 2*

4.4.4 Comparison of LCI analysis methods

Differences in LCI z-score results were seen in the two control populations. Data collection and test procedures were the same in both studies and laboratory standard operating procedures had not changed over time. The analysis of MBW tests is performed in custom made software (TestPoint, Capital Equipment Corp., Billerica, MA, USA). Minor updates were made to the software during the time periods between cohort 1 and 2 studies, to improve operability. Although these updates should not change the final result of the analysis, it has been reported that minor changes in software settings can impact on MBW outcomes[146]. Both software versions used in the cohort 1 and 2 analyses were available, so it was possible to compare results in subjects using both versions. If a higher LCI was seen in the 'new' (used in cohort 2 analysis) versus 'old' (used in cohort 1) software, the discrepancy in LCI could therefore be attributed to change in software between the two studies.

4.4.4.1 Differences in software for MBW analysis

To investigate any differences in LCI using different versions of software, ten MBW tests in healthy control subjects from each cohort were analysed in both 'old' (version a) and 'new' (version b) software, as shown in Table 4.7. The differences were compared using the Wilcoxon paired samples test. There was no significant difference in LCI analysed in either software version for healthy control subjects in cohort 1 or cohort 2. In fact, there was a trend for lower LCI values from software version *b*, the version used for analysis in cohort 2. The direction of this difference is the opposite to the discrepancy noted in this study, where cohort 2 control subjects had a higher LCI than cohort 1 controls. The discrepancy in LCI z-score when comparing the control populations from cohorts 1 and 2 could not, therefore, be attributed to software adjustments made between the two study time periods.

Table 4.7: Multiple breath washout software comparison in both cohorts

	Version a	Version b	p value
LCI cohort 1 (SD)	6.75 (0.39)	6.73 (0.37)	0.84
LCI cohort 2 (SD)	7.97 (2.02)	7.89 (2.07)	0.35

Legend: Lung clearance index (LCI) results from analysis in each software version were compared with the Wilcoxon paired samples test. Abbreviations: SD=standard deviation. There was no significant difference between software versions in either cohort

4.5 Discussion

The main aim of this study was to compare lung function in NBS children with CF to a cohort of children clinically diagnosed with CF a decade earlier.

4.5.1 Review of hypothesis and principal findings

The hypothesis of this chapter was:

"Despite the deterioration in lung function from age two to preschool, MBW, sRaw and spirometry outcomes remain significantly better in newborn screened preschool children with CF when compared to children clinically diagnosed a decade earlier."

The principal findings have proved the hypothesis for all primary lung function outcomes in this study. LCI, s*R*tot and $FEV_{0.75}$ were all significantly better in cohort 2 when compared to cohort 1.

4.5.2 Comparison of this study to other reports

The main finding of this study was the improvement of lung function in children with CF diagnosed a decade apart. It was previously discussed in chapter 1 that the Wisconsin randomised trial of NBS[6] did not show improved lung function in those with an earlier

diagnosis of CF, but the results may have been influenced by a higher rate of *Pseudomonas aeruginosa* infection in the NBS group. This study adds to the evidence of other observational studies suggesting improved pulmonary health in children with CF over the last ten years.

LCI was the most sensitive discriminator of disease, with very few children with abnormal airway resistance and spirometry indices in either cohorts. There is much interest at present in using LCI as an end point in clinical trials and monitoring tool in children with CF as a non-invasive, non-irradiating measure. This interest developed out of studies showing the high sensitivity of LCI as a marker of disease, but these observations were made in studies now more than ten years old in children clinically diagnosed with CF. This study shows that if LCI is to be used as an outcome measure in prospective clinical trials, power calculations cannot be based on historical studies, as there are far fewer children with abnormal results than in studies of those diagnosed with CF a decade earlier. This finding will be discussed further in Chapter 7.

4.5.3 Strengths and limitations of the study

The main strength of this study was the ability to directly compare two cohorts of children with CF diagnosed a decade apart. To the author's knowledge no other group has been able to make this comparison of lung function measures in preschool children with the same study design and methodology. The study inclusion and exclusion criteria were identical and there were only minor differences in the mode of recruitment for healthy controls. Both cohorts of children were tested at the Great Ormond Street Hospital lung function laboratory and the protocols for measurement of lung function had not changed over time.

Both the cohort 1 and 2 studies included a contemporaneous healthy control group for comparison. The importance of studying a control group and possible misinterpretation of lung function tests using reference equations has been previously described[33]. In this study, a difference was shown in mean LCI and sR_{tot} z-score of the control populations of cohorts 1 and 2. This made a direct comparison of differences between children with CF and healthy controls in the two cohorts difficult to interpret. To account for this, the control group for each lung function primary outcome was used to define abnormality as the upper or lower limit of normal (Section 4.4.3). This analysis identified a similar number of subjects with an abnormal result in cohort 2 as the z-

score conversion, and the difference in proportion of those with abnormal results was not statistically significant. Furthermore, a regression analysis to compare the mean difference between CF and control subjects (cohort 1 versus cohort 2) showed that NBS children had better LCI when the difference between control populations was taken into account. Both methods of analysis point towards improved lung function in cohort 2, strengthening the conclusions.

Reasons for the difference between LCI and sR_{tot} control means between cohorts 1 and 2 were not clear in this study. There were minor differences in recruitment between studies; preschool children in cohort 1 were recruited from local schools and playgroups, as opposed to a postnatal ward in cohort 2. Exclusion criteria were identical between the two studies, and the two methods of recruitment would not be expected to result in different populations, but it remains possible that this was the cause of the discrepancy. For LCI, there were no methodological differences between cohorts in data collection; in fact the same mass spectrometer, pneumotachometer and connectors were used in both studies. Departmental standard operating procedures had not changed over time. The only difference was that the location of MBW equipment had changed between studies, but this would not be expected to result in a shift in MBW outcomes. In section 4.4.4 I investigated whether any MBW analysis software changes between cohorts resulted in a higher LCI value, but again there were no significant differences between either software versions. I was therefore unable to identify the cause of the healthy control differences seen between cohort 1 and cohort 2 studies.

Despite efforts to match the two cohorts by age, children with CF in NBS cohort 2 were significantly older than those in cohort 1 at test date, by around 0.5 years. As the overall study design was longitudinal and the children followed from infancy, some were approaching their sixth birthday when the preschool follow-up study began. There were more children in the CF group, and recruitment at the beginning of the study was concentrated on these children to ensure they were not lost to follow up. As CF is a progressive disease, lung function can be expected to worsen with age. Despite being older, these children still had better lung function than cohort 1, and the difference may have been underestimated with inclusion of children of a higher age. The rate of change of lung function over the preschool time period was, however, shown to be minimal in Chapter 3, and therefore the six month discrepancy between the two cohorts is unlikely to have much influence on the results, although any difference would *reduce* the chance of finding improved lung function in NBS cohort 2.

The main limitations in this study are the inherent changes over time in which children in the two cohorts were managed. There are many potential differences during this period, such as centralisation of care, segregated clinics, earlier start of treatment, more intensive therapy and increased use of treatments such as mucolytic therapy. Therefore, this study compares lung function in two cohorts not only diagnosed a decade apart, and by different methods, but also with very different management strategies. How much these differences contribute to the change in lung function is not possible to ascertain, and is outside of the scope of this thesis. Superior lung function in cohort 2 cannot be attributed to newborn screening *per se*, but does reflect an improvement over the time between the study periods. However, if the NBS cohort had been no better or even inferior than cohort 1, despite modern advances in treatment, this would have cast grave doubts on the value of screening.

The lung function protocols for both cohorts were examined to investigate any change in methodology during the time between the two studies. At present there are no agreed guidelines for the measurement of specific airway resistance. In a multi-centre study collating reference data for specific airway resistance, Kirkby et al.[55] showed methodological variation between centres and stated that the reference values are only applicable to studies employing a similar measurement approach. The test conditions matched those described in this article for the cohort 1 and 2 studies when reviewing sR_{aw} protocols, and any differences minimised as children were tested in the same centre. However, without strict guidelines there may have been unintentional and minor discrepancies in measurement techniques, but with the passage of time it is not possible within the scope of this thesis to examine this further.

4.5.4 Meaning of the study, conclusions and future research

This chapter adds to the overall aim of the thesis, to describe the evolution of lung function in NBS children with CF, by showing a clear improvement in lung function at preschool age over a ten year period. The children we treat with CF today thus have better pulmonary health than historical cohorts. The main question that remains is, what factors are responsible for this improvement? As discussed above, it is not possible to determine the extent to which each of the many management changes over ten years have contributed to the improvement seen. Some of the predictors of lung function in the LCFC clinically diagnosed cohort have been described[125], and data from

the AREST-CF cohort explore which factors, including inflammatory markers, infection and structural change, are associated with poorer lung function in NBS children with CF.

The previous two chapters have demonstrated that lung function, although improved to that seen in those clinically diagnosed ten years ago, is abnormal by the preschool years in NBS children with CF. Using measurements made in infancy from these children, the next chapter will go on to explore whether infant lung function or clinical parameters can predict preschool lung function in the NBS cohort of children.

5 Predictors of preschool lung function in newborn screened children with CF

5.1 Introduction

As new therapies become available for the treatment of CF, especially including those that can correct the basic CFTR defect,[147, 148] there has been a shift over recent years towards introducing these treatments in ever younger children. However these molecules are expensive, result in increased treatment burden for families, and have side effects that could potentially be harmful to infants, particularly during early lung development, when any adverse effect on airway obstruction will be lifelong. Important questions that remain are when the optimal time point to start these therapies occurs, explored in Chapter 3, but also whether any measure in infancy can predict those who will decline. This would identify a select group of children to target such treatments or more intensive therapy, and would further allow us to select infants for randomised controlled trials for whom a beneficial effect is most likely demonstrable.

The preschool children described in chapters three and four were part of the LCFC longitudinal study of NBS children with CF (section 2.3). In summary, at three months, lung function for all tests was significantly poorer than a contemporaneous healthy control population[36], but at one and two years of age, LCI and FRC_{pleth} remained stable and a significant improvement was seen in $FEV_{0.5}$ over time with treatment in specialist centres[127, 129]. At two years, only 7% had abnormal $FEV_{0.5}$, 15% abnormal LCI and 19% abnormal FRC_{pleth}. Excellent somatic growth was also seen, with no group differences seen between subjects with CF and controls. The determinants of lung function were reported, and significant associations seen between a history of *Pseudomonas aeruginosa* (higher FRC_{pleth}) and those receiving additional antibiotics or positive cough swab (lower $FEV_{0.5}$) at one year, and a greater improvement in $FEV_{0.5}$ in subjects who had not isolated *Pseudomonas aeruginosa*. However there were no associations with CF treatments, such as courses of intravenous antibiotics. These studies are discussed in detail in section 1.4.3.2, and their results suggest that novel treatments for CF could be deferred until after infancy.

This chapter will describe the evolution of lung function in children with CF from diagnosis to the preschool years, tracking lung function over time in cohort 2, andspecifically investigate whether any measures (anthropometric, functional or

clinical) in infancy can predict worse preschool lung function, and thus a high risk group.

5.2 Hypothesis, aims and objectives

5.2.1 Hypotheses

The hypotheses were:

a) Infant lung function measures ($FEV_{0.5}$, FRC_{pleth} and LCI z-scores) and nutritional markers (height, weight and BMI z-scores) cannot predict preschool lung function (LCI, s*R*tot and $FEV_{0.75}$ z-scores) in NBS children with CF

b) Clinical markers of infection (isolation of bacteria on respiratory samples), cannot predict preschool lung function, and markers of disease severity (for example courses of intravenous antibiotics) in infancy will not be significantly associated with preschool lung function primary outcomes (LCI, s*R*tot and $FEV_{0.75}$ z-scores)

5.2.2 Aim

The aim of the study was to describe the evolution of lung function from diagnosis to the preschool years in NBS children with CF, and to report whether characteristics in infancy (anthropometric, lung function, bacterial infection and markers of disease severity) can predict preschool lung function

5.2.3 Objectives

The objectives were:

a) To describe the anthropometric characteristics (height, weight and body mass index) from birth to preschool age in subjects with CF and their association with lung function at three to six years of age (primary outcomes LCI, s*R*tot and $FEV_{0.75}$ z-score)

b) To examine whether there is an association between infant lung function measures ($FEV_{0.5}$, LCI and FRC_{pleth}) with the preschool lung function primary outcomes used in this study

c) To investigate the association between clinical markers in infancy, such as bacterial isolates, and markers of disease severity, such as courses of intravenous antibiotics, known to be associated with lung function decline in older children

5.3 Methods

5.3.1 Subjects and measurements

The recruitment process and methodology for the LCFC infant study is described in section 2.3, and for the preschool NBS follow up study in section 2.4. Data from infants were collected as part of an earlier study (Principal Investigator Professor Janet Stocks), and not by myself.

5.3.2 Statistical analysis

Presentation of summary data and group comparison analyses were performed as described in section 3.3.2. Paired t-tests were used to investigate the change in lung function outcomes between each test occasion. Regression analysis was used to investigate the association of subject anthropometric and clinical characteristics, and infant lung function outcomes with preschool lung function. This was limited to children with CF as predictors such as disease severity and medical treatments were not applicable to healthy subjects. Measures known to influence lung function were specifically chosen as independent variables, and are detailed in Table 5.1. Isolation of *Pseudomonas aeruginosa* (PA)*, Staphylococcus aureus* (SA)*, Haemophilus influenzae* (HI) *and Stenotrophomonas maltophilia* (SM) were investigated as the positive isolates found in this cohort. The following independent variables were investigated:

- Subjects with CF 'ever' isolating each organism
- Age of first acquisition of each organism (continuous variable)
- To examine the significance of early isolation further, isolation before the first six months of life, between six months and two years, and between two year and preschool test were investigated as categorical variables (although these time periods were arbitrary and analysis post-hoc)

Table 5.1: Independent variables used for regression analysis

Anthropometric variables:

- Birthweight z-score
- Height, weight and BMI z-score at 3 month, 1 year, 2 year and preschool test occasion
- Change in weight z-score from birth to each test occasion (birth to 3 months, birth to 1 year and birth to 2 years)

Clinical variables:

- Genotype (p.Phe508del homozygous or 'other')
- Pancreatic sufficiency
- Diagnosis of meconium ileus
- Total number of courses of intravenous antibiotics from birth to preschool test
- Number of intravenous antibiotics in categories (none, 1-2 and ≥3) from birth to preschool test
- Use of mucolytic treatment 'ever' and in the twelve months preceding preschool test occasion

Bacterial isolates (SA, PA, HI, SM):

- Isolation 'ever' for each pathogen
- Age of first acquisition of each pathogen
- Acquisition of each pathogen <6 months of age, between 6 months and 2 years and between 2 years and preschool test occasion

Infant lung function primary outcomes:

- LCI z-score at 3 month, 1 year and 2 year test occasions
- $FEV_{0.5}$ z-score at 3 month, 1 year and 2 year test occasions
- FRC_{pleth} z-score at 3 month, 1 year and 2 year test occasions

Legend: Independent variables used for simple linear regression analysis to investigate associations with each preschool lung function primary outcome.
Abbreviations: SA=Staphylococcus aureus; PA=Pseudomonas aeruginosa; HI=Haemophilus influenzae; SM=Stenotrophomonas maltophilia; LCI=lung clearance index; $FEV_{0.5}$=forced expired volume in 0.5 seconds

As LCI z-score was the most sensitive marker of abnormality in the 67 children with CF tested in the preschool years, it was chosen as the primary outcome, and modelled first as a dependent variable. s*R*tot z-score and $FEV_{0.75}$ z-score were then investigated as secondary outcome dependent variables.

Simple linear regression was performed first to examine the effect of individual characteristics and infant lung function (independent variables) on each preschool lung function measure (LCI, sRtot and FEV0.75 z-scores), with each primary lung function outcome modelled separately as dependent variables. Multiple linear regression was then used to investigate the combined interactions for independent variables.

The multiple linear regression models are presented in tables. A description of the headings are as follows:

- 'n' represents the number of subjects included in the model
- 'Coefficients' describe unstandardized slope coefficients ('B') for each variable, measured in their natural units. 'B' represents the change in the dependent variable for every one unit change in the independent variable. 'SE' is the standard error of 'B', used to determine whether 'B' is significantly different from zero. The 'p-value' indicates whether the independent variable in question is a statistically significant predictor for the dependent variable. In the multiple regression models, the 'p-value' is calculated after adjusting for the other independent variables. The 'Constant' values (intercept) are presented in the multiple regression models; the value of the dependent variable when all the independent variables are zero.
- The 'Model summary' includes 'R', the Pearson correlation coefficient between the values predicted by the regression model and the actual values of the dependent variable. It is a measure of the strength of the linear association between the independent and dependent variables and the model fit, ranging from zero to one; higher values indicate a stronger association. The 'Adjusted R2' is the proportion of the variance accounted for by the regression model that would be expected in the population. The 'p value' indicated whether the model is statistically significant.

Models were examined to ensure the assumptions of regression were met; linearity, no multicollinearity or highly influential points, and that errors (residuals) were homoscedastic and approximately normally distributed. Statistical significance was taken as $p<0.05$. To select the most appropriate regression model, forward selection and backward elimination procedures were used. This involved sequentially entering variables into the model to investigate whether the overall model fit was improved

(more variance explained). If a variable did not improve the model, it was then removed or 'eliminated'. The final models therefore consist of the relative contribution of independent variables significantly associated with the dependent variable, with the maximum variance explained. An evaluation of binary classifiers is also presented for preschool LCI z-score using infant lung function tests and *Pseudomonas aeruginosa* status as predictor variables.

5.4 Results

5.4.1 Subject characteristics

67 subjects with CF and 41 healthy controls with prior infant lung function results had the tests repeated at preschool age (three to six years). The background characteristics of cohort 2 children with CF at the preschool test occasion were presented in section 3.4.2.

Table 5.2 summarises the age and anthropometric characteristics at each infant lung function test occasion (three months, one year and two years) and at the preschool test occasion. Weight, height and BMI z-scores were significantly poorer than controls in subjects with CF at three months of age, then showed improvement at one and two years, and BMI z-score remained stable at preschool test. Figure 5.1 illustrates the trends of growth parameters graphically from infancy to preschool age by test occasion.

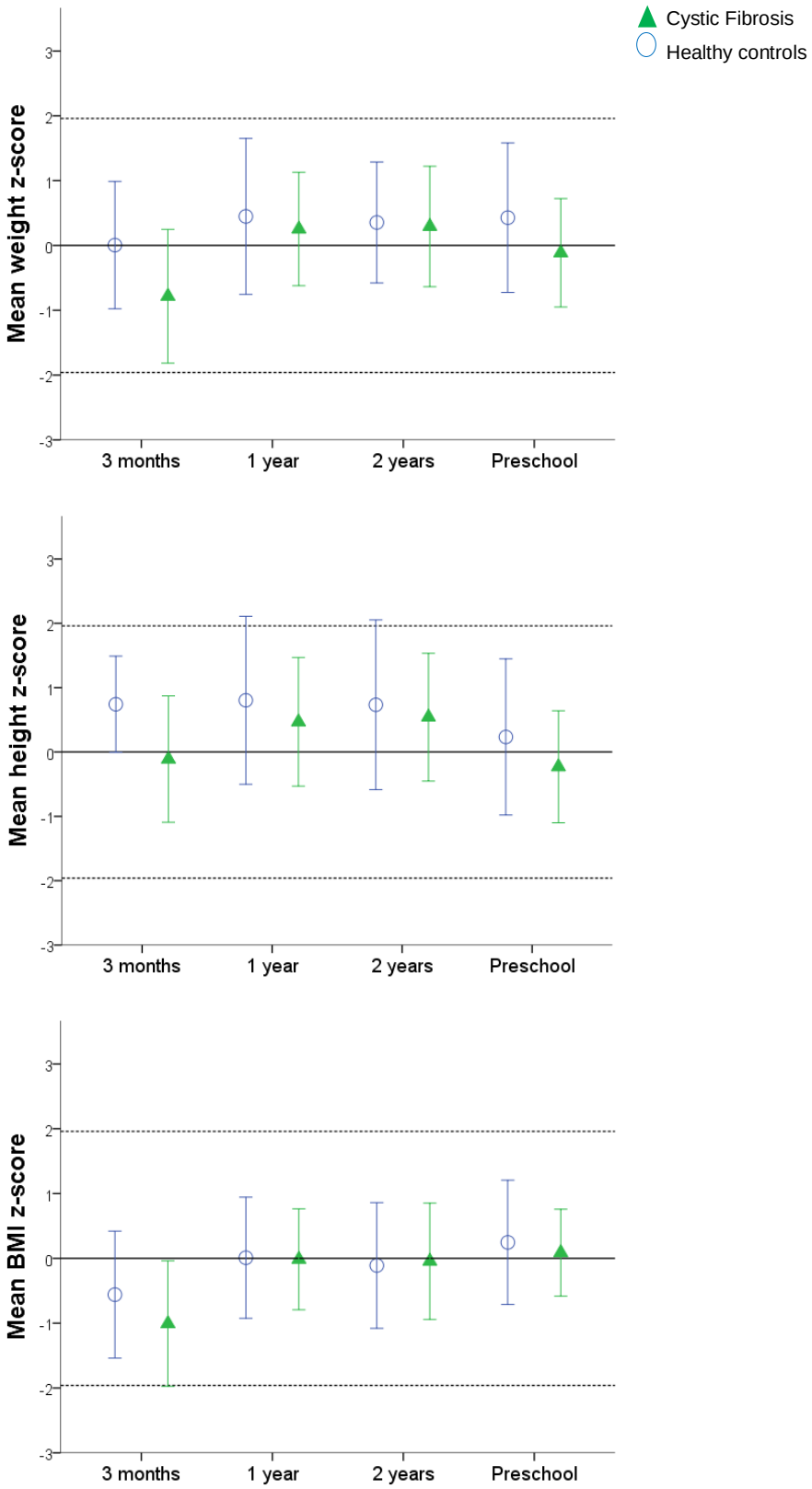
Table 5.2: Background characteristics of subjects at each lung function test from infancy to preschool age

	3 months			1 year			2 year			Preschool (3-6 years)		
	CF[a]	**HC[b]**	**Δ CF-HC [95% CI]**	**CF[c]**	**HC[d]**	**Δ CF-HC [95% CI]**	**CF[e]**	**HC[f]**	**Δ CF-HC [95% CI]**	**CF[g]**	**HC[h]**	**Δ CF-HC [95% CI]**
Age at test (y)	0.23 (0.08)	0.25 (0.07)	-0.02 [-0.05;0.01]	1.01 (0.11)	1.02 (0.13)	-0.01 [-0.06;0.04]	1.82 (0.14)	1.86 (0.15)	-0.04 [-0.12;0.04]	4.76 (0.75)	4.41 (0.62)	0.35 [0.08;0.63]*
Weight z-score#	-0.78 (1.03)	0.01 (0.98)	-0.79 [-1.21;-0.37]***	0.25 (0.88)	0.45 (1.20)	-0.19 [-0.62;0.23]	0.29 (0.93)	0.35 (0.93)	-0.06 [-0.52;0.39]	-0.11 (0.84)	0.43 (1.15)	-0.54 [-0.92; -0.16]**
Height z-score#	-0.11 (0.98)	0.74 (0.74)	-0.85 [-1.23;-0.47]***	0.47 (0.10)	0.80 (1.30)	-0.34 [-0.80;0.13]	0.54 (0.99)	0.73 (1.32)	-0.19 [-0.73;0.35]	-0.23 (0.87)	0.23 (1.21)	-0.46 [-0.90; -0.03]*
BMI z-score#	-1.01 (0.97)	-0.56 (0.98)	-0.45 [-0.85;-0.05]*	-0.01 (0.78)	0.01 (0.93)	-0.02 [-0.38;0.33]	-0.04 (0.90)	-0.11 (0.97)	0.07 [-0.38;0.51]	0.09 (0.67)	0.25 (0.96)	-0.16 [-0.47; 0.15]

*Legend: Results presented as mean (SD), and mean difference (Δ) [95% CI for the difference] children with CF minus healthy controls (HC). Abbreviations: y=years; #=calculated using to British 1990 reference[138]. *indicates statistical significance of p<0.05, **p<0.01 and ***p<0.001, highlighted in red. Numbers of subjects: a=64, b=35, c=62, d=35, e=54, f=24, g=67, h=41.*

Weight, height and BMI z-scores were lower in children with CF at 3 months, showed improvement at 1 and 2 years, but height and weight then deteriorated by preschool age, although differences were minor.

Figure 5.1: Trends in somatic growth at each test occasion for subjects with CF and healthy controls



Legend: Mean somatic growth z-scores at each test occasion for children with CF (green triangles) and healthy controls (blue circles). Error bars represent the standard deviation, solid line 0 z-scores and dotted lines +/-1.96 z-scores.

5.4.2 Clinical characteristics of subjects with CF

Clinical characteristics of cohort 2 children with CF in were described in section 3.4.3. Table 5.3 summarises treatment characteristics of children with CF at one year, two years and preschool age. Data were available for 64 subjects as three children transferred to other CF centres after two years of age where ethical approval for data collection was not valid.

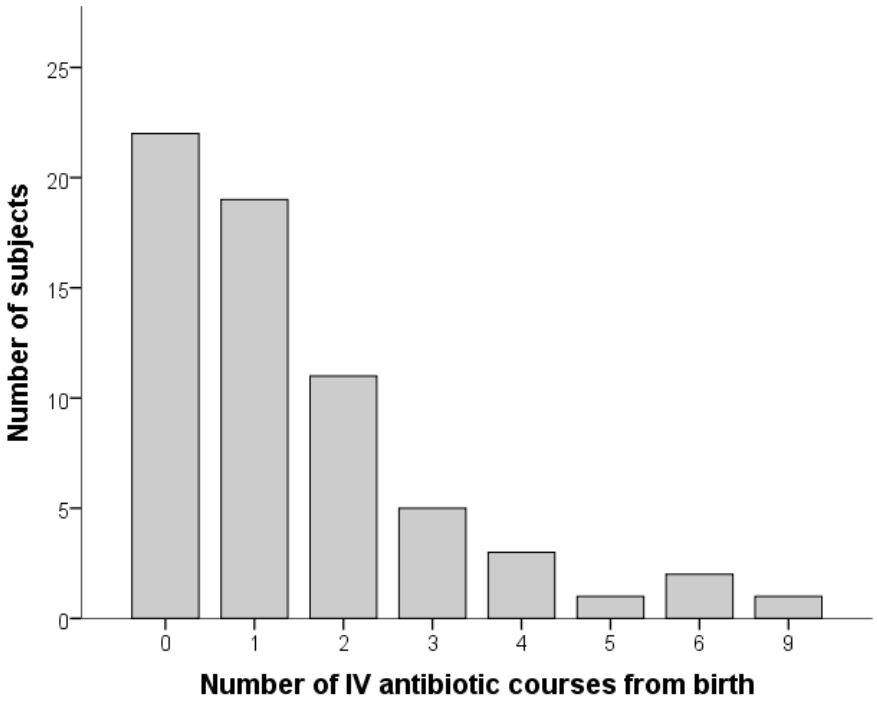
The proportion of children receiving treatment for gastro-oesophageal reflux disease remained relatively stable throughout the study (34-42%). The number of children on oral antibiotic prophylaxis (mostly flucloxacillin, some co-amoxiclav) decreased from 88% at one year to 55% at preschool test. An increase in the number of subjects receiving other treatments was seen at sequential study visits for all other therapies. Long term nebulised antibiotic therapy was defined as more than three months, and children received either colistin, alternate month tobramycin or alternating colistin and tobramycin (each month on, month off). The use of nebulised antibiotics increased from 14% at one year to 23% of subjects at three to six years. Few children received mucolytic therapy (rhDNase or 7% hypertonic saline) in infancy (8% at one year and 17% at two years) but numbers were higher by the preschool test occasion (38%). Intravenous antibiotic courses are presented in Figure 5.2 as number from birth, and were also seen to increase in frequency over time, with 30 children (47%) who had received one or two courses and 12 (19%) more than three courses by preschool age. The number of courses ranged from zero to nine.

Table 5.3: Summary of treatment histories in children with CF

Clinical characteristic	1 year	2 years	Preschool
Treatment for GORD	27 (42%)	22 (34%)	26 (41%)
Antibiotic prophylaxis	56 (88%)	40 (63%)	35 (55%)
Long term nebulised antibiotic	9 (14%)	12 (19%)	15 (23%)
URSO	0 (0%)	3 (5%)	12 (19%)
Nebulised rhDNase	4 (6%)	6 (9%)	20 (31%)
Nebulised hypertonic saline	2 (3%)	5 (8%)	11 (17%)
Any mucolytic therapy	5 (8%)	11 (17%)	24 (38%)
Long term azithromycin	0 (0%)	2 (3%)	5 (8%)
Treatment for ABPA	0 (0%)	0 (0%)	1 (2%)

Legend: Data presented as number of children receiving each treatment (%) for the 64 subjects at each time point. Abbreviations: GORD=gastro-oesophageal reflux disease, URSO=ursodeoxycholic acid, rhDNase=recombinant human deoxyribonuclease, ABPA=allergic bronchopulmonary aspergillosis.
Apart from the number of subjects receiving treatment for GORD (which remained stable) and antibiotic prophylaxis (where numbers decreased with time), an increase in the number of subjects receiving each treatment was seen from one year to preschool test.

Figure 5.2: Number of intravenous antibiotic courses by the preschool test occasion



Legend: Number of intravenous antibiotic courses are presented from birth to the preschool test occasion

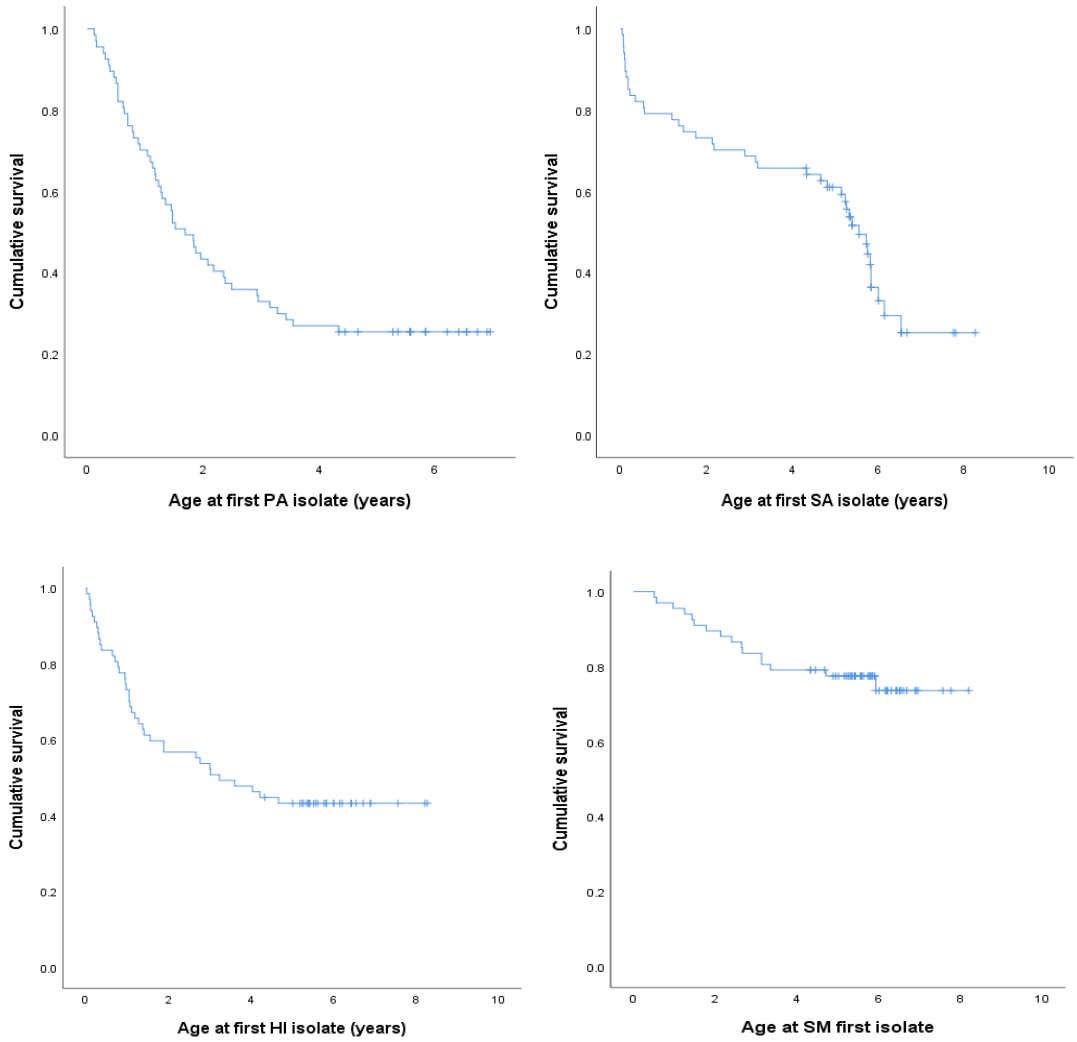
Table 5.4 summarises microbiological isolates from cough swabs, sputum or BAL in children with CF at one year, two year and preschool test occasion. Data are presented as having 'ever' isolated each organism. The number of children isolating the main infecting CF bacterial organisms at this age increased as expected over the study period. One child isolated MRSA at preschool age. No other organisms were isolated which are known to cause significant infection in CF; no children isolated non-tuberculous *Mycobacteria*, *Achromobacter, Serratia* or *Burkholderia* species. Infections were rarely chronic in the 12 months preceding each test occasion (as defined by the Leeds criteria[139]); two children had chronic *Pseudomonas aeruginosa* infection at one and two year tests, and only one child at preschool testing. If the Leeds criteria are extrapolated to other isolates, one child had chronic *Staphylococcus aureus* at two year test, and three by preschool testing. No child had chronic *Haemophilus influenzae, Stenotrophomonus maltophilia* or MRSA infection during the study period. Survival curves for each organism are also shown in Figure 5.3.

Table 5.4: Summary of microbiological isolates in children with CF

Organism	1 year	2 years	Preschool
Pseudomonas aeruginosa	17 (27%)	30 (47%)	50 (75%)
Staphylococcus aureus	17 (27%)	19 (30%)	40 (60%)
Haemophilus influenzae	20 (31%)	24 (38%)	38 (57%)
Stenotrophomonas maltophilia	3 (5%)	6 (9%)	16 (24%)
MRSA	0 (0%)	0 (0%)	1 (2%)
Aspergillus fumigatus	4 (6%)	3 (5%)	4 (6%)

Legend: Data presented as number of subjects (%) having 'ever' isolated each organism at one year, two year and preschool test occasions. Abbreviations: MRSA=methicillin resistant Staphylococcus aureus.
The number of children isolating each organism increased with age.

Figure 5.3: Survival curves for isolation of each bacterial organism



Legend: Cumulative survival curves for isolation of each bacterial organism in children with CF. Straight blue lines represent subjects isolating the organism and crossed blue lines those that did not. Abbreviations: PA=pseudomonas aeruginosa, SA=staphylococcus aureus, HI=haemophilus influenzae, SM=stenotrophomonas maltophilia

The age at first isolation for the five infecting organisms is shown in Table 5.5. *Pseudomonas aeruginosa, Staphylococcus aureus* and *Haemophilus influenzae* were isolated relatively early on average (1.05-1.38 years) when compared to *Stenotrophomonas maltophilia* (2.26 years), but the age range at first isolation was wide for all organisms. To investigate this relationship further, age at acquisition was catergorised into isolation before six months of age (early infancy), between six months and two years (later infancy) and between two years and preschool test (after infancy).

Table 5.6 illustrates the number of subjects that isolated each organism in these three categories.

Table 5.5: Age at first isolate of bacterial organisms in children with CF

Organism	Age at first growth, years (range)
Pseudomonas aeruginosa	1.21 (0.12 – 4.34)
Staphylococcus aureus	1.38 (0.04 – 5.76)
Haemophilus influenzae	1.05 (0.02 – 4.67)
Stenotrophomonas maltophilia	2.26 (0.51 – 5.92)

Legend: Age at first growth for the most common bacterial organisms isolated is presented as median years and range. Stenotrophomonas maltophilia was acquired later than the other three organisms, but the age range at first acquisition was wide for all isolates.

Table 5.6: Number of subjects with bacterial isolates in early infancy, late infancy and after infancy

	< 6 m	6m - 2y	2y – PS
Staphylococcus aureus	16 (25%)	7 (11%)	17 (27%)
Pseudomonas aeruginosa	8 (13%)	27 (42%)	12 (19%)
Haemophilus influenzae	11 (17%)	18 (28%)	9 (14%)
Stenotrophomonas maltophilia	0 (0%)	7 (11%)	9 (14%)

Legend: Data presented as number of subjects (% of total cohort with clinical data). Abbreviations: m=months; y=years; PS=preschool

5.4.3 Lung function from infancy to preschool age

5.4.3.1 Feasibility of lung function tests

67 subjects with CF and 41 healthy controls had successful measurements at preschool age (three to six years) and at least one valid infant test occasion (at three months, one year or two years of age). The proportion of these subjects with unsuccessful tests for each of the lung function primary outcomes at infant and preschool test occasions is shown in Table 5.7. A small proportion of children had unsuccessful tests at any time point, the highest was 15% at preschool test for $FEV_{0.75}$ and three month FRC_{pleth}.

Table 5.7: Proportion of children with unsuccessful results at each test occasion for lung function primary outcomes

	Cystic Fibrosis (n=67)				***Healthy Controls (n=41)***			
Test	**3m**	**1y**	**2y**	**PS**	**3m**	**1y**	**2y**	**PS**
Total n	64	62	54	67	35	35	24	41
LCI	4 (6%)	0 (0%)	1 (2%)	1 (1%)	4 (11%)	5 (14%)	0 (0%)	2 (5%)
$FEV_{(t)}$	2 (3%)	3 (5%)	6 (11%)	5 (7%)	1 (3%)	2 (6%)	3 (13%)	6 (15%)
FRC_{pleth}	10 (15%)	3 (5%)	6 (11%)	-	4 (11%)	3 (9%)	2 (8%)	-
s*R*tot	-	-	-	3 (4%)	-	-	-	3 (7%)

Legend: Values expressed as subjects with CF and healthy controls without a successful result for each lung function test, and as a percentage of the total number of children (total n) attending that test occasion. Abbreviations: m=months, y=years, PS=preschool, n=number of subjects. $FEV_{(t)}$ represents $FEV_{0.5}$ at three months, one year and two year tests and $FEV_{0.75}$ at preschool test.
A low proportion of children had unsuccessful tests at any time point.

5.4.3.2 Lung function results

Table 5.8 summarises lung function results for the primary outcome measures in infancy and at preschool test occasion in children with CF and healthy controls followed up at preschool age. In subjects with CF, LCI z-score was not significantly different to healthy controls at three month test, but progressive ventilation inhomogeneity was then seen, from mean [95% CI] z-score 0.73 [0.15;1.30] higher at one year, 0.89 [0.49;1.29] at two years and 1.47 [0.85; 2.08] z-scores higher at preschool test in subjects with CF. Importantly, the mean LCI z-score (SD) at preschool age was 2.14 (1.84) in subjects with CF, the only lung function outcome to be outside the normal range (≥1.96 z-scores) at any test occasion over the course of the study.

$FEV_{(t)}$ was significantly poorer in children with CF compared to controls at three months (mean z-score [95% CI] -0.88 [-1.28;-0.47]), but then showed a progressive improvement over infancy; at one year mean z-score was -0.61 [-1.03;-0.20] lower and by two years there was no difference between those with CF and controls. However a deterioration was seen at preschool testing with mean z-score 0.54 [-0.98; -0.10] lower in children with CF. FRC_{pleth} was significantly higher in subjects with CF at three months 0.86 [0.36;1.36], indicating hyperinflation, but like $FEV_{(t)}$ it improved over infancy; at one year mean z-score was 0.64 [0.12;1.17] higher, and at two years there was no significant difference to controls. Children with CF had evidence of increased airway resistance when compared to controls, with s*R*tot significantly poorer (mean z-score [95% CI] 0.72 [0.32; 1.13] higher) at preschool test. Figure 5.4 summarises the lung function trends graphically at each infant and preschool test occasion. There was wide distribution of LCI z-score over the preschool years, as shown in Figure 5.5. Individual patient trajectories are illustrated in Figure 5.6. There is considerable variation in each trajectory, and a distinct pattern is not seen.

Table 5.8: Lung function in subjects with CF and healthy controls at infant and preschool test occasions

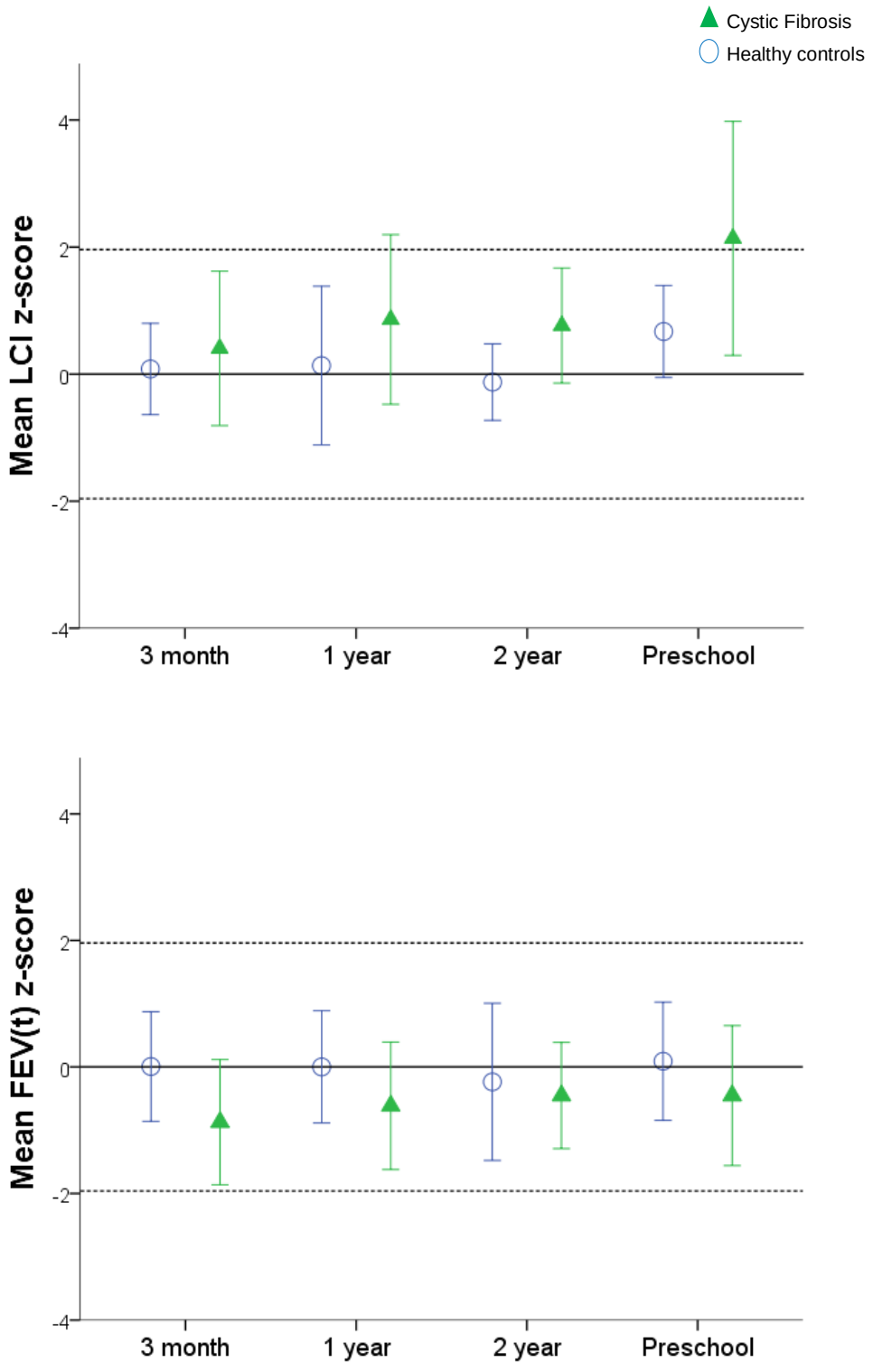
	3 months			1 year			2 year			Preschool (3-6 years)		
	CF	**HC**	**Δ CF-HC [95% CI]**	**CF**	**HC**	**Δ CF-HC [95% CI]**	**CF**	**HC**	**Δ CF-HC [95% CI]**	**CF**	**HC**	**Δ CF-HC [95% CI]**
LCI z-score[#]	0.41[a] (1.21)	0.08[b] (0.72)	0.32 [-0.14;0.80]	0.86[c] (1.34)	0.13[d] (1.25)	0.73 [0.15;1.30][*]	0.76[e] (0.91)	-0.13[f] (0.60)	0.89 [0.49;1.29][***]	2.14[g] (1.84)	0.67[h] (0.72)	1.47 [0.85; 2.08][***]
$FEV_{(t)}$ z-score[#]	-0.87[i] (0.99)	0.01[j] (0.87)	-0.88 [-1.28;-0.47][***]	-0.61[k] (1.01)	0.00[l] (0.89)	-0.61 [-1.03;-0.20][**]	-0.45[m] (0.84)	-0.24[n] (1.24)	-0.22 [-0.72;0.29]	-0.45[o] (1.12)	0.09[p] (0.93)	-0.54 [-0.98; -0.10][*]
FRC_{pleth} z-score[#]	0.77[q] (1.09)	-0.09[r] (1.11)	0.86 [0.36;1.36][**]	0.76[s] (1.25)	0.12[t] (1.11)	0.64 [0.12;1.17][*]	0.85[u] (1.31)	0.18[v] (1.33)	0.67 [-0.00;1.35]	-	-	-
s*R*tot z-score[#]	-	-	-	-	-	-	-	-	-	0.35[w] (0.99)	-0.37[x] (1.04)	0.72 [0.32; 1.13][**]

Legend: Results presented as mean (SD), and the mean difference (Δ) [95% CI] between children with CF minus healthy controls (HC). FRC_{pleth} is an infant test and sRtot a preschool test. Abbreviations: Δ=mean difference, $FEV_{(t)}$ represents $FEV_{0.5}$ for infant tests and $FEV_{0.75}$ for preschool test. [*]indicates statistical significance of p<0.05, [**]p<0.01 and [***]p<0.001. Significant differences are highlighted in red. Number of subjects: a=60, b=31, c=62, d=30, e=53, f=24, g=66, h=39, i=62, j=34, k=59, l=33, m=48, n=21, o=62, p=35, q=50, r=31, s=59, t=32, u=48, v=22, w=65, x=38. [#]=calculated from published reference equations[31, 32, 49, 55, 75].

LCI z-score was not significantly different in subjects with CF compared to controls at three months but then showed a progressive deterioration, and at preschool test was the only parameter outside the normal range (≥1.96 z-scores) at any test occasion during the study

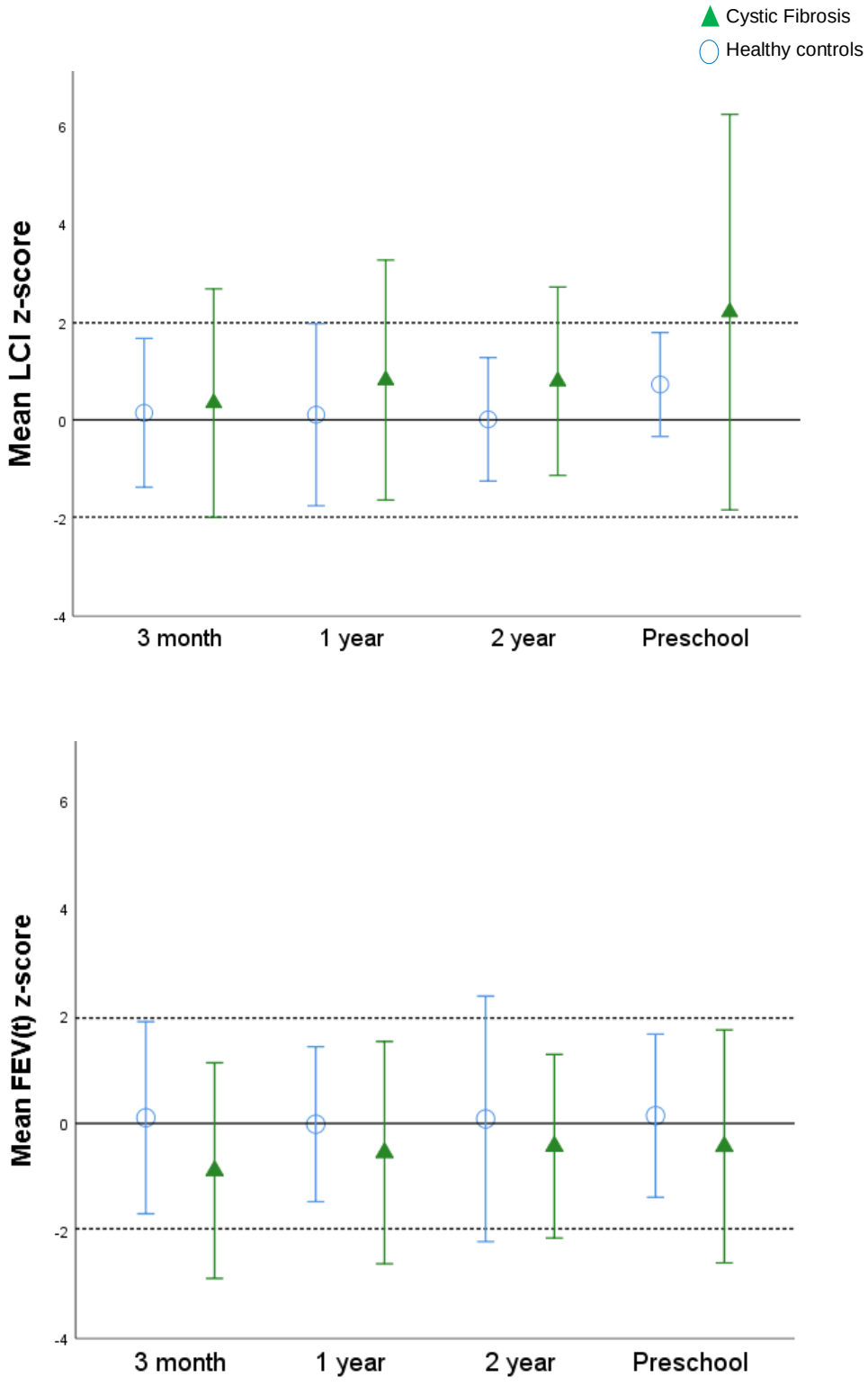
Figure 5.4: Lung function z-scores at each test occasion in children with CF and healthy controls

a)



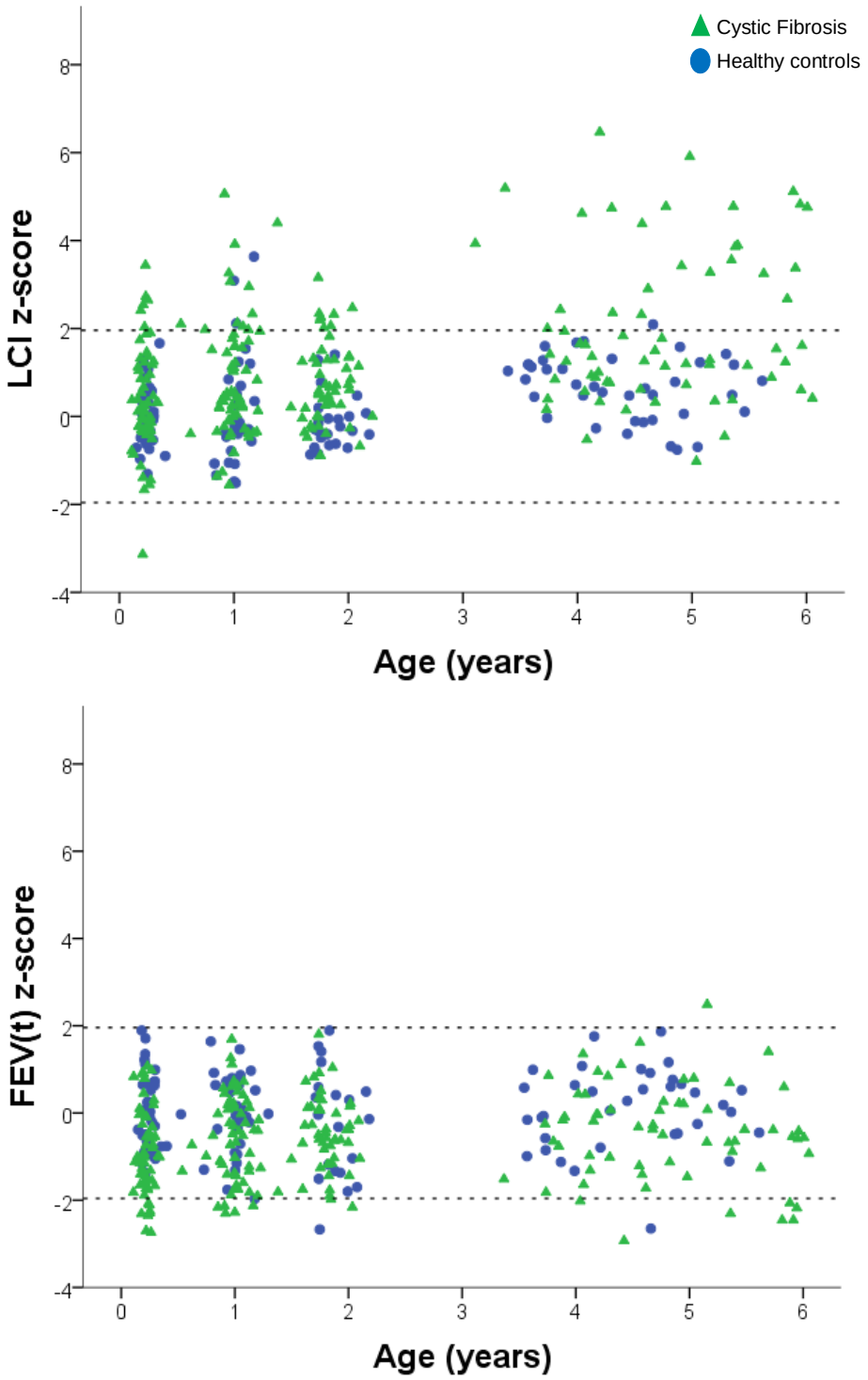
Legend: a) Mean group lung function z-scores for children completing any test occasion, children with CF (green triangles) and healthy controls (blue circles). Error bars represent the standard deviation, solid line 0 z-score and dotted lines +/-1.96 z-scores. $FEV_{(t)}$ represents $FEV_{0.5}$ in infancy and $FEV_{0.75}$ at preschool test.
Apart from a sharp increase in LCI from two year to preschool test, lung function is stable in CF subjects

b)



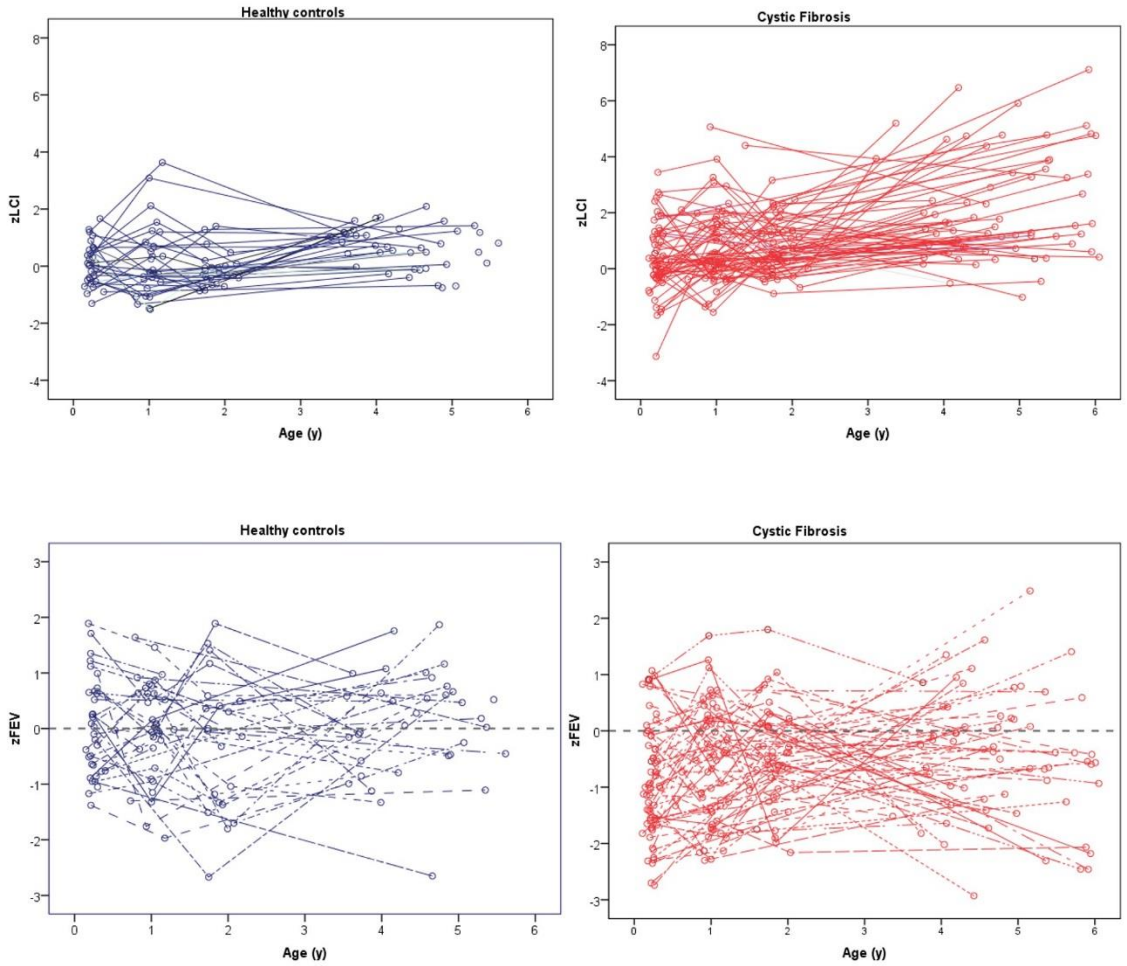
Legend: b) Mean lung function z-scores at each test occasion for children with CF (green triangles) and healthy controls (blue circles), limited to children with data all four test occasions. Error bars represent the standard deviation, solid line 0 z-score and dotted lines +/-1.96 z-scores. $FEV_{(t)}$ represents $FEV_{0.5}$ in infancy and $FEV_{0.75}$ at preschool test.

Figure 5.5: Lung clearance index and forced expired volume z-scores plotted against age in children with CF and healthy controls



Legend: Lung clearance index (LCI) and forced expired volumes ($FEV_{(t)}$) z-scores at each test occasion for children with CF (green triangles) and healthy controls (blue circles). Dotted lines represent +/-1.96 z-scores (limits of normality). $FEV_{(t)}$ represents $FEV_{0.5}$ in infancy and $FEV_{0.75}$ at preschool test.
There is wide distribution of LCI z-score over the preschool years

Figure 5.6: Individual patient trajectories for lung clearance index and forced expired volume z-scores



Legend: Trajectories for individual patients with CF (in red) and healthy controls (in blue). Abbreviations: zLCI= lung clearance index z-score, zFEV=forced expired volume (in 0.5 seconds 0-2.5 years of age and 0.75 seconds 2.5-6 years of age) z-score, y=years. Subject curves are extremely variable with no clear tracking pattern seen

Table 5.9 summarises the mean group difference in subjects with CF between each lung function test occasion, so the change in mean lung function can be observed. LCI and $FEV_{(t)}$ z-score both showed significant improvement from three month to one year test, but the most significant change was in LCI from two years to preschool test, with mean increase [95% CI] of 1.32 [0.82;1.92] z-scores.

Table 5.9: Mean z-score change between test occasions for each lung function primary outcome

	Mean difference	95% CI of the difference	p-value
LCI			
3m to 1y	0.43 (1.33)	0.07;0.78	**0.02**
1y to 2y	0.08 (1.40)	-0.32;0.48	0.69
2y to PS	1.32 (1.73)	0.82;1.92	**<0.001**
$FEV_{(t)}$			
3m to 1y	0.28 (0.97)	-0.02;0.54	**0.03**
1y to 2y	0.14 (1.05)	-0.17;0.45	0.37
2y to PS	-0.01 (1.44)	-0.44;0.42	0.96

Legend: Mean difference in z-score for each lung function outcome between infant and preschool tests. $FEV_{(t)}$ represents $FEV_{0.5}$ in infancy and $FEV_{0.75}$ at preschool test.
Abbreviations: m=month, y=year.
LCI z-score showed the most significant change from two year to preschool test

5.4.3.3 Abnormal results

Table 5.10 summarises the proportion of children with CF with abnormal results at each test occasion for the lung function primary outcomes. Abnormal results were defined as ≥1.96 z-scores for LCI, s*R*tot and FRC_{pleth} and ≤-1.96 z-scores for $FEV_{(t)}$. The proportion of children with an abnormal test was low on all test occasions (between 4 and 19%) for all primary outcomes, apart from LCI z-score at preschool age, where 39% of subjects had an abnormal result. Of the 26 children with an abnormal preschool LCI z-score, four also had an abnormal LCI z-score at three months, six (10%) were also abnormal at one year and six also had an abnormal LCI z-score at two years. Only two children had an abnormal LCI z-score on all four test occasions. Of the 7 subjects with CF with an abnormal $FEV_{0.75}$ at preschool test, two also had an abnormal $FEV_{0.5}$ at three months, no subjects also had an abnormal $FEV_{0.5}$ at one year, and only one subject also had an abnormal $FEV_{0.5}$ at two year test. No subjects had an abnormal $FEV_{(t)}$ z-score on all four test occasions. Of the nine children with an abnormal FRC_{pleth} z-score at two years, two also had an abnormal FRC_{pleth} z-

score at three months, and two at one year. No subjects had an abnormal FRC_{pleth} z-score on all three test occasions.

Table 5.10: Proportion of children with abnormal results at each test occasion for lung function primary outcomes in subjects with CF

	3 months	1 year	2 year	Preschool
Abnormal LCI z-score	7 (12%)[a]	11 (18%)[b]	8 (15%)[c]	26 (39%)[d]
Abnormal $FEV_{(t)}$ z-score	8 (13%)[e]	5 (8.5%)[f]	2 (4%)[g]	7 (11%)[h]
Abnormal FRC_{pleth} z-score	8 (16%)[i]	8 (14%)[j]	9 (19%)[k]	-
Abnormal s*R*tot z-score	-	-	-	5(8%)[l]

Legend: Data presented as number of subjects (%) with abnormal lung function primary outcome results at each test occasion, and includes infant and preschool tests. LCI is the lung clearance index; $FEV_{(t)}$ represents $FEV_{0.5}$ at three month, one year and two year tests and $FEV_{0.75}$ at preschool test; FRC_{pleth} is plethysmographic FRC (an infant test) and sR_{tot} specific airway resistance (a preschool test). Total subject numbers for each outcome: a=60, b=62, c=53, d=66, e=62, f=59, g=48, h=62, i=50, j=59, k=48, l=65.

The proportion of subjects with abnormal tests was low for all outcomes, apart from a high proportion with abnormal lung clearance index at preschool test

5.4.4 Predictors of preschool lung function in NBS children with CF

5.4.4.1 Predictors of preschool LCI z-score in subjects with CF

Anthropometric variables

On univariable regression analysis, the only anthropometric variable significantly associated with preschool LCI z-score was BMI z-score at preschool test (regression coefficient [95% CI] -0.8 [-1.4;-0.1], p=0.02). For every 1 z-score increase in BMI, LCI z-score is predicted to be 0.8 z-scores lower. Preschool height and weight z-score

were not significantly associated with preschool LCI z-score. No associations were found between birthweight z-score or height, weight and BMI z-score at any infant test occasion. As somatic growth outcomes were significantly lower than controls at three months in subjects with CF, but then showed improvement to one and two years (section 5.4.1) and greater infant weight gain is associated with higher $FEV_{(t)}$[149], change in weight z-score from birth to each infant lung function test occasion was also investigated. There was also no association between change in somatic growth (from birth to three months, birth to one year and birth to two years) with preschool LCI z-score. 'Catch up' growth was not significantly associated with preschool LCI z-score.

Clinical variables

There were no significant associations between homozygous p.Phe508del genotype, pancreatic insufficiency or presentation with meconium ileus and preschool LCI z-score on univariable analysis. There were also no significant associations between the use of mucolytic therapy, medication for gastro-oesophageal reflux disease ('ever' or in the twelve months preceding preschool test), or number of intravenous antibiotic courses with preschool LCI z-score. Treatments reflecting disease severity were not significant determinants of preschool LCI z-score.

Bacterial isolates

Bacterial isolates from birth to preschool test, and age of first acquisition for each organism are summarised in section 5.4.2. 50 children (77%) had isolated PA by preschool test occasion. Isolation of PA 'ever' was not significantly associated with preschool LCI z-score. A significant association was found however between age at first acquisition of PA and preschool LCI z-score (regression coefficient [95% CI] -0.31 [-0.57;-0.05], p=0.02). For every year earlier that a child in acquired PA prior to preschool test, LCI z-score at 3-6 years was 0.3 z-scores higher; later acquisition was significantly associated with a better preschool LCI z-score. Isolation of PA by six months of age was significantly associated with higher (worse) preschool LCI z-score when compared to later or no acquisition (regression coefficient [95% CI] 2.00 [0.52;3.49], p<0.01); isolating PA by six months of age is associated with a higher preschool LCI z-score.

40 children (62%) had isolated SA, 38 (58%) HI and 16 (25%) SM by preschool test occasion. Neither isolation of any of the three organisms 'ever' nor age at first isolation were significantly associated with preschool LCI z-score.

Infant lung function primary outcomes

No significant association was found between three month or one year LCI z-score and preschool LCI z-score, but LCI z-score at two years was highly significantly associated with preschool LCI z-score (regression coefficient [95% CI] 0.89 [0.36;1.43], p=<0.01). No associations between $FEV_{0.5}$ z-score at three month, one year and two year tests and preschool LCI z-score were found by univariable regression analysis. For $FRC_{pleth,}$ z-score at one year was significantly associated with preschool LCI z-score by univariable analysis (regression coefficient [95% CI] 0.40 [0.01;0.79], p=0.04. However there was no association between FRC_{pleth} at two years and preschool LCI z-score.

Table 5.11 summarises the results of simple linear regression analysis for each independent variable. Significant associations with preschool LCI z-score are highlighted in red.

Table 5.11: Determinants of preschool lung clearance index z-score by univariable regression analysis

	Mean coefficient			*Mean coefficient*	
	B	**95% CI**		**B**	**95% CI**
Anthropometric variables:			***Bacterial isolates:***		
z-Birthweight	-0.06	-0.53;0.42	**SA 'ever'**	0.54	-0.38;1.47
3m z-weight	0.03	-0.43;0.50	**SA 1st age**	-0.05	-0.28;0.17
1y z-weight	-0.17	-0.72;0.38	**SA <6m**	0.24	-0.93;1.41
2y z-weight	-0.15	-0.72;0.41	**SA 6m-2y**	0.27	-1.31;1.84
PS z-weight	-0.37	-0.91;0.17	**SA 2y-PS**	0.94	-0.21;2.09
3m z-height	0.20	-0.29;0.68	**PA 'ever'**	0.72	-0.27;1.71
1y z-height	0.17	-0.31;0.65	**PA 1st age**	-0.31	-0.57;-0.05*
2y z-height	0.23	-0.29,0.76	**PA <6m**	2.00	0.52;3.49**
PS z-height	0.04	-0.48,0.57	**PA 6m-2y**	0.41	-0.70;1.52
3m z-BMI	-0.12	-0.61;0.38	**PA 2y-PS**	0.49	-0.86;1.85
1y z-BMI	-0.51	-1.11;0.10	**HI 'ever'**	0.17	-0.72;1.05
2y z-BMI	-0.48	-1.05;0.09	**HI 1st age**	-0.11	-0.34;0.12
PS z-BMI	-0.77	-1.43;-0.12*	**HI <6m**	0.69	-0.63;2.02
Δ z-weight birth-3m	0.05	-0.38;0.47	**HI 6m-2y**	0.37	-0.77;1.51
Δ z-weight birth-1y	-0.14	-0.57;0.29	**HI 2y-PS**	0.28	-1.15;1.70
Δ z-weight birth-2y	-0.04	-0.47;0.39	**SM 'ever'**	-.64	-0.41;1.70
			SM 1st age	0.02	-0.31;0.35
Infant lung function outcomes:			**SM <6m**	-	-
3m z-LCI	0.15	-0.30;0.60	**SM 6m-2y**	0.71	-0.78;2.20
1y z-LCI	0.32	-0.03;0.67	**SM 2y-PS**	0.59	-0.75;1.93
2y z-LCI	0.89	0.36;1.43**			
3m z-$FEV_{0.5}$	-0.30	-0.78;0.17	***Clinical variables:***		
1y z-$FEV_{0.5}$	-0.11	-0.62;0.39	**Genotype**	0.36	-0.56;1.29
2y z-$FEV_{0.5}$	-0.04	-0.70;0.63	**P.Insufficiency**	0.85	-0.47;2.16
3m z-FRC_{pleth}	0.21	-0.30;0.71	**Meconium ileus**	-0.20	-1.67;1.29
1y z-FRC_{pleth}	0.40	0.01;0.79*	**IV courses birth**	0.01	-0.24;0.26
2y z-FRC_{pleth}	0.33	-0.09;0.75	**Mucolytic 'ever'**	0.23	-0.67;1.13
			Mucolytic at PS	0.28	-0.62;1.18

Legend: Genotype was categorised as p.Phe508del homozygous (yes/no), 'IV courses birth' = total number of intravenous antibiotic courses; mucolytic at PS=use of mucolytic therapy in the 12 months before preschool test. Bacterial isolates 'ever' represents subjects who had ever isolated that organism; '1st age' represents age of first acquisition. No subjects isolated SM by 6 months so this variable was not included. Abbreviations: z=z-score; m=months; y=years; PS=preschool; Δ=change in; P.insufficiency=pancreatic insufficiency.
*Significant associations between independent variables and preschool LCI z-score are highlighted in red, *represents p<0.05, **p<0.01*

Multiple linear regression models for preschool LCI z-score

Associations between clinical variables (presentation with meconium ileus, pancreatic insufficiency, genotype, mucolytic treatment 'ever' and intravenous antibiotic courses) and preschool LCI z-score were investigated by multiple linear regression analysis. No significant associations were found with these variables and preschool LCI z-score. These results were in agreement with those previously reported in LCFC infants, where clinical variables and treatments were also not associated with infant lung function outcomes at any test occasion[36, 129]. These variables were therefore taken out of the multiple regression model.

Nutritional outcomes were then entered into a model together; no association was found between height, weight or BMI z-scores at any test occasion (3 month, 1 year, 2 years or preschool) and preschool LCI z-score.

When bacterial isolates 'ever' for PA, SA, HI and SM were entered into the model, there were no significant associations with preschool LCI z-score. Age of acquisition for each bacterial isolate were then added to the model. Despite isolation of PA 'ever' having no association with preschool LCI z-score, age of acquisition of PA was the only significant predictor of preschool LCI z-score (mean regression coefficient [95% CI] -0.27 [-0.53;-0.01], p=0.04).

When categorical age of acquisition for each bacterial isolate (<6 months, 6 months-2 years and 2 years-preschool test) were entered into a model together, isolation of PA by six months of age remained the only significant predictor of preschool LCI z-score, with mean regression coefficient [95% CI] 1.62 [0.02;3.23], p=0.02. However only eight subjects had isolated PA by six months.

To then investigate whether infant lung function tests could predict preschool LCI z-score after adjustment for other variables, lung function results ($FEV_{0.5}$, LCI and FRC_{pleth} z-scores) for each test occasion were then added to the model. First all three tests at three months were added, then three month tests were removed and all one year tests added, then one year tests removed and all two year tests added. Two year LCI z-score was the only infant test significantly associated with preschool LCI z-score, with mean regression coefficient [95% CI] 0.88 [0.29;1.47], p=0.004.

Table 5.12 shows the final multiple regression models for preschool LCI z-score. When two year LCI z-score and age of acquisition of PA were entered into a model

together, two year LCI z-score remained a significant predictor of preschool LCI z-score, the adjusted R^2 increased from 0.16 to 0.17 (an improved model fit), but age of acquisition of PA was no longer significant in this model. A similar effect was found with categorical age of acquisition of PA.

Table 5.12: Final multiple regression models for preschool lung clearance index z-score

n=53	***Coefficients***			***Model summary***		
	B	**95% CI**	**p-value**	**R**	**Adjusted R^2**	**p-value**
Constant	1.40	0.77;2.03	0.00	0.42	0.16	**0.002**
2y z-LCI	0.89	0.36;1.43	**0.002**			

n=50	***Coefficients***			***Model summary***		
	B	**95% CI**	**p-value**	**R**	**Adjusted R^2**	**p-value**
Constant	2.00	1.16;2.84	0.00			
2y z-LCI	0.81	0.30;1.33	**0.003**	0.45	0.17	**0.004**
PA age	-0.23	-0.50;0.04	0.09			

n=50	***Coefficients***			***Model summary***		
	B	**95% CI**	**p-value**	**R**	**Adjusted R^2**	**p-value**
Constant	0.93	-0.04;1.91	0.06			
2y z-LCI	0.81	0.28;1.35	**0.004**			
PA <6m	1.14	-0.44;2.74	0.15	0.47	0.15	**0.02**
PA 6m-2y	0.50	-0.63;1.63	0.38			
PA 2y-PS	1.09	-0.33;2.50	0.13			

Legend: Abbreviations: m=months; y=years; PS=preschool, z=z-score, B=unstandardized coefficient, n=number of subjects with data for all independent variables reported in the multiple regression model.

When age of PA acquisition is added to the model with two year LCI z-score, the model fit is improved

5.4.4.2 Predictors of preschool s*R*tot z-score in subjects with CF

Using preschool s*R*tot z-score as the dependent variable, simple and multiple regression analyses were used to investigate significant associations with the independent variables in the same process as preschool LCI z-score. s*R*tot was considered a secondary outcome as it identified less children with abnormality (8%) than preschool LCI z-score (39%), but was still significantly different to controls at preschool test occasion with mean z-score [95% CI] 0.72 [0.32; 1.13] higher in subjects with CF.

The only significant association between anthropometric measures and s*R*tot z-score on univariable analysis was height at three months (regression coefficient [95% CI] 0.26 [0.01;0.51], but the effect size was small and, albeit statistically significant, is of minimal clinical significance. Subsequent height z-scores at one and two years were not significantly associated with preschool s*R*tot z-score.

As was seen for preschool LCI z-score, PA infection by six months and 2 year LCI z-score were significantly associated with preschool s*R*tot (regression coefficients [95% CI] 1.17 [0.44;1.90], p<0.01 and 0.34 [0.05;0.64], p=0.03 respectively. Isolation of HI 'ever' was also a significant determinant of s*R*tot (0.65 [0.18;1.12], p<0.01), but early isolation (<6 months) was not significantly associated. Isolation of SM between six and 24 months and SA between 2 year and preschool test also reached significance, but isolation of these organisms 'ever' was not significantly associated with preschool s*R*tot z-score. Results of univariable analysis for s*R*tot z-score are summarised in Table 5.13.

Table 5.13: Determinants of preschool specific airway resistance z-score by univariable regression analysis

	Mean coefficient			*Mean coefficient*	
	B	**95% CI**		**B**	**95% CI**
Anthropometric variables:			***Bacterial isolates:***		
z-Birthweight	0.19	-0.07;0.45	**SA 'ever'**	0.20	-0.30;0.70
3m z-weight	0.21	-0.04;0.45	**SA 1st age**	0.00	-0.12;0.12
1y z-weight	0.21	-0.08;0.51	**SA <6m**	0.17	-0.42;0.75
2y z-weight	0.00	-0.31;0.31	**SA 6m-2y**	0.80	-0.01;1.61
PS z-weight	-0.15	-0.45;0.15	**SA 2y-PS**	0.81	0.25;1.36**
3m z-height	0.26	0.01;0.51*	**PA 'ever'**	-0.00	-0.56;0.56
1y z-height	0.19	-0.07;0.45	**PA 1st age**	-0.05	-0.20,0.09
2y z-height	0.14	-0.16;0.43	**PA <6m**	1.17	0.44;1.90**
PS z-height	-0.08	-0.36;0.21	**PA 6m-2y**	0.34	-0.13;0.81
3m z-BMI	0.11	-0.16;0.37	**PA 2y-PS**	0.36	-0.31;1.03
1y z-BMI	0.13	-0.21;0.46	**HI 'ever'**	0.65	0.18;1.12**
2y z-BMI	-0.13	-0.45;0.18	**HI 1st age**	-0.08	-0.20;0.04
PS z-BMI	-0.26	-0.63;0.11	**HI <6m**	0.41	-0.23;1.09
Δ z-weight birth-3m	0.04	-0.18;0.26	**HI 6m-2y**	0.99	0.47;1.50***
Δ z-weight birth-1y	0.00	-0.24;0.23	**HI 2y-PS**	1.09	0.36;1.81*
Δ z-weight birth-2y	-0.06	-0.29;0.17	**SM 'ever'**	0.55	-0.05;0.50
			SM 1st age	-0.08	-0.26;0.09
Infant lung function outcomes:			**SM <6m**	-	-
3m z-LCI	-0.08	-0.31;0.15	**SM 6m-2y**	0.88	0.10;1.65*
1y z-LCI	-0.02	-0.22;0.18	**SM 2y-PS**	0.27	-0.47;0.10
2y z-LCI	0.34	0.05;0.64*			
3m z-$FEV_{0.5}$	-0.14	-0.40;0.12	***Clinical variables:***		
1y z-$FEV_{0.5}$	0.11	-0.17;0.38	**Genotype**	-0.23	-0.73;0.26
2y z-$FEV_{0.5}$	-0.08	-0.38;0.38	**P.Insufficiency**	0.04	-0.67;0.76
3m z-FRC_{pleth}	0.19	-0.09;0.46	**Meconium ileus**	-0.08	-0.88;0.72
1y z-FRC_{pleth}	0.13	-0.09;0.35	**IV courses birth**	-0.02	-0.16;0.12
2y z-FRC_{pleth}	0.22	-0.02;0.45	**Mucolytic 'ever'**	-0.11	-0.61;0.39
			Mucolytic at PS	-0.01	-0.52;0.50

Legend: Genotype was categorised as p.Phe508del homozygous (yes/no), 'IV courses birth' = total number of intravenous antibiotic courses; mucolytic at PS=use of mucolytic therapy in the 12 months before preschool test. For bacterial isolates 'ever' represents subjects who had ever isolated that organism; '1st age' represents age of first acquisition. No subjects isolated SM by 6 months so this variable was not included. Abbreviations: z=z-score; m=months; y=years; PS=preschool; Δ=change in; P.insufficiency=pancreatic insufficiency; IV=intravenous.

*Significant associations between independent variables and preschool sRtot z-score are highlighted in red, *represents p<0.05, **p<0.01 and ***p<0.01*

Multiple linear regression models for preschool s*R*tot z-score

Multiple linear regression analysis was performed to investigate the relationship between measures in infancy and preschool s*R*tot z-score using the same step-wise method as described above for LCI z-score. No significant associations were found between preschool s*R*tot z-score and clinical variables (presentation with meconium ileus, pancreatic insufficiency, genotype, mucolytic treatment 'ever' and intravenous antibiotic courses) or anthropometric variables (height, weight and BMI z-score) on any test occasion.

When isolation of pathogens 'ever' were investigated, isolation of HI 'ever' was significantly associated with preschool s*R*tot z-score with mean regression coefficient [95% CI] 0.65 [0.19;1.18], p=0.01. The age at which any bacterial isolate was acquired did not have a significant effect. For categorical age of acquisition, isolation of SA between two year and preschool test, HI between six months and two years and SM between six months and two years all reached significance.

For any lung function test in infancy (at three months, one year and two years), two year LCI z-score was the only variable to have a significant association with preschool s*R*tot z-score; mean regression coefficient [95% CI] 0.37 [0.02;0.71], p=0.04.

5.4.4.3 Predictors of preschool $FEV_{0.75}$ in subjects with CF

Univariable associations with preschool $FEV_{0.75}$ z-score were investigated in the same process as preschool LCI and s*R*tot z-score. $FEV_{0.75}$ was considered a secondary outcome as it identified less children with abnormality (11%) than preschool LCI z-score (39%), but was still significantly different to controls at preschool test occasion with mean z-score [95% CI] -0.54 [-0.98; -0.10] lower in subjects with CF.

On univariable regression analysis, both one year and preschool BMI z-scores were significantly associated with preschool $FEV_{0.75}$ z-score (regression coefficient [95% CI] 0.49 [0.14,0.84], p=<0.01; and 0.61 [0.22;0.99], p=<0.01 respectively). Use of mucolytic 'ever' was also a significant determinant (-0.63 [-1.17;-0.09]), p=0.02.

Several significant associations were seen with bacterial isolates, the most highly significant of which was with isolation of SM 'ever' (-1.10 [-1.68,-0.52], p=<0.001. HI 'ever' also reached significance between two year (-0.59 [-1.14;-0.04], p=0.04) and preschool test (-0.81 [-1.59;-0.03], p=0.04). PA 'ever' was not significantly associated with preschool $FEV_{0.75}$, but isolation between two years and preschool test was (-0.98 [-1.68;-0.29], p=0.01).

For infant lung function measures, LCI z-score at one year was a significant (but weak) determinant of preschool $FEV_{0.75}$ (-0.22 [-0.43;-0.02], p=0.04), but no association was seen with two year LCI z-score. $FEV_{0.5}$ at three months was significantly associated with preschool $FEV_{0.75}$ (0.41 [0.13;0.69], p=<0.01). The univariable regression analysis is summarised in Table 5.14.

Table 5.14: Determinants of preschool forced expired volume by univariable regression analysis

	Mean coefficient			*Mean coefficient*	
	B	**95% CI**		**B**	**95% CI**
Anthropometric variables:			***Bacterial isolates:***		
z-Birthweight	-0.09	-0.39;0.21	**SA 'ever'**	-0.32	-0.89;0.25
3m z-weight	-0.01	-0.28;0.27	**SA 1st age**	0.04	-0.09;0.18
1y z-weight	0.12	-0.21;0.45	**SA <6m**	-0.08	-0.76;0.60
2y z-weight	-0.01	-0.37;0.34	**SA 6m-2y**	-0.34	-1.23;0.56
PS z-weight	0.28	-0.06,0.62	**SA 2y-PS**	-0.15	-0.80;0.50
3m z-height	-0.25	-0.52;0.30	**PA 'ever'**	-0.43	-1.07;0.21
1y z-height	-0.21	-0.50;0.07	**PA 1st age**	0.02	-0.14;0.19
2y z-height	-0.10	-0.45;0.26	**PA <6m**	0.08	-0.73;0.88
PS z-height	-0.03	-0.36;0.30	**PA 6m-2y**	0.21	-0.36;0.79
3m z-BMI	0.19	-0.09;0.48	**PA 2y-PS**	-0.98	-1.68;-0.29**
1y z-BMI	0.49	0.14,0.84**	**HI 'ever'**	-0.59	-1.14;-0.04*
2y z-BMI	0.07	-0.29;0.43	**HI 1st age**	0.06	-0.09;0.20
PS z-BMI	0.61	0.22;0.99**	**HI <6m**	0.20	-0.65;1.04
Δ z-weight birth-3m	0.11	-0.15;0.36	**HI 6m-2y**	-0.32	-0.94;0.30
Δ z-weight birth-1y	0.12	-0.15;0.39	**HI 2y-PS**	-0.81	-1.59;-0.03*
Δ z-weight birth-2y	0.04	-0.23;0.32	**SM 'ever'**	-1.10	-1.68,-0.52***
			SM 1st age	0.15	-0.04;0.35
Infant lung function outcomes:			**SM <6m**	-	-
3m z-LCI	0.08	-0.17;0.34	**SM 6m-2y**	-0.89	-1.76;-0.03*
1y z-LCI	-0.22	-0.43;-0.02*	**SM 2y-PS**	-0.98	-1.74;-0.21**
2y z-LCI	-0.33	-0.70;0.05			
3m z-$FEV_{0.5}$	0.41	0.13;0.69**	***Clinical variables:***		
1y z-$FEV_{0.5}$	0.24	-0.06;0.55	**Genotype**	0.05	-0.53;0.62
2y z-$FEV_{0.5}$	0.00	-0.44;0.43	**P.Insufficiency**	-0.67	-1.50;0.16
3m z-FRC_{pleth}	-0.07	-0.37;0.23	**Meconium ileus**	-0.19	-1.15;0.77
1y z-FRC_{pleth}	0.13	-0.14;0.39	**IV courses birth**	0.00	-0.16;0.16
2y z-FRC_{pleth}	-0.15	-0.42;0.11	**Mucolytic 'ever'**	-0.63	-1.17;-0.09*
			Mucolytic at PS	-0.39	-1.00;0.18

Legend: Genotype was categorised as p.Phe508del homozygous (yes/no), 'IV courses birth' = total number of intravenous antibiotic courses; mucolytic at PS=use of mucolytic therapy in the 12 months before preschool test. For bacterial isolates 'ever' represents subjects who had ever isolated that organism; '1st age' represents age of first acquisition. No subjects isolated SM by 6 months so this variable was not included. Abbreviations: z=z-score; m=months; y=years; PS=preschool; Δ=change in; P.insufficiency=pancreatic insufficiency.

*Significant associations between independent variables and preschool $FEV_{0.75}$ z-score are highlighted in red, *represents p<0.05, **p<0.01 and ***p=<0.001*

Multiple linear regression models for preschool $FEV_{0.75}$ z-score

Multivariable regression analyses were used to investigate the combined effects of determinants of preschool $FEV_{0.75}$ z-score. When clinical variables were entered into a model, both pancreatic insufficiency and mucolytic 'ever' had a significant association with $FEV_{0.75}$ z-score. None of the anthropometric measures (height, weight or BMI z-score at three month, one year, two year and preschool test occasion) were significantly associated with preschool $FEV_{0.75}$ z-score.

When isolation of pathogens 'ever' were entered into a multiple linear regression model, HI 'ever' and SM 'ever' were both significantly associated with preschool $FEV_{0.75}$ z-score. Age of acquisition of each pathogen showed no significant associations in a multiple regression model together, but categorical age of acquisition of HI between two year and preschool test was significant and SM between six month and two year, and SM between two year and preschool all had significant associations with preschool $FEV_{0.75}$ z-score.

To examine the relationship between infant lung function tests and preschool $FEV_{0.75}$ z-score, all infant lung function tests first at three months, then at one year and then at two years were entered into a regression model. None of the infant lung function tests at any test occasion had a significant association with preschool $FEV_{0.75}$ z-score.

5.4.4.4 Evaluation of binary classifiers to predict preschool lung clearance index

In addition to regression analysis, an evaluation of binary classifiers can be used for measures in infancy to predict those at preschool age. As LCI z-score was the most sensitive marker of abnormality in the 67 children with CF tested in the preschool years, it was chosen as the outcome variable for sensitivity analysis. In children with CF, infant lung function results (LCI, $FEV_{0.5}$ and FRC_{pleth} z-scores) were classified as normal or abnormal. Abnormal tests for LCI and FRC_{pleth} were >1.98 z-scores and for $FEV_{0.5}$ <-1.98 z-scores. *Pseudomonas aeruginosa* status was also included (a positive isolate being an abnormal result). Two year LCI z-score had the highest positive predictive value (71%) for preschool LCI z-score, and negative predictive value of 73%.

Table 5.15: Positive and negative predictive values of infant lung function measures and *Pseudomonas* status for preschool lung clearance index

Variable	Positive predictive value	Negative predictive value
3 month LCI z-score	57%	63%
1 year LCI z-score	60%	65%
2 year LCI z-score	75%	73%
3 month $FEV_{0.5}$ z-score	38%	62%
1 year $FEV_{0.5}$ z-score	20%	58%
2 year $FEV_{0.5}$ z-score	71%	63%
3 month FRC_{pleth} z-score	25%	63%
1 year FRC_{pleth} z-score	63%	64%
2 year FRC_{pleth} z-score	33%	67%
PA < 6 months	40%	68%
PA 6 months-2 years	43%	72%
PA 2 years-preschool	50%	70%

Legend: Positive and negative predictive values for infant lung function measures and Pseudomonas status for preschool lung clearance index z-score. Abbreviations: LCI=lung clearance index, $FEV_{0.5}$=forced expired volume in 0.5 seconds, FRC_{pleth}=plethysmographic functional residual capacity, PA=pseudomonas aeruginosa isolate. Two year LCI z-score had the highest positive and negative predictive values for preschool LCI z-score

5.5 Discussion

5.5.1 Principal findings

The main results of this study are as follows:

a) In NBS subjects followed up at preschool age, LCI z-score was not significantly different in subjects with CF compared to controls at three months, but then showed a progressive deterioration, and at preschool test was the only parameter outside the normal range (≥1.96 z-scores) at any test occasion during the study. $FEV_{(t)}$ remained stable throughout infancy and at preschool test occasion

b) The only significant association of infant lung function measures and preschool lung function primary outcomes was between two year LCI z-score and preschool LCI and s*R*tot z-scores. Otherwise infant measures did not predict preschool lung function

c) For bacterial infection, earlier age of acquisition of PA was a determinant of both preschool LCI z-score, with isolation in the first six months of life having a significant effect on univariable regression analysis, but this relationship was not significant on multiple regression analysis when two year LCI z-score was accounted for. Isolation of HI 'ever' was a significant determinant of preschool s*R*tot and $FEV_{0.75}$ z-score, and isolation of SM 'ever' was a significant determinant of preschool $FEV_{0.75}$ z-score

5.5.2 Review of hypotheses

The hypotheses were:

a) *"Infant lung function measures ($FEV_{0.5}$, FRC_{pleth} and LCI z-scores) and nutritional markers (height, weight and BMI z-scores) cannot predict preschool lung function (LCI, sRtot and $FEV_{0.75}$ z-scores) in NBS children with CF"*

This hypothesis was incorrect, as there was a significant association between two year LCI z-score and preschool LCI and s*R*tot z-scores. However, nutritional outcomes were not significant predictors of preschool lung function.

b) *"Clinical markers of infection (isolation of bacteria on respiratory samples), cannot predict preschool lung function, and markers of disease severity (for example courses of intravenous antibiotics) in infancy will not be significantly associated with preschool lung function primary outcomes (LCI, sR_{tot} and $FEV_{0.75}$ z-scores)"*

This hypothesis was proven as no significant associations were found between clinical markers in infancy and preschool lung function in the multiple regression models.

5.5.3 Comparison of this study to other reports

This study shows that lung function changes in NBS infants are mild and short-lived until preschool age when LCI gives a sensitive signal of abnormality. However, as two year LCI z-score predicts later pulmonary function, lung damage is likely to occur before this point. This will be discussed further in the final chapter of this thesis.

There were no other significant associations between lung function measures in early infancy and preschool lung function outcomes. The fact that infant lung function, with the exception of two year LCI, does not predict preschool measures is not unexpected considering the transient abnormalities of functional measures seen in NBS infants earlier in the study. This suggests that preschool determinants are post-natal (rather than ante-natal) and supports an argument for starting new therapies at an early age. There was a progressive deterioration seen in LCI z-score with each test when compared to healthy controls; the mean difference was 0.32 z-scores at three months, 0.73 z-scores at one year, 0.89 z-scores at two years and 1.47 at preschool test. This is the first study to report the progressive decline of LCI from infancy to preschool age when compared to healthy controls, and is in keeping with a Canadian study showing progressive deterioration of LCI in the same subjects with CF on repeated measurement of LCI over the 3-6 year age range[144].

In the regression analyses, a significant association was seen between two year LCI z-score and preschool LCI z-score, when two year LCI z-score was analysed as a continuous variable. However, as shown in Table 5.10, only eight infants had abnormal two year LCI z-score; six of whom had abnormal LCI z-score at both two year and preschool test. Therefore, if the cut off of +1.96 z-scores to define abnormality was used to enrol children with CF to interventional trials at two years of age, very few subjects would be included, but this may represent a small subgroup that could be targeted for intervention or more aggressive treatment. Although out of the scope of this thesis, the rate of decline in LCI may be a more important marker for inclusion in clinical trials, particularly as this and the Canadian study show a progressive deterioration with age.

An association was seen with early *Pseudomonas aeruginosa* infection and preschool LCI, and isolation before six months of age was significantly associated with higher preschool LCI z-score on univariable analysis. On multiple regression analysis any association between *Pseudomonas aeruginosa* and preschool lung function was lost

when two year LCI was accounted for. It must be noted that there were only eight subjects who isolated this organism by six months. The results reported in this chapter are an exploratory analysis of associations between measures in infancy and preschool lung function, but with such small numbers in the aforementioned groups, the study is underpowered. The AREST-CF group have reported progression of structural[136] and functional[41] abnormalities in NBS infants isolating this organism. In an earlier publication of LCFC clinically diagnosed children, Kozlowska and colleagues found early infection with *Pseudomonas aeruginosa* before any lung function test (infancy or preschool) preceded a decline in lung function. However, LCI was not reported in this study. It may be that the early developing lung is particularly susceptible to infection with *Pseudomonas aeruginosa* in the first few months of life, and irreversible damage may occur, but this is not reflected in tests of infant or preschool lung function in cohort 2.

5.5.4 Limitations of the study

Limitations relating to the collection of clinical data are presented in the final chapter of this thesis. Although associations between lung function and, for example, bacterial isolates and CF treatments were investigated, it is recognised that numbers were small in many of these categories and exploration of any association therefore limited.

A further limitation of the study was the period between two year and preschool test. The age range for preschool follow was wide, and therefore reporting the average time-point when lung function became abnormal was limited. However it is difficult to justify repeated measures in young children, and it would place an unnecessary burden on the families who had already attended for infant testing under sedation on at least one occasion. All test occasions are relatively time consuming, and increasing the number of measurements could lead to reduced follow-up or withdrawal from the study. It was not possible therefore to describe response to treatment or natural variability of lung function in these children.

5.5.5 Meaning of the study, conclusions and future research

This study has described the evolution of lung function in a NBS cohort of UK children under six years of age, and investigated factors in early life that may predict decline in pulmonary function. The implications of these findings for clinical monitoring, enrolment in interventional trials and timing of new therapies is explored in the discussion chapter of this thesis. The final results chapter explores predictors of preschool lung function further, focusing on infant measures of lung structure and inflammation.

6 Measures of infant lung structure and inflammation as predictors for preschool pulmonary function

6.1 Introduction

In addition to the measurement of lung function, markers of lung structure and inflammation can be used to monitor disease in young children with CF. Their use remains controversial in infancy, with concerns around the effect of ionising radiation on the developing lung if HRCT is used, and the invasive nature of sampling for inflammatory markers using bronchoscopic assisted BAL. However if either could predict lung health at a later age, and identify children who are at risk of decline, surveillance using these methods would be justified.

A 'gold standard test' can be considered as one that has a high sensitivity for the presence of disease and high specificity; one that does not identify abnormality in those without disease, the ideal being 100% for both. In young children with CF, this gold standard is likely a test that easily identifies an abnormality that has detrimental consequences on long term health. At present, there is no agreed gold standard to identify early lung disease in children with CF. Abnormal markers (whether from lung function, CT or BAL) have unknown long term outcomes, and the choice of which exact marker to follow unclear at present.

The AREST-CF group have demonstrated that CT abnormalities can be detected in a high proportion of infants with CF from three months of age[3], and in the first year of life, bronchial dilatation (32% of subjects) and air trapping (69%) were both detectable in NBS infants[135]. This group reported cross-sectional correlations between lung function (LCI) and CT abnormalities in infants and preschool children[78]. LCI was insensitive to structural disease in infants, but by preschool age LCI correlated with total disease extent. However, at the time of writing, this group have not described whether CT outcomes measured in infancy can predict lung function in later life.

The relationship between inflammatory markers in BAL and lung function has also been reported in the AREST-CF cohort. Infants with detectable free neutrophil elastase, a marker of neutrophilic inflammation, in BAL at three months of age had significantly poorer lung function in the first two years of life than those who did not[41]. They went on to show that neutrophil elastase activity at three months was associated with persistent bronchial dilatation on two or more sequential CT scans at one and

three years of age[135]. No associations were found with other BAL inflammatory markers and later lung function outcomes. When neutrophil elastase measured in the first two years of life was investigated as a determinant for school age lung function, only a univariable association was found which did not remain significant after adjustment for respiratory pathogens detected on BAL[133]. However, early school age spirometry ($FEV_{0.75}$) was reported in this study; they have not yet reported longitudinal relationships between inflammatory markers in infancy and later LCI.

The LCFC NBS cohort of children with CF described in this thesis also underwent CT and BAL at one year of age. A report of CT abnormalities has previously been published[94]. Bronchial dilatation and air trapping were seen in 26% and 42% of infants respectively at one year of age. However, changes observed on chest CT scan were so mild that scoring was poorly reproducible between two observers apart from air-trapping (strong agreement), bronchial dilatation and total CT score (fair agreement). The aforementioned studies from both the AREST-CF and LCFC groups are discussed in detail in section 1.4.3.2.

As the ability of CT and BAL inflammatory markers measured in infancy to predict later lung function in preschool children has not been previously reported, and in view of the apparent differences between the AREST-CF and LCFC cohorts, this chapter will investigate the relationship between markers of lung structure and inflammation at one year and preschool lung function.

6.2 Hypotheses, aims and objectives

The hypotheses were:

1. Inflammatory markers from BAL at one year cannot predict preschool lung function (LCI, s*R*tot and $FEV_{0.75}$) at three to six years of age

2. Abnormalities on CT (air trapping, bronchial dilatation and total CT score) cannot predict preschool lung function

The aims were to describe associations between CT score and BAL inflammatory markers in infancy with preschool lung function in NBS children with CF. The objective was to investigate the relationship between CT and BAL parameters measured at one year in the LCFC cohort of NBS children and preschool lung function primary outcome

measures (LCI, s*R*tot and $FEV_{0.75}$ z-scores) measured at three to six years in the same children.

6.3 Methods

The LCFC infant study design and outcome measures are described in section 2.3. To summarise the relevant tests investigated in this chapter, at one year of age infants underwent lung function testing using the RVRTC technique, whole body plethysmography and MBW, with primary outcomes of $FEV_{0.5}$, FRC_{pleth} and LCI respectively. Infants were tested when well; defined as free from respiratory symptoms for three weeks before the test date. The detailed methodology of infant lung function measurement in this longitudinal study has been previously published[36, 127]. In addition, a CT scan and bronchoscopy were performed within two weeks of lung function tests if infants remained clinically stable. Data from infants were collected as part of an earlier study (Principal Investigator Professor Janet Stocks), and not by myself.

Volume controlled thin section chest CT was performed under general anaesthesia, as described by Thia et al.[94]. In brief, inspiratory and expiratory scans were obtained using controlled ventilation. Anonymised scans were scored independently by two specialist paediatric radiologists (Drs Alan Brody and Alistair Calder), blinded to subject clinical characteristics. The Brody-II CF-CT scoring system was utilised[98]. Air trapping, bronchial dilatation and total CT score were used in the present chapter as the primary outcomes due to the poor intra- and inter-observer agreement for other CT score parameters previously reported[94]. The mean of the two operator scores for each of these parameters were used. Abnormal scores were defined as a total CT score greater than 12 (maximum possible 243), any evidence of bronchial dilatation (maximum possible score 72), or any air trapping (maximum 27)[94, 126].

Flexible bronchoscopy was performed after the CT scan under the same general anaesthetic. A 2.8 mm bronchoscope was inserted via an endotracheal tube. ERS taskforce guidelines for BAL in children were followed[110]; four aliquots of normal saline at a volume of 1ml/kg were instilled and retrieved using low pressure suction, three from the right middle lobe and one from the lingula. The first returned aliquot from each lobe was sent for bacterial and fungal culture. Subsequent samples from the right middle lobe were pooled, centrifuged and the supernatants frozen at -80°C. Cytokines were measured from pooled samples using the Meso Scale Discovery® Multi- Array technology (MesoScale Discovery, Gaithersburg, MD, USA). Assays for markers

known to reflect pulmonary inflammation were chosen, namely interleukin- (IL) 6, IL-8, IL-10, tumour necrosis factor alpha (TNFα), monocyte chemoattractant protein-1 (MCP-1) and neutrophil elastase (NE). Free NE activity was measured using an adapted ELISA (enzyme-linked immunosorbent assay) technique[3]. The lower limits of detection were 0.03 pg/ml for IL-10, 0.04pg/ml for IL-8 and TNFα, 0.06 pg/ml for IL-6, 2pg/ml for MCP-1 and 200ng/ml for NE. Inflammatory marker analysis was completed in the Great Ormond Street Hospital microbiology laboratory.

At three to six years of age the same children were invited to return for preschool lung function testing (MBW, whole body plethysmography and spirometry) as described in the current study (section 2.6), with primary outcomes of LCI, s*R*tot and $FEV_{0.75}$.

6.3.1 Statistical analysis

The sample size was determined by the numbers previously recruited, so no power calculation was carried out. Published reference equations were used to express anthropometric data[138], infant lung function[31, 75, 150] and preschool lung function[49, 75, 151] results as z-scores, and were summarised as the mean and standard deviation. Group comparisons of subjects with and without CT and/or BAL data were investigated with student t-tests, Chi-squared or Fisher's exact test as appropriate. CT scores and BAL inflammatory markers were non-normally distributed and expressed as median and interquartile range, or range. Cytokine concentrations were log transformed to allow visual inspection and investigation of any association with preschool lung function using linear regression analysis. Variation exists in what is considered to be a normal cytokine profile, so each inflammatory marker was modelled as a continuous independent variable to investigate whether high (or low) levels were associated with preschool lung function outcomes.

Preschool lung function primary outcome measures (LCI, s*R*tot and $FEV_{0.75}$) were modelled separately. An evaluation of binary classifiers is also presented for preschool LCI z-score using infant CT scores and presence of neutrophil elastase in BAL as predictor variables. Statistical analyses were conducted using SPSS software (IBM SPSS Statistics for Windows, Version 24.0). Statistical significance was taken as $p<0.05$.

6.4 Results

6.4.1 Subject characteristics

Of the 67 children with CF with preschool follow up, 48 had HRCT and 49 had BAL (one child had CT performed outside of the study scoring period). Data for inflammatory markers were not available for 16 infants due to a processing error, in that these samples of BAL were frozen before centrifuge. This is required before supernatant is extracted, therefore it was not possible to use the data for these subjects. Preschool growth and lung function characteristics of subjects with and without CT/BAL are summarised in Table 6.1 (those with BAL were those for whom inflammatory marker data were available). Children with BAL were significantly younger at preschool test than those without, but there were no significant differences in growth or lung function results between the two groups. Clinical characteristics are shown in Table 6.2, and were similar to those described in the full cohort (sections 3.4.3 and 5.4.2). Only one child at one year test and one child preschool test had chronic PA in the CT group and no children had chronic PA by one year, and only one at preschool test in the BAL group (Leeds criteria[139]).

Table 6.1: Comparison of preschool growth and lung function in those with and without computed tomography and broncho-alveolar lavage

	With CT (n=48)	Without CT (n=19)	Mean difference [95% CI] with-without	With BAL (n=33)	Without BAL (n=34)	Mean difference [95% CI] with-without
Age diagnosis (w)	4.4 (3.7)	5.0 (6.3)	-0.56 [-3.04;1.91]	3.9 (2.8)	5.3 (5.7)	-1.41 [-0.36;0.80]
Age preschool test (y)	4.65 (0.69)	5.03 (0.86)	-0.38 [-0.78;0.03]	4.24 (0.51)	5.26 (0.60)	-1.02 [-1.29;-0.74]***
Weight z-score	-0.13 (0.79	-0.08 (0.96)	-0.05 [-0.51;0.41]	-0.02 (0.85)	-0.20 (0.83)	0.18 [-0.23;0.59]
Height z-score	-0.26 (0.82)	-0.15 (1.01)	-0.11 [-0.59;0.36]	-0.19 (0.85)	-0.27 (0.91)	0.08 [-0.35;0.51]
BMI z-score	0.08 (0.67)	0.12 (0.68)	-0.04 [-0.41;0.33]	0.20 (0.66)	-0.02 (0.67)	0.22 [-0.11;0.54]
LCI z-score	2.09 (1.88)[a]	2.25 (1.80)[b]	-0.15 [-1.16;0.85]	1.67 (1.62)[g]	2.56 (1.96)[h]	-0.87 [-1.76;0.02]
s*R*tot z-score	0.39 (1.08)[c]	0.24 (0.72)[d]	0.15 [-0.40;0.70]	0.23 (0.89)[i]	0.46 (1.08)[j]	-0.23 [-0.72;0.26]
$FEV_{0.75}$ z-score	-0.44 (1.11)[e]	-0.48 (1.13)[f]	0.04 [-0.60;0.69]	-0.32 (1.06)[k]	-0.59 (1.15)[l]	0.27 [-0.29;0.85]

Legend: Data presented as mean (SD) and mean difference [95% CI]. Abbreviations: w=weeks; y=years. Number of subjects as follows: a=47, b=19, c=47, d=18, e=46, f =16, g=32, h=34, i=31, j=34, k=31, l=31.

Age at preschool test was significantly lower in subjects who underwent bronchoalveolar lavage compared to those who did not, but there were no differences in preschool lung function or nutritional outcomes

Table 6.2: Clinical characteristics of children with CF assessed at preschool age with one year broncho-alveolar lavage and computed tomography

	With CT	With BAL
Age at diagnosis, median weeks (IQR)	3.4 (1.4)	3.3 (1.3)
Male	22 (45%)	12 (36%)
Mean birthweight z-score (SD)	-0.47 (0.85)	-0.35 (1.12)
p.Phe508del homozygous	28 (58%)	21 (64%)
p.Phe508del heterozygous	15 (31%)	11 (33%)
Meconium ileus presentation	5 (10%)	6 (18%)
Pancreatic insufficient	43 (89%)	30 (90%)
Isolation of PA by 1y test	14 (30%)	12 (36%)
Isolation of PA by PS test	3 (69%)	24 (75%)
IV antibiotic courses by 1y test, median (range)	0 (0-3)	0 (0-3)
IV antibiotic courses by PS test, median (range)	1 (0-6)	1 (0-6)

Legend: Results presented as number of subjects (%) and median (IQR) or (range) unless otherwise stated. Data for isolation of Pseudomonas aeruginosa (PA) and number of IV antibiotic courses (presented from birth) were available for 46/48 subjects with CT and 32/33 subjects with BAL. Abbreviations: y=year, PS=preschool, IV=intravenous, CT=computed tomography, BAL=broncho-alveolar lavage. Clinical characteristics in children who underwent computed tomography and broncho-alveolar lavage were similar to those described in the full cohort

6.4.2 Lung function, CT score and BAL results

Table 6.3 and Table 6.4 present a summary of infant and preschool lung function, CT score and BAL inflammatory markers in subjects with CF. As described in the full cohort in Chapter 5, lung function at one year was in the normal range. There was minor elevation of LCI and FRC_{pleth}, and lower $FEV_{0.5}$ in both CT and BAL groups. By preschool age, mean (SD) LCI was elevated at 2.09 (1.89) z-scores in those with CT. In the BAL group with inflammatory marker data, mean preschool LCI was lower than the CT group at 1.69 (1.62) z-scores. Mean s*R*tot and $FEV_{0.75}$ were within +/-1.96 z-scores in both CT and BAL groups.

48 children followed up at preschool age had data for one year HRCT. 41 (85%) had evidence of 'any' structural change (score greater than zero for any Brody II subscore), but all abnormalities were extremely minor, as previously reported. Air trapping was present in 21 infants (44%), however all but two infants had a score less than 4 out of a maximum possible 27 for air trapping. Bronchial dilatation was seen in 13 (27%), but again the extent of this was minimal; the highest score was three out of a possible 72. Total CT score was abnormal (greater than 12) in only one infant (whose total score was 15.5 out of a maximum of 243). Median scores and ranges for each parameter are presented in Table 6.4

49 infants underwent flexible bronchoscopy. A visual secretion score was determined by the bronchoscopist, ranging from one (no secretions) to five (copious secretions). 46 subjects had available data; 22 (48%) had no visible secretions, and 24 (52%) had secretions present. 49 had BAL fungal culture; *Aspergillus fumigatus* was detected in five (10%) and *Candida Albicans* in two (4%). 51 subjects had BAL samples processed for bacterial culture; two isolated *Pseudomonas aeruginosa*, two *Staphylococcus aureus* and five *Haemophilus influenzae* at the time of bronchoscopy. No subjects isolated *Stenotrophomonas maltophilia*. In the 33 subjects with inflammatory marker data, only seven (21%) had detectable neutrophil elastase activity.

Table 6.3: One year and preschool lung function in CF subjects with computed tomography and broncho-alveolar lavage data

1year lung function: mean (SD) z-score	**With CT**	**With BAL**
Lung clearance index	0.77 (1.30)[a]	1.11 (1.43)[b]
Plethysmographic FRC	0.75 (1.19)[c]	0.96 (1.18)[d]
Forced expired volume 0.5s	-0.60 (1.06)[e]	-0.54 (1.05)[f]
Preschool lung function: mean (SD) z-score		
Lung clearance index	2.09 (1.88)[g]	1.69 (1.61)[h]
Specific airway resistance	0.39 (1.08)[i]	0.23 (0.88)[j]
Forced expired volume 0.75s	-0.44 (1.11)[k]	-0.32 (1.06)[l]

Legend: Results are presented as mean (SD) z-score for CF subjects with computed tomography and broncho-alveolar lavage data. Number of subjects: a=48, b=32, c=45, d=30, e=45, f=30, g=47, h=32, i=47, j=31, k=46, l=31. Children who underwent broncho-alveolar lavage had better lung function than those who underwent CT

Table 6.4: One year computed tomography scores and broncho-alveolar lavage inflammatory marker results

Computed tomography score: median (range), n=48	
Total score	2.00 (0-15.5)
Air trapping	0.00 (0-5.5)
Bronchial dilatation	0.00 (0-3)
Broncho-alveolar lavage inflammation: n (%) or median (IQR), n=33	
Detectable free neutrophil elastase activity	7 (21%)
Neutrophil elastase concentration, ng/ml	119.5 (89.5)
IL-6, pg/ml	33.5 (91.8)
IL-8, pg/ml	373.3 (1162.4)
IL-10, pg/ml	1.4 (3.3)
TNFα, pg/ml	1.8 (4.1)
MCP-1, pg/ml	132.6 (369.7)

Legend: Results are presented as median (IQR or range). Detectable free neutrophil elastase activity = >200ng/ml. Computed tomography scores represent the mean of two scorers. Broncho-alveolar lavage data presented for those with inflammatory marker results. Abbreviations: n=number of subjects; IL=interleukin; TNFα=tumour necrosis factor alpha; MCP-1=monocyte chemoattractant protein-1; ng=nanograms; pg=picograms; ml=millilitres.
Overall, very few children had abnormal results for either computed tomography score or inflammatory markers

6.4.3 Associations between CT score, BAL inflammatory markers and preschool lung function

Associations between CT score, inflammatory marker data and preschool lung function were investigated by linear regression analysis. Due to the spread of the inflammatory marker data, values were log transformed to allow inspection of the data and investigate any linear relationship. For each CT outcome, associations were described as to whether each subscore (bronchial dilatation, air trapping and total score) was above zero as a categorical variable (yes/no), and also as a continuous variable so the

extent of abnormality for each subscore could be investigated. Table 6.5 shows univariable linear regression analysis of one year CT and BAL results to predict each preschool lung function primary outcome measure. There were no significant associations between BAL inflammatory markers, including free neutrophil elastase activity, and preschool lung function. For CT, the presence of air trapping at one year was significantly associated with both LCI and s*R*tot z-scores, and air trapping score (the extent of air trapping) highly significantly associated with LCI z-score, with mean regression coefficient [95% CI] 0.72 [0.40;1.01], p<0.001. For every one point increase in air trapping score at one year, preschool LCI is expected to increase on average by 0.72 z-scores. Total CT score was also significantly associated with preschool LCI z-score, mean regression coefficient [95% CI] 0.33 [0.18;0.47].

Table 6.5: Univariable regression analysis of computed tomography score and inflammatory markers with preschool lung function primary outcomes

	PS z-LCI	PS z-s*R*tot	PS z-$FEV_{0.75}$
CT markers	n=47	n=47	n=46
Bronchial dilatation (Y/N)	0.39 [-0.85;1.63]	-0.05 [-0.76;0.67]	-0.13 [-0.89;0.64]
Bronchial dilatation score	0.72 [-0.16;1.60]	-0.25 [-0.76;0.26]	-0.16 [-0.70;0.39]
Air trapping (Y/N)	1.57 [0.55;2.60]**	0.77 [0.16;1.37]*	-0.12 [-0.79;0.56]
Air trapping score	0.72 [0.40;1.01]***	0.21 [-0.06;0.42]	-0.12 [-0.35;0.10]
Any abnormality (Y/N)	1.03 [-0.50;2.57]	-0.37 [-1.26;0.53]	0.40 [-0.52;1.33]
Total score	0.33 [0.18;0.47]***	0.06 [-0.04;0.16]	-0.08 [-0.18;0.02]
BAL markers	n=32	n=31	n=31
IL6	0.00 [-0.90;0.91]	-0.16 [-0.66;0.34]	-0.39 [-0.98;0.20]
IL8	-0.39 [-1.21;0.43]	-0.40 [-0.85;0.05]	-0.08 [-0.63,0.47]
IL10	0.10 [-0.96;1.15]	0.04 [-0.49;0.58]	-0.42 [-1.16;0.31]
TNF	-0.06 [-0.98;0.86]	-0.24 [-0.75;0.26]	-0.15 [-0.74,0.43]
MPC	0.43 [-0.67;1.54]	-0.37 [-0.97;0.22]	-0.24 [-0.97;0.49]
Neutrophil elastase (NE)	-0.43 [-1.7;0.88]	-0.61 [-1.4,0.19]	-0.11 [-0.94;0.72]
Detectable free NE (Y/N)	0.12 [-1.35;1.59]	-0.60 [-1.39,0.19]	-0.28 [-1.21;0.67]

*Legend: Results are presented as mean regression coefficient [95% CI]. **represents p<0.01 and *** p<0.001. Computed tomography parameters investigated as presence or absence (Y/N) and extent (continuous score). Inflammatory marker coefficients and 95% CI presented as log_{10} transformed data. Abbreviations: n=number of subjects, PS=preschool, CT=computed tomography, BAL=broncho-alveolar lavage, IL=interleukin; TNFα=tumour necrosis factor alpha; MCP-1=monocyte chemoattractant protein-1; ng=nanograms; pg=picograms; ml=millilitres.*

Significant associations on univariable regression analysis were seen between preschool lung clearance index z-score and air trapping on computed tomography at one year of age. Total computed tomography score predicted preschool lung clearance index z-score, and air trapping was also associated with preschool specific airway resistance z-score

6.4.4 Evaluation of binary classifiers to predict preschool lung clearance index

In addition to regression analysis, an evaluation of binary classifiers can be used for CT and BAL outcomes in infancy to predict lung function at preschool age. As LCI z-score was the most sensitive marker of abnormality in the 67 children with CF tested in the preschool years, it was chosen as the outcome variable for sensitivity analysis. In children with CF, CT outcomes (presence of bronchiectasis, air trapping, abnormal total CT score and any abnormality on CT) and BAL outcomes (presence of neutrophil elastase) were classified as abnormal predictor variables. Preschool LCI >1.98 z-scores was categorised as abnormal. Abnormal total CT score had the highest positive predictive value (100%) for preschool LCI z-score, but negative predictive value of 32%. Presence of air trapping had a 50% positive predictive value for abnormal preschool LCI, and negative predictive value of 78%.

Table 6.6: Positive and negative predictive values of CT score and neutrophil elastase for preschool lung clearance index

Variable	Positive predictive value	Negative predictive value
Bronchiectasis present	38%	67%
Air trapping present	50%	78%
Abnormal total CT score	100%	32%
Any CT abnormality	35%	71%
Presence of neutrophil elastase	29%	75%

Legend: Positive and negative predictive values of CT and BAL measures for abnormal preschool lung clearance index z-score

6.5 Discussion

6.5.1 Review of hypotheses

In relation to BAL inflammatory markers, the hypotheses was:

1. *"Inflammatory markers from BAL at one year cannot predict preschool lung function (LCI, sRtot and FEV0.75) at three to six years of age"*

This hypothesis was proven as there was no association between any BAL inflammatory markers (including neutrophil elastase concentration or detectable free neutrophil elastase activity) with any of the preschool lung function primary outcome measures (LCI, s*R*tot or $FEV_{0.75}$ z-score).

In relation to CT score, the hypothesis was:

1. *"Abnormalities on CT (air trapping, bronchial dilatation and total CT score) cannot predict preschool lung function"*

The results disproved this hypothesis as significant associations were found between air trapping and total CT score as predictors for preschool LCI z-score. No associations were found between CT outcomes and s*R*tot or $FEV_{0.75}$ z-scores.

6.5.2 Comparison of this study to other reports

As was true for lung function, only minor abnormalities were seen in CT and BAL markers at one year in NBS infants with CF. This is further evidence that in this cohort, children are relatively well during infancy as defined by several different methods of disease monitoring. It is not surprising that there were few associations between these minor abnormalities to predict lung function at preschool age. However, despite this, air trapping and total CT score on one year CT showed significant associations with preschool lung function in this study. The association between the presence and extent of air trapping at one year CT and preschool LCI z-score is interesting as both markers reflect the early abnormalities seen in CF lung disease of small airways with

mucus plugging and obstruction[24]. In this sense, there may be a signal of initial subtle abnormalities leading to a deficit in function later in life, only detected by a marker sensitive to early disease such as LCI. Alternatively, both measures may detect, for example, small amounts of mucus in the airways, but it is not possible to ascertain which is true from this data. Follow up of these children to school age and adulthood will determine whether this relates to later structural or functional abnormality, or worse disease course.

The AREST-CF group reported cross sectional correlation of LCI and HRCT findings from infancy to school age[78]. No association was found in infancy between LCI and PRAGMA-CF scored CT, but by preschool and school age a correlation was found between LCI and total disease extent on CT, which included air trapping score. Although not yet published, it would be interesting to see whether any structural abnormality, such as air trapping in infancy, is also associated with later deficits in function in the AREST cohort, as seen in the subjects described in this thesis. It is likely that intervention studies in the very young require long term end points to determine treatment effects, such as school age spirometry, and this will be discussed in more detail in Chapter 7.

The AREST-CF group found a strong association between BAL neutrophil elastase and CT abnormalities; presence of neutrophil elastase at three months was associated with a higher chance of persistent airway dilatation of seven fold by a year and four fold by three years[135]. In the current study, no association was found between one year neutrophil elastase (or any other inflammatory marker) and preschool lung function. However the limited number of infants with any elevation in inflammatory markers reduces the power of this study to determine any significant relationship. The children that did have inflammatory marker data at one year had an overall lower mean LCI z-score than the full cohort (1.69 compared to 2.14), and are likely to represent those with milder disease. It is therefore difficult to draw any solid conclusions regarding BAL biomarkers and lung function in the LCFC cohort.

Earlier reports from the AREST-CF group describe bronchial dilatation in 32% and air trapping in 69% of NBS infants with CF at one year[135] using a locally derived CT score, comparable to bronchial dilatation (26%) and air trapping (42%) in the full cohort of the LCFC at one year using the Brody II score[128]. Since these publications, a quantitative score has been developed and used in the AREST-CF studies, (PRAGMA-CF)[99], which allows expression of the 'extent' of each CT finding as a percentage of the lung. A more recent publication using this scoring system reports bronchial dilatation present in 20% and air trapping in 58% in infants aged three months to two years[78]. The 'extent'

of bronchiectasis was only 0.1% and air trapping 1.3%, so despite a high proportion having abnormality on CT, the degree of this is mild, as found in the LCFC cohort. It is also important to note that some of the CT abnormalities were found to regress in the AREST-CF series, so a single or three slice scan may miss those with intermittent changes (of which the importance is unknown), or identify a subject with an abnormality that may resolve.

6.5.3 Strengths and limitations of the study

There was a relatively high rate of follow up of those with one year CT and preschool lung function, and there were no significant differences in lung function between those with and without CT data. CT scans were performed using a standardised imaging protocol with carefully controlled ventilation and were scored in duplicate by experienced scorers, and BAL markers processed in a research laboratory with skilled knowledge of current techniques.

The AREST-CF group have reported cross-sectional associations between LCI and CT score, infection and inflammatory markers[78, 130, 134], longitudinal lung structure and inflammation[135], and how infection in infancy relates to school-age spirometry[133]. This is the first study to report how markers of lung structure and inflammation in infancy can predict lung function in the preschool years.

Limitations of this study were that not all subjects in the infant cohort underwent CT and BAL, and not all subjects with CT and BAL had lung function data at preschool age, due to loss to follow up or unsuccessful preschool lung function tests. This led to a reduction in the number of subjects to investigate associations between one year CT (48 subjects) and inflammatory markers (33 subjects) with preschool lung function. Inflammatory marker data was limited further due to the processing error of supernatant. The AREST-CF publications report BAL and CT data in up to 127 children from diagnosis to three years of age[135], surpassing the numbers of the LCFC and resulting in a better powered study.

As described in detail by Thia et al.[94] agreement between the two CT scorers was only reproducible for three of the seven scoring categories in the Brody II CF-CT scoring system. Therefore only three measures (bronchial dilatation, air trapping and total CT score) were able to be used as primary outcomes, and to investigate associations with preschool lung function. The Brody II score was designed to describe abnormalities in

CF lung disease, but as changes are very mild in young subjects this may explain why agreement between scorers was not as strong as expected. This study was designed as an exploratory analysis of associations between one year lung structure and inflammation with preschool lung function. There were very few subjects with either abnormal CT or inflammatory marker data, therefore the chance of finding any true association with preschool lung function was relatively low.

6.5.4 Meaning of the study, unanswered questions and future research

In this study, no associations was found between BAL inflammatory markers at one year and preschool lung function. However number of subjects with BAL data were reduced due to a sample processing error, and those subjects overall had a lower mean LCI z-score than the full cohort. Neutrophil elastase may still be a useful marker in infancy, as a strong association with later bronchiectasis was found in the AREST-CF reports[135], and a future report of the ability of infant BAL inflammatory markers to predict preschool lung function in their cohort is key to clarify this relationship. Furthermore, with the wealth of data in the AREST-CF cohort, it would be interesting to investigate whether similar relationships between air trapping and preschool LCI are also found in their subjects, as shown in the LCFC cohort described in this chapter. With only minor abnormalities seen in any marker at one year, interventional studies using these outcome measures should be aimed at children in later life where changes are more clearly detected.

7 Discussion

7.1 Summary of principal findings

The overall aim of this thesis was to describe the evolution of lung function from diagnosis to six years of age in a NBS cohort of children with CF managed with standard UK care. This was in order to determine whether lung function remained stable with standard treatment to the preschool years. It serves to inform two crucial questions in children of this age group; whether new treatments for CF could be deferred to a later age, and whether interventional studies of CF treatment in young children using lung function outcomes should be powered by results in NBS children, rather than the much larger number who were clinically diagnosed. Furthermore, the relationship between infant clinical, structural, functional, inflammatory and infection markers with preschool lung function was explored, to investigate whether a subgroup of children could be potentially identified for enrolment in such studies, or for more intensive therapy. To address these questions, the primary and secondary hypotheses addressed in Chapter 1 were as follows:

Primary hypotheses:

1. NBS children with CF managed with standard UK care will have stable lung function at three to six years of age, which will be similar to contemporaneous healthy controls

Secondary hypotheses:

1. NBS children will have better lung function than preschool children with a clinical diagnosis of CF born a decade earlier

2. Infant lung function measures and nutritional markers cannot predict preschool lung function in NBS children with CF

3. Clinical markers of infection (isolation of bacteria on respiratory samples), and markers of disease severity (for example courses of intravenous antibiotics) in infancy cannot predict preschool lung function

4. Inflammatory markers measured in BAL in infancy cannot predict lung function at three to six years of age

5. Abnormalities on CT (air trapping, bronchial dilatation and total CT score) measured in infancy cannot predict preschool lung function

The principal findings of this thesis in addressing the primary and secondary hypotheses were:

- Contrary to primary hypothesis 1, the stability or improvement of lung function seen in infancy in LCFC NBS children with CF at two years was not completely maintained at three to six years of age. This was shown by LCI z-score which was outside the normal range at preschool testing. The other preschool lung function primary outcomes (s*R*tot and $FEV_{0.75}$), whilst significantly poorer than controls, remained within the normal range

- In agreement with secondary hypothesis 1, NBS preschool children had significantly better lung function than those clinically diagnosed with CF born a decade earlier for all of the lung function primary outcomes

- Contrary to secondary hypothesis 2, a significant association was found between two year LCI z-score and preschool LCI and s*R*tot z-score

- No association was found between infant lung function, nutritional measures, clinical markers or BAL inflammatory markers and preschool lung function in agreement with secondary hypotheses 2, 3 and 4

- Contrary to secondary hypothesis 5, a significant association was found between air trapping and total score on one year CT and preschool LCI z-score

7.2 Strengths and limitations of the study

7.2.1 Strengths

The main strengths of the study were that lung function measurements were made by an experienced team and in the same lung function laboratory. There was follow up of both children with CF and contemporaneous controls over a long time period. This was also true in cohort 1 diagnosed ten years earlier, with no identifiable differences in lung function measurement methodology over this time period. The vast majority of lung function data were collected by the author and two research assistants, but other experienced researchers at the UCL Great Ormond Street Institute of Child Health lung function laboratory were available to over-read data, and the resulting meticulous quality control was a major strength of this study.

There was a good retention rate of preschool children tested as infants, and the subject numbers met criteria to adequately power the study for the three lung function outcome measures. Where available, international standards and guidelines for lung function testing were followed. Serial measurements in the same children with a relatively short time period between each test occasion resulted in good data capture showing how lung function evolves in this population. The tests (MBW, sR_{aw} and spirometry) were performed in the same order in cohort 2 as in cohort 1 to ensure the two studies were comparable. The employment of three lung function outcomes minimised the chance of missing any abnormality when assessing the underlying physiology in preschool children with CF. The importance of this was illustrated in an earlier LCFC report of cohort 1 undergoing school age CT and LCI[126], where of nine children with normal LCI, five children had abnormal CT, and of nine with normal CT, five had abnormal LCI. Children were also tested when well and clinically stable in an attempt to eliminate the effect of a pulmonary exacerbation on lung function results in subjects with CF.

The cooperation of the LCFC enabled accurate records of background characteristics, clinical status and infection to be collected and adherence to standardised treatment protocols. The database of infant lung function, structure and BAL markers enabled detailed investigation of significant predictors of preschool lung function.

A further strength was recruitment of contemporaneous healthy controls who were well matched to subjects with CF. This was particularly important when it was observed that controls in cohort 2 had slightly higher values of LCI z-score than expected, and their inclusion allowed the difference between subjects with CF and controls to be

expressed. The importance of including a control group has been well documented[152], and illustrated by the lack of control subjects in the AREST-CF study of lung function in infants leading to difficult interpretation of forced expiratory volumes in their cohort[41]. Expressing results as z-scores means that a comparison can be made to other studies using the same equipment and software as used to generate the z-score data. However in the publication of LCI z-scores used in this thesis[75], differences were still seen between international centres collecting healthy control data, and there are therefore still some limitations when relying on z-scores alone. Addressing these international differences is important, but out of the scope of this thesis.

7.2.2 Limitations

7.2.2.1 Study design

The cohort 2 preschool follow up study was significantly powered to show a difference between children with CF and healthy controls. However, when investigating predictors of preschool lung function, larger numbers would have increased the power of the subgroup analyses. Nearly all of the infants diagnosed with CF in London during the infant study recruitment period were enrolled in the study. A wider study in the UK would have been time-intensive, travel for families from outside London problematic, and clinical data collection more difficult.

Although the overall feasibility of lung function tests was high in the preschool age group, there was a difference in success rates between lung function tests; 91% for MBW, 82% for specific airway resistance and 77% for spirometry. A difference was also seen between age groups and, as expected, older children had higher success rates than younger children. Only 56% of subjects less than three years of age completed the full study protocol on their first visit, 54% between four and five years, but all subjects over five years completed all three tests. For MBW, 89% of children under three obtained valid data, rising to 90% between four and five years and 100% over five years. This is an important consideration when designing trials in preschool children; particularly if interventions are to be targeted in the younger age group, as it may not be possible to obtain data in all subjects enrolled.

Recruitment in the preschool follow up of cohort 2 was targeted to those with infant lung function, structure and inflammatory marker data, in order to investigate the relationship between these outcomes and preschool lung function. Families had been recruited during the infant phase of the LCFC study and consented to participation in the follow up study. However, there was likely a larger population of preschool children at each centre that could have attended for lung function testing, and been recruited to increase the power of the study. Although they would not have infant data, more information would be gained for preschool pulmonary function, and the results of such a study would be a more accurate reflection of preschool patients, and relevant to the whole clinic population. The ethical approval of the study did not include examination of records of those who had been approached but declined to participate in the study, and did not allow recruitment of new subjects.

The choice of lung function outcome measures was discussed in section 1.5.1. Ideally the outcomes in this study would be compared to a 'gold standard' test that defines disease. As is true for most respiratory diseases, no such standard exists for CF. A comparison to structural changes on CT or markers of inflammation on BAL were not therefore possible in the LCFC cohort. The AREST-CF group perform all three of these measures annually as part of their surveillance protocol. In a report from this cohort in preschool and school-age children, LCI had a positive predictive value (83-86%) but poor negative predictive value (50-55%) to detect the presence of bronchial dilatation on CT performed at the same time using the PRAGMA-CF score[78]. This study was reported after data collection for the current LCFC preschool study had been completed. The decision not to include measures of structure and inflammation in the current study protocol was in view of the fact that only mild and poorly reproducible CT changes were seen in the LCFC cohort at one year, and lung function was stable at two years of age. The Collaboration unanimously felt that CT could not be justified, and would have led to a more arduous study visit, or multiple visits for the child and their family, and potential subsequent attrition of subjects. Practically, BAL and CT are not performed as part of routine clinical surveillance in children with CF in London. Inclusion of these measures would mean exposure to radiation, and a general anaesthetic, and could therefore not be justified in order to solely compare to lung function results. However, as LCI was unexpectedly abnormal by preschool age, structural abnormalities could also have been more prominent, and CT could potentially be a useful outcome measure in further studies of NBS preschool children with CF. Inclusion of CT in preschool follow up of cohort 2 would also help to define any differences or similarities between the LCFC and AREST-CF cohorts and whether

results are comparable between the two CF populations. The role of CT beyond the first year of life merits further consideration in light of the present data.

7.2.2.2 Longitudinal comparison of infant and preschool lung function tests

The LCFC longitudinal study of cohort 2 was designed to describe the evolution of lung function from diagnosis of CF to preschool age. However the physiological tests that can be measured in infancy (less than two years of age) differ to those that are practical in three to six year old children. Infants were sedated and asleep for lung function tests, and underwent measurement of plethysmographic FRC. Older awake children will not tolerate breathing against a closed shutter, so it was not possible to measure this in preschool children. sR_{aw}, performed in preschool children, requires active tidal breathing and therefore cannot be measured in infancy. It was possible, however, to perform a measurement of forced expiration in both infants and preschool subjects, but different volumes are reported ($FEV_{0.5}$ versus $FEV_{0.75}$) and the technique for acquiring each test differs. The raised volume technique is used in infants, and active forced spirometry in those older than three years. Whilst similar measures, the tests are therefore not directly comparable, and it cannot be assumed that $FEV_{0.5}$ and $FEV_{0.75}$ measure the function of the same airway generations at the respective ages. MBW testing was also performed in both age groups but methodology again differs. Infants are tested when sedated in the supine position, whereas children over three are tested sitting upright. Smaller volume (and therefore dead space) masks are used in infants, but a mask interface was used for both age groups. It has been clearly documented that LCI is higher (and FRC lower) in the supine compared to sitting position[153, 154], and that LCI measured supine correlates better with CT outcomes that when measured sitting[153]. These differences are important to consider when interpreting changes in LCI between infants and preschool children, or relating abnormalities in infancy of both MBW and CT outcomes to preschool LCI measured in the upright position.

7.2.2.3 Blinding of lung function results

As part of the cohort 2 preschool study, clinicians treating children with CF at the respective centres were sent a report with the results of pulmonary function tests, and whether any result was abnormal. Therefore the treating clinician was not blinded to

the results of testing. Although all centres in the study followed a common treatment protocol, this may have been adjusted in light of an earlier lung function report and confound results in certain patients. When the longitudinal design of the study is considered; treatment change in response to an abnormal infant test may have influenced the preschool results. As shown in chapters 4 and 5 and previous LCFC reports[129], lung function was normal at two years, abnormal in preschool children but much improved in cohort 2 when compared to cohort 1. It is still unclear whether this was due to the introduction of screening, rapid progression of lung function from two to preschool age, or more intensive monitoring such as pulmonary function tests.

7.2.2.4 Loss of subjects to follow up

The recruitment accrual for the NBS preschool cohort 2 study is summarised in Figure 3.1. 67 children with CF had preschool lung function data out of 92 tested as infants, and 41 healthy controls out of 62. Of particular interest were children with CF who withdrew from the study. Some of the families listed time constraints, or that their child was too anxious or did not want to participate. It may have been that these children had more frequent visits to hospital because they were more unwell, and their parents wanted to avoid additional tests. Another important group were children who were too unwell for testing (eight subjects with CF) and therefore met exclusion criteria despite multiple attempts at recruitment. Again, these children are likely to have poor lung function if frequently unwell, but it was not possible to include their results in the study. If a further follow up study at school age is planned, if willing it would be important to include these children despite lack of preschool lung function data, to get a representative sample of the full cohort.

7.2.2.5 Collection of clinical data

Clinical data such as bacterial isolates, courses of intravenous antibiotics and medical treatments were recorded in this study to investigate associations with preschool lung function. The information collected was targeted to those known to influence respiratory disease in CF. Data collected reflected reported therapies, but the study was not designed to measure adherence. Although data collection from each centre was as comprehensive as possible, it was recorded retrospectively (an acknowledged weakness of the study) from clinical records held at each centre, and cross checked

with annual assessment summaries and, if available, local databases. In the infant study, clinical record forms were used at every clinical encounter (~2-3 monthly outpatient clinic visits). The difficulties with collecting such information in busy clinics, and incomplete records was apparent in the LCFC infant study[155]. It would have been preferable to continue to collect accurate information at each clinical visit up to preschool test occasion, but this was not possible due to time constraints in clinics, the vast amount of data that would have to be summarised, and concerns over incomplete recording of data without dedicated research staff (which would fall outside the preschool study period). Ideally, longitudinal clinical data would have been collected prospectively at each clinic visit for children enrolled in the study and stored in a database for analysis.

This was a multicentre study, and all tertiary centres participating in LCFC studies managed patients according to an agreed clinical protocol. It was not possible however to guarantee complete adherence to this protocol and therefore there may have been some variation in treatment of certain subjects that could potentially influence preschool lung function results.

Although the majority of care for CF subjects was provided by their specialist CF centre, children also had access to medical care via a 'local' hospital closest to their home for emergencies, and a general practitioner. Whilst most cough swab or sputum samples were taken and processed when children attended clinic at their specialist centres, when acutely unwell and if more convenient for the family to deliver them to their local hospital or general practitioner, processing may have taken place there. Communication between local and specialist centres around positive bacterial isolates is likely to be good, and recorded in the specialist centre records, but this was another potential source of loss of data. Furthermore, the analysis of microbiological samples in non-specialised laboratories may have resulted in species misidentification, another source of error.

Routine surveillance for respiratory pathogens for subjects in this study was by collection of OPS in children who are either not productive of, or cannot expectorate, sputum (the majority of infants and preschool children), with the exception of BAL collected at one year. A recent study compared oropharyngeal swabs, induced sputum (with hypertonic saline) and BAL samples in children with CF[109]. This study reported a much higher rate of pathogen identification using sputum induction and BAL compared to oropharyngeal swabs. The AREST-CF group perform BAL at diagnosis and

annually for subjects in their cohort, and therefore a higher rate of detection of pathogens is likely when compared to identification by oropharyngeal swabs used in the LCFC study. However, studies have shown significant inter-lobar differences in BAL fluid in children with CF, particularly bacterial distribution[156], and six lobe lavage superior to one or two lobe BAL for the identification of pathogens[157].

7.2.2.6 Extrapolation of results to other cohorts

LCI z-score was the only lung function outcome to be abnormal at preschool age in this study. MBW data were collected using custom made equipment, using SF_6 as the tracer gas and a mass spectrometer for detection of gas concentrations. There is currently much interest in using LCI in clinical monitoring and as an outcome measure in clinical trials, which becomes increasingly possible when using commercial devices. Care must be taken when comparing the results of this study with data collected in other cohorts using different methodological approaches, as the reference values are tracer gas and device specific.

78% of children had a positive isolate for *Pseudomonas aeruginosa* by preschool test in this study. This high rate was seen despite the majority of samples collected by OPS, apart from one year BAL. It is routine in the UK and part of the shared LCFC centre protocol to start prophylactic antistaphylococcal antibiotics from diagnosis until at least two years of age. It is possible that the high rate of *Pseudomonas aeruginosa* could be secondary to such treatment protocols, and this is currently being tested in the START-CF trial (http://www.cfstart.org.uk). Therefore extrapolation of results to cohorts not employing routine antistaphylococcal prophylaxis must be done cautiously, as with any other discrepancy in standard clinical care.

In chapter 4, no difference was noted in height, weight or BMI z-score between children with CF in cohorts 1 and 2. This was unexpected as other studies, in particular the Wisconsin randomised trial of NBS[118] showed nutritional benefit in screened compared to unscreened children with CF. As poor nutrition can be associated with deficits in lung function, the abnormalities in anthropometric parameters of children in this study must be considered when comparing to other cohorts with better (or worse) nutritional outcomes.

7.3 Meaning of the study

7.3.1 Abnormal lung function in NBS preschool children

Previous reports of the children described in this study showed lung function was in the normal range at two years of age in the LCFC cohort of NBS infants. In this study, LCI was the only lung function outcome measured at preschool age to show deterioration, and by this time was outside of the normal range. LCI was also the most sensitive outcome to abnormality, with 39% of children with CF having an abnormal result, compared with only 7% abnormal s*R*tot and 11% abnormal $FEV_{0.75}$. This was in agreement with other reports of preschool NBS children; although using N_2 washout as opposed to SF_6 washout in this study, the AREST-CF group report 55% of preschool children had an abnormal LCI[134] and an even higher proportion in a Canadian study (66%)[144].

It was discussed in section 1.3.1.2 how outcomes from MBW testing are more sensitive to early lung disease than spirometry. Non-uniform airway disease results from mucus obstruction, inflammation and airway remodelling, reflected in inhomogeneous ventilation and therefore abnormal LCI. It is clear that these processes are likely to be present in the preschool population of CF subjects in this study. Nearly all children with an abnormal s*R*tot or $FEV_{0.75}$ z-score also had an abnormal LCI z-score (4/5 and 5/7 respectively); giving evidence that LCI is the most useful measure in this age group but other lung function tests still offer valuable information. The feasibility of MBW, in agreement with other reports, was high in this study, with a valid result being obtained from 91% of children on their first test occasion.

The use of MBW as a clinical monitoring tool should be explored further in preschool children with CF, but only long term data will show if acting on an abnormal LCI has any effect on later outcomes. Commercial devices using N_2 washout are more portable than the mass spectrometer and O_2, the tracer gas used, is readily available. Therefore monitoring, if indicated, could be implemented in routine clinical management. In the cohort 1 LCFC studies, LCI was shown to predict later decline; with a strong correlation with school age MBW and spirometry[51], and to abnormalities on school age CT[126]. However, this study used SF_6 as a tracer gas and cannot be directly applied to LCI measured by N_2 washout devices. Chapters 3 and 4 showed that, although improved from their counterparts diagnosed ten years earlier, LCI was still abnormal in the NBS cohort 2. Further follow up of cohort 2 will determine whether

preschool LCI has the same predictive ability as that shown in cohort 1, but it is likely that a similar correlation will be seen, just with fewer abnormal subjects at school age follow up.

It is yet to be defined exactly what constitutes a clinically meaningful change in LCI, and whether acting on that change has long term benefits in CF lung disease. A recent report investigating how LCI can monitor treatment response in preschool children during a CF pulmonary exacerbation[158] gives important information to this point. LCI was seen to deteriorate with symptoms of an exacerbation relative to baseline, and a significant treatment effect with antibiotic therapy was seen using LCI as the outcome measure, but not in FEV_1. Further studies will give more insight on how changes in LCI can help guide clinical decision in preschool children, but an abnormal result along with pulmonary symptoms in this population should prompt closer monitoring and consideration of a change in treatment in routine clinical care.

7.3.2 Interventional trials in preschool children with CF

There is still much debate as to whether LCI can be used as a robust outcome measure in clinical trials of young children with CF. The European CF Society Clinical Trial Network Standardisation Committee advocated LCI as an outcome measure in those with mild CF disease[159], but a Cystic Fibrosis Foundation Workshop Report did not encourage the use of LCI in clinical trials due to gaps in knowledge of the appropriate tracer gas, equipment and standardisation across systems[160]. Several trials have been performed to date using LCI as an outcome measure in children with CF, but few incorporated preschool children or infants. Trials investigating modulator therapies and hypertonic saline have recruited children over six years of age. A double blind crossover study of ivacaftor and placebo in patients with at least one p.Gly551Asp -*CFTR* mutation and normal FEV_1 showed a significant improvement in LCI compared to placebo (mean change in LCI from baseline [95% CI] was -2·16 [-2·88 to -1·44], p<0.0001)[161]. A further study investigated the combination of ivacaftor/lumacaftor in children over six years using LCI as an outcome measure (which had only shown a small albeit significant improvement in FEV_1 in those over 12 years of age[148]). LCI improved on average [95% CI] by -0.88 units [-1.40 to -0.37], p=0.001[162]. A third crossover trial comparing isotonic and hypertonic saline reported improved mean LCI in the hypertonic saline group by 1.16 units [95% CI 0.26 to 2.05], p=0.016, again in children over six years[81]. In these reports, no significant change in spirometry

outcomes were seen, and using a sensitive measure of abnormality in young children, such as LCI, allows a much greater power of the study. Of note, currently the FDA has approved modulator therapy in very young children on demonstrating safety and reduction of sweat chloride as a biomarker, without mandating a direct demonstration of efficacy[163]. It is possible from the data reported here to speculate that in fact efficacy may be directly measurable from the slope of rise in LCI, even in very young children.

Two studies to date report a treatment effect in infants and preschool children. The first is a randomised controlled trial of isotonic versus hypertonic saline in children of median age (range) 2.6 years (0.34-4.95). LCI z-score remained stable in the isotonic saline group at 0.81 (95% CI -0.40 to 2.02) but was decreased on average by 1.19 z-scores (95% CI -2.46 to 0.06) in the hypertonic saline group. A significant treatment effect was reported for LCI z-score of 2.01 (95% CI 0.26 to 3.76), p=0.02. However numbers were small in each group (13 and 12 in each arm respectively). An early report of the GOAL (G551D Observational Study) in children aged three to five years has been published recently; nine subjects with CF treated with ivacaftor undergoing serial measurements of LCI at baseline, one month and six months[164]. A 24% improvement from baseline at one month and 25% at six months was seen (exceeding the between test reproducibility of 15% in preschool children[165]).

Although the studies listed above used different equipment, the data presented in this thesis show there is a signal of abnormality in NBS preschool children managed with standard UK CF care. Therefore this work supports the inclusion of UK preschool children with CF as subjects in interventional trials if LCI is used as an outcome measure. A significant treatment effect, if present, could be detected by change in LCI in an appropriately powered study.

In chapter 3, it was estimated that 60 children with CF and 40 controls would detect a difference in SD score of 0.7 (or greater) in the primary lung function outcomes (LCI, sRtot and $FEV_{0.75}$) with 80% power at the 5% significance level. Results in this cohort for sRtot and $FEV_{0.75}$ z-score were within the normal range. If the power calculation is performed using only LCI z-score as the outcome measure, from these data only 33 subjects per group would be needed to reach the same power and significance. If undertaken solely in the UK, a multi-centre study of preschool children would likely be needed to enrol a sufficient number of subjects, but is practically possible. This, along with the high feasibility of MBW in this age group, gives further evidence for the inclusion of preschool children in interventional trials.

Lung clearance index has the potential to be an effective patient-centered outcome. It is a safe, non-invasive test that can be undertaken in an outpatient setting[166]. The high feasibility in preschool children shows it is well tolerated. Lung function monitoring is a common practice for children with CF and their families. A raised LCI is relatively easy to understand for families as a measure of early disease that may not be apparent with symptoms or clinical signs. If it is shown to detect early disease and predict later decline in patients with CF, it can enable families and clinicians to choose a more intensive treatment course, or encourage them to keep up with recommended therapies. When longer term consequences of a raised LCI are known, and acting on an abnormal result can improve outcome, it can reduce delays in patient care that would be harmful to their wellbeing otherwise.

7.3.3 Predicting preschool lung function and optimal timing of new therapies

The LCFC study was designed longitudinally to describe the evolution of lung function in children with CF that we see and treat today. Chapters 5 and 6 reported the relationship between measures in infancy (lung function, infection, inflammation and structure) and preschool lung function. The study showed that lung function changes in NBS infants are mild and short-lived until preschool age when LCI gives a sensitive signal of abnormality. No tests of lung function in infancy were significant predictors of preschool lung function, with the exception of two year LCI z-score. The fact that most infant measures do not predict preschool outcomes is not unexpected considering the transient abnormalities of function seen in NBS infants earlier in the study.

For the whole group, there was a progressive deterioration in LCI z-score with each test from diagnosis when compared to healthy controls; the mean difference was 0.32 z-scores at three months, 0.73 z-scores at one year, 0.89 z-scores at two years and 1.47 at preschool test. This is the first study to report the progressive decline of LCI from infancy to preschool age when compared to healthy controls. The strong association between two year and preschool LCI (and s*R*tot) is an important one. Although two year LCI is on average normal in cohort 2 subjects with CF, it can predict those who show further decline by the preschool years. Closer analysis of the RCT of hypertonic saline in children under six years[82] show that in subjects less than one year,

LCI was normal at baseline and did not change after treatment, all though numbers were small. Of concern is that by two years of age pathophysiological processes are apparent in some subjects who continue to deteriorate into later life. The data in this thesis suggest that the causative factors for later decline occur before two years of age. Therefore trials of treatments in young children with CF could be targeted before two years to preserve lung function. It is also proposed that the optimal timing of starting new treatments, such as modulator therapies that can potentially correct CFTR defects, is before children reach two years of age.

7.4 Unanswered questions and future research

This longitudinal study reported new data of the pulmonary status of NBS preschool children with CF, and explored which measures in infancy could potentially predict later lung function. This was clearly impaired on preschool follow up as shown by abnormal LCI z-score. The most important unanswered question from this data is what implication abnormal tests have on the progression of respiratory disease in children from this cohort. The need for long-term follow up is clear. The natural history of, and relationship between, infant and preschool measures of lung function, structure and disease course in later child- and adult-hood are crucial to define whether early interventions can prevent later decline. Tracking of lung function has been shown in cohort 1 of the LCFC study; with preschool LCI having a positive predictive value of 95% for any abnormal school age lung function result[51]. Nearly all subjects with abnormal preschool LCI went on to have abnormal school age lung function. Preschool LCI was also highly correlated with structural changes on CT at school age in cohort 1. Whether these relationships are also seen in cohort 2 remains to be defined, but if they are, this makes the significance of these early changes even greater.

This study showed an important association between two year and preschool LCI, suggesting that pulmonary insults occur in early infancy and have a significant later impact on lung function. Therefore there is a persuasive argument for targeting new therapies and trials of current treatment in the first two years of life. Sensitive measures of abnormality need to be employed; the lung function data in this study show that LCI is likely to be the most useful outcome measure at least in preschool children. As MBW is a non-invasive and inexpensive test, many CF centres now have access to commercial devices and are introducing regular monitoring into their clinics. Although CT was not performed at preschool age in the children described in this

thesis, the AREST-CF data show that structural change is present from an early age and progresses[135]. Quantitative CT[167] and new techniques in MRI[168] are being reported and outcomes from these imaging modalities could serve as end-points that do not involve radiation of the developing lung. Potential strategies in the care of children with CF in the future will therefore include studying current treatments and CFTR modulators with these outcome measures when introduced soon after diagnosis.

Both the LCFC and AREST-CF cohorts have started to define important predictors of disease progression in children under six years of age with CF. There are, however, many unanswered questions of the interplay between lung structure, function, infection and inflammation and their ability to predict an individual's risk of later disease. Clinical research in CF at present is heavily weighted towards disease modulators, and this is justified by the potential to modify disease from diagnosis. However the cost of these potentially life long agents is high, and long term side effects unknown. Also, side-effects in very young children may be unpredictable, as shown by the increased prevalence of liver function abnormalities in preschool children treated with ivacaftor[169]. Further observational studies and long term follow-up in children under six years of age will further define predictors of later disease, and are crucial to identify those who will benefit the most from early therapy.

7.5 Conclusion

NBS in children with CF lends the opportunity to identify and ideally prevent decline in pulmonary health. The overall aim of this thesis was to describe the evolution of lung function from diagnosis to the preschool years in a NBS cohort of children. Secondary aims were to investigate which measures in infancy can predict pulmonary function in preschool children. It can now be concluded that despite normal lung function at two years of age, children in this cohort showed a significant decline in the preschool years. As seen in clinically diagnosed children, although improved from those diagnosed ten years earlier, LCI remained the most sensitive marker of abnormality in those NBS. When considering the evolution of lung function, LCI at two years of age was the most significant predictor of preschool lung function. These findings therefore suggest that interventional trials of current and new therapies can utilise LCI as a marker to define significant treatment effects in preschool children managed with standard UK care. By two years of age, a signal exists for future decline, and therefore the optimal timing for introduction of disease modifying therapies is in infancy, before two years of age.

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Appendices

A1: Carer information sheet for:

a) Carer(s) of children with CF

b) Carer(s) of healthy control children

A2: LCFC standard treatment protocol

A3: Testing day reward chart for participants

A4: Consent forms and carer testing day questionnaire for:

a) Carer(s) of children with CF

b) Carer(s) of healthy control children

A5: Clinician questionnaire

A6: Acceptability criteria and software protocol for MBW analysis

A7: Laboratory protocol for specific airway resistance testing and quality control

A1: Carer study information booklet

a) Study information leaflet for carers of children with CF

Parent Information Booklet

Great Ormond Street Hospital for Children NHS Foundation Trust

Maintaining the momentum:

Will newborn screened babies with Cystic Fibrosis still have normal lung function when they go to school?

REC Number: 12/LO/1668 Chief Investigator: Dr Paul Aurora

PART 1: Essential elements of the study

We are approaching you as a parent of a child that has been diagnosed with CF who has taken part in a previous study (NREC Number: _09H071314) to find out the best ways of detecting early lung disease in babies who have been diagnosed with cystic fibrosis (CF) through the national UK newborn screening programme.

We would like to invite you to take part in another research study to find out about how lung function progresses through the pre-school years in the same children who have been diagnosed with cystic fi-brosis (CF) through the national UK newborn screening programme. On this occasion, all tests are per-formed while your child is awake and they can eat and drink normally prior to and after the test.

Before you decide whether to take part, you need to understand why the research is being done and what it would involve for you and your child. Please take time to read the information in this document carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. If you would like your child to take part once you have understood what the project is about, we will ask you to sign a consent form. You will be given a copy of this consent form to keep.

What is the purpose of this study?

This is a follow-up study which will enable us to compare results obtained with those previously and to the results for healthy children. We will also be testing new equipment which will help other CF centres elsewhere to use the sensitive tests that we have developed in London. This study will also allow us to identify children with CF who will benefit most from intensified therapy.

Do we have to take part in this study?

You do not have to take part in this research project if you don't want to. It is up to you to decide. If you decide not to take part, this will not affect your child's general care in the CF clinic in any way. We will describe the study and go through this information sheet with you. You will then be given the sheet to take away and think about whether you would like to be involved in this study. If you would like to par-ticipate, we will the ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason. Again, this will not affect the care your child receives at the hospital in any way. Many of the tests included in the study are part of annual reviews undertaken at Great Ormond Street Hospital (GOSH). If you decide not to take part in the main study we would like you to consider whether you would be willing for us to use the results of the routine tests you have dur-ing normal clinic visits as these will also help us to understand more about CF in babies diagnosed by newborn screening.

What will we have to do if we take part?

Whether or not you take part in this study, we will see your child regularly in clinic (just as is the case normally), but if your child takes part in this study, you and your child will have to come in for an extra visit to have the tests described below. At GOSH most of the tests are part of our routine care.

We will invite you to Great Ormond St Hospital when your child is around 3-6years of age to measure his/ her lung function. On this occasion, all tests are performed while your child is awake. Each visit will take about 2 hours in total. You can take your child home as soon as the test is complete.

What are the tests in the study?

1. Breathing tests

Breathing tests for children less than 6 years old are very safe and painless. You can stay with your child while they are being done. These are highly specialised tests which can only be performed at Great Ormond Street Hospital for children. We will arrange the timing of these visits to suit your family and all travel ex-penses will be refunded.

Your child will be asked to do the breathing tests by taking deep breaths and blowing through special equipment. All tests are performed while your child is awake and they can eat and drink normally prior to and after the tests. The tests do not upset the children, who usually enjoy the process. It usually only takes about an hour to do all the tests but we generally allow two hours to ensure both you and your child are comfortable and relaxed. During the visit a research doctor will examine your child and ask a few health questions.

a. Multiple breath washout test

This test is designed to find out how evenly your child breathes and will be performed in two ways. The child breathes in a special air mixture through a face mask which contains a small amount of a gas called Sulphur Hexafluoride (or SF_6). SF_6 is 'inert' which means that it does not cross from your child's lungs into the blood stream, and has no taste or smell. The gas has been used safely at GOSH in babies and young children for the past 10 years and is used at many other specialist hospitals throughout the world. The child breathes this mixture for a few minutes so that it mixes through the lungs. After a few minutes the gas mix-ture is swapped for normal air and we measure how quickly the child breathes (or "washes") out the SF_6 from the lungs. Children with normal lungs quickly wash out SF_6 whereas children with early lung disease take longer to clear the gas from their lungs. This is similar to the test that was performed when your child was approximately 3 months, 1 year and 2 years old.

b. Airway Resistance

The second test measures how big the airways are. In order to do this the child sits in a special cubicle (which looks a bit like a public telephone box) and breathes air through a face mask. The cubicle is transparent and has a door which is closed for 2-3 minutes so that we can record the small pressure changes that occur while your child is breathing.

c. Forced expiration

This last breathing test measures how quickly your child can breathe out and also reflects airway size. Your child is asked to wear a nose clip (which looks a bit like a swimming nose clip), breathe in deeply and then breathe out as hard and fast as they can through a plastic tube which they hold in their mouth. To help them do this, we use a variety of specially made computer games. Children with lung disease cannot blow out as much air or as quickly as children with normal lungs.

2. Other investigations:

While you are at the laboratory, we will also weigh and measure your child, and ask you a few questions about your family and your child's health. We would also like to know about your views and experiences of being a part of this study. We will ask you to fill out a questionnaire once at the end of the study.

Any other measurements or results of tests which will be used for this study are all part of your child's routine care at your CF centre.

What is the questionnaire for?

It is important that we know how parents feel about being asked to take part in studies such as this. We would also like to know about your views and experiences of being a part of this study. We will ask you to fill out a questionnaire once at the end of the study.

Are there any risks or discomfort for my child?

Breathing tests: We do not believe there are any risks or discomfort associated with performing these breathing tests in children. On the contrary, experience has shown that most children of pre-school age, enjoy their visit to the lung function laboratory.

Time burden: Attending appointments for the testing sessions could cause some inconvenience as you may need to come in for an additional appointment, but we will ensure that any inconvenience is limited by booking appointments that are suitable to both you and your child where possible.

PART 2: Additional Information to be read before you decide whether to participate or not.

Why is this study important?

Children with Cystic Fibrosis are more prone to chest infections and repeated infections lead to lung damage. These infections may occur very early in life and some can go unnoticed (because the child does not have an obvious cough or other symptoms). It is very important that the effects of such infections are detected and treated rapidly, using tests like the ones in this study, to prevent irreversible damage to the lungs which may limit the child's physical ability and lifestyle.

Who will this study help?

We cannot promise that participating in this study will help your child specifically, but the information we obtain will help improve the treatment of all children born with CF in the future. It will help us to understand more about CF lung disease in the first few years of life, and which test or combination of tests is likely to be most useful in detecting early changes in the lungs.

For your child specifically, the advantages of taking part in the study are that s/he will be monitored very closely throughout the period of the study both by her/his specialist CF team and by the research team. Your child will also have the opportunity to have specialised assessments such as pre-school lung function (breathing) tests. Such tests are not widely available yet, but have been shown to be accurate and reliable in monitoring lung growth and identifying early problems, which can then be treated more promptly. If any problems are picked up as a result of the tests you will be informed and your child's treatment changed if and as necessary.

Results from all these tests will be sent to your consultant who will then be able to discuss them with you.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and any information about you or your child will be handled in strictest confidence and will only be used in a way that will not allow you or your child to be identified. Your GP will be informed of your involvement in the study with your consent.

What will happen if we don't want to carry on with the study?

You can withdraw from the study at any time without having to explain why. You would continue to attend clinic regularly when cough swabs would be taken, and lung function tests would be scheduled between 3-6 years of age as is current routine practice at GOSH.

What will happen when the study stops?

Once your child is six years old, we will not require you to attend for any additional visits, but would like permission to continue to track your child's clinical progress (from the information we get at routine clinic visits) so that we can assess how well these early tests predict future outcome at school age. This would not involve any extra effort from you or your family as it would be based on routine medical records.

How will I learn about the results of this study?

We can send you a summary of the study once all the results have been analysed (approximately 2015). We will be giving talks about the results to other doctors and nurses around the world and will display the findings on the CF Trust's website .

Who is organising and funding the research?

Action Medical Research is funding this research, which also has approval from the UK Cystic Fibrosis Trust.

Who has reviewed this Study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity.

This study has been reviewed and given favourable opinion by the Bloomsbury Research Ethics Committee who consider that it is addressing an important question regarding treatment of children with CF and that there will be minimal risk to you or your child if you participate.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to your Specialist CF consultant, CF nurse or one of the researchers who will do their best to answer your questions. Their contact numbers are at the end of this information sheet. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital PALS service on 020 7829 7862. In the extremely unlikely event that something does go wrong and you or your child are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against The Institute of Child Health / Great Ormond Street Hospital but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

Who can I talk to about this study?

Your Specialist CF consultant or any of the research team will be more than happy to talk to you about this study. Their contact details are below:

[redacted]

You can also contact the CF Trust, with whom we keep in close touch.

Thank you for reading this leaflet, we look forward to seeing you again soon.

Cystic Fibrosis our focus

Great Ormond Street Hospital

action medical research for children

b) Study information leaflet for carers of healthy control children

Parent Information Booklet

Great Ormond Street Hospital for Children NHS Foundation Trust

Maintaining the momentum:

Will newborn screened babies with Cystic Fibrosis still have normal lung function when they go to school?

REC Number: 12/LO/1668 Chief Investigator: Dr Paul Aurora

PART 1: Essential elements of the study

We are approaching you as a parent of a child that has been diagnosed with CF who has taken part in a previous study (NREC Number: _09H071314) to find out the best ways of detecting early lung disease in babies who have been diagnosed with cystic fibrosis (CF) through the national UK newborn screening programme.

We would like to invite you to take part in another research study to find out about how lung function progresses through the pre-school years in the same children who have been diagnosed with cystic fibrosis (CF) through the national UK newborn screening programme. On this occasion, all tests are performed while your child is awake and they can eat and drink normally prior to and after the test.

Before you decide whether to take part, you need to understand why the research is being done and what it would involve for you and your child. Please take time to read the information in this document carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. If you would like your child to take part once you have understood what the project is about, we will ask you to sign a consent form. You will be given a copy of this consent form to keep.

What is the purpose of this study?

This is a follow-up study which will enable us to compare results obtained with those previously and to the results for healthy children. We will also be testing new equipment which will help other CF centres elsewhere to use the sensitive tests that we have developed in London. This study will also allow us to identify children with CF who will benefit most from intensified therapy.

Do we have to take part in this study?

You do not have to take part in this research project if you don't want to. It is up to you to decide. We are sending you this information sheet so you can think about whether you would like to be involved. We will then phone you to discuss this further and answer any questions you may have. You will have further opportunities to ask questions if you bring your child for the breathing tests. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw from this study at any time, without giving a reason.

If we take part, what will this involve?

We will invite you to Great Ormond St Hospital when your child is around 3-6years of age to measure his/her lung function. On this occasion, all tests are performed while your child is awake. Each visit will take about 2 hours in total. You can take your child home as soon as the test is complete.

What are the tests in the study?

1. Breathing tests

Breathing tests for children less than 6 years old are very safe and painless, but are only available at a few centres round Britain. We therefore need you to come to Great Ormond Street Hospital for Children (GOSH) for these tests. We will arrange the timing of these visits to suit your family and all travel expenses can be refunded. You can stay with your child all the time that they are being tested.

Your child will be asked to do the breathing tests by taking deep breaths and blowing through special equipment. All tests are performed while your child is awake and they can eat and drink normally prior to and after the tests. The tests do not upset the children, who usually enjoy the process. It usually only takes about an hour to do all the tests but we generally allow two hours to ensure both you and your child are comfortable and relaxed. During the visit a research doctor will examine your child and ask a few health questions.

We use three tests to see how the lungs are developing and growing:

a. Multiple breath washout test

This test is designed to find out how evenly your child breathes and will be performed in two ways. The child breathes in a special air mixture through a face mask which contains a small amount of a gas called Sulphur Hexafluoride (or SF_6). SF_6 is 'inert' which means that it does not cross from your child's lungs into the blood stream, and has no taste or smell. The gas has been used safely at GOSH in babies and young children for the past 10 years and is used at many other specialist hospitals throughout the world. The child breathes this mixture for a few minutes so that it mixes through the lungs. After a few minutes the gas mix-ture is swapped for normal air and we measure how quickly the child breathes (or "washes") out the SF_6 from the lungs. Children with normal lungs quickly wash out SF_6 whereas children with early lung disease take longer to clear the gas from their lungs. This is similar to the test that was performed when your child was approximately 3 months, 1 year and 2 years old.

b. Airway Resistance

The second test measures how big the airways are. In order to do this the child sits in a special cubicle (which looks a bit like a public telephone box) and breathes air through a face mask. The cubicle is transparent and has a door which is closed for 2-3 minutes so that we can record the small pressure changes that occur while your child is breathing.

c. Forced expiration

This last breathing test measures how quickly your child can breathe out and also reflects airway size. Your child is asked to wear a nose clip (which looks a bit like a swimming nose clip), breathe in deeply and then breathe out as hard and fast as they can through a plastic tube which they hold in their mouth. To help them do this, we use a variety of specially made computer games. Children with lung disease cannot blow out as much air or as quickly as children with normal lungs.

2. Other investigations:

While you are at the laboratory, we will also weigh and measure your child, and ask you a few questions about your family and your child's health. We would also like to know about your views and experiences of being a part of this study. We will ask you to fill out a questionnaire once at the end of the study.

What is the questionnaire for?

It is important that we know how parents feel about being asked to take part in studies such as this. We would also like to know about your views and experiences of being a part of this study. We will ask you to fill out a questionnaire once at the end of the study.

Are there any risks or discomfort for my child?

Breathing tests: We do not believe there are any risks or discomfort associated with performing these breathing tests in children. On the contrary, experience has shown that most children of pre-school age, enjoy their visit to the lung function laboratory.

Time burden: Attending appointments for the testing sessions could cause some inconvenience as you may need to come in for an additional appointment, but we will ensure that any inconvenience is limited by booking appointments that are suitable to both you and your child where possible.

Lung function testing

PART 2: Additional Information to be read before you decide whether to participate or not.

Why is this study important?

Children with Cystic Fibrosis are more prone to chest infections and repeated infections lead to lung damage. These infections may occur very early in life and some can go unnoticed (because the child does not have an obvious cough or other symptoms). It is very important that the effects of such infections are detected and treated rapidly, using tests like the ones in this study, to prevent irreversible damage to the lungs which may limit the child's physical ability and lifestyle.

Who will this study help?

As a parent of a healthy child, participating in this study will not be of any direct benefit to your child, but the information we obtain will help improve the treatment of children with CF. It will help us to understand more about CF lung disease in the first few years of life, and which test, or combination of tests, is likely to be most useful in detecting early changes in the lungs.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and any information about you or your child will be handled in strictest confidence and will only be used in a way that will not allow you or your child to be identified. Your GP will be informed of your involvement in the study with your consent.

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the Bloomsbury Research Ethics Commitee who consider that it is addressing an important question re-garding treatment of children with CF and that there will be minimal risk to you or your child if you partici-pate.

What if there is a problem?

In the extremely unlikely event that something does go wrong and you or your child are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensa-tion but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Who can I talk to about this study?

All members of the pre-school lung function team will be happy to explain the tests and answer your questions.

You may phone Dr Julie Duncan, who is carrying out this research, at Great Ormond Street Hospital on [redacted]

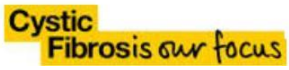
If you cannot get through on this line, then contact our secretary, Jana Varma (Portex Unit) on [redacted]

Dr Paul Aurora who is organising the study can also contacted via the above telephone numbers.

Finally the Patient Advice and Liaison Service (PALS) can be contacted on [redacted]

Thank you for reading this leaflet, we look forward to seeing you again soon.

Research supported by:



A2: Standard treatment protocol

Standardised treatment for children with CF

Prior to commencing this study, a standardised treatment protocol, as described below, was developed and agreed upon by all participating consultants. This was used throughout the duration of the study. Following diagnosis, all infants commenced on multivitamins, pancreatic supplements (if pancreatic insufficient as determined from faecal elastase levels) and prophylactic flucloxacillin (25mg/kg twice daily). The extent of adherence to protocol was checked both by regular review of prospectively completed Case Record Forms (CRFs) and by discussions at collaborative meetings of the LCFC. Cough swabs were taken routinely at clinic visits (minimum every 2−3 monthly) and additionally when the infant was symptomatic. All centres in the UK encourage daily chest physiotherapy to infants and children with CF. Within the London CF Collaboration (LCFC), parents/carers of CF infants and children are taught an appropriate airway clearance technique. Physiotherapy is carried out as appropriate to the child's age and condition and reviewed frequently in conjunction with medical treatment.

1. Infection with Pseudomonas aeruginosa (PsA)

a) First growth- Monthly cough swabs were collected while on treatment.

- Well infant (based on clinical judgement)
  - Home therapy with 3 weeks of oral Ciprofloxacin (15mg/kg twice daily) and
  - 3 months of nebulised Colistin (Colomycin: 1 million units twice daily).
- Unwell infant (based on clinical judgement)
  - Hospital admission for 2 weeks of intravenous antibiotics;
  - Intravenous Ceftazidime (50mg/kg three times daily) and intravenous Tobramycin (10mg/kg once daily), though this choice could be modified by results from culture and sensitivity;
  - Also started on 3 months of nebulised Colistin, initiated whilst in hospital.

b) Re-growth during the initial 3 month treatment period (whilst still on Colistin)

- Well infant
    - Further 3 weeks of oral Ciprofloxacin.
- Unwell infant
    - Hospital admission for intravenous antibiotics and a further 3 months of nebulised Colistin. If second course of intravenous antibiotics was inappropriate, 3 weeks of oral Ciprofloxacin was given instead.

c) Regrowth at the end of 3 weeks ciprofloxacin or 3 months nebulised Colistin

- Intravenous antibiotics, and either
    - a further 3 months of nebulised Colistin, or
    - 28 days of TOBI (300mg twice daily) followed by 3 months of Colistin.

d) Regrowth after IVs and at least 6 months of nebulised Colistin

- Try 28 days nebulised TOBI™ and then continuous nebulised Colistin 1 million units twice daily for a further six months. In practice this is unlikely to arise during the study.

(e) Regrowth > 6 months from first growth

- Treat as for first growth.

(f) Chronic Pseudomonas Infection

Defined for analysis purposes by the Leeds criteria:

Never	never cultured
Free	cultured previously but not in last year
Intermittent	cultured in < 50% of samples in past year
Chronic	cultured in > 50% of samples in past year

2. Infection with Methicillin Sensitive Staphylococcus Aureus (MSSA)

(a) First growth

• Well infant

– Oral Augmentin duo (400/57) 0.3mL/kg twice daily for 2−4 weeks, or an equivalent dose of Co-amoxiclav syrup (0.25mL/kg of 250/62 strength) three times daily for 2−4 weeks based on clinical judgment.

• Unwell infant

– Hospital admission for 2 weeks of intravenous antibiotics;

– Intravenous Tobramycin once daily and intravenous Teicoplanin 10mg/kg for 2 doses twelve hours apart then 10mg/kg once daily.

(b) Regrowth less than 6 months from first growth

– Oral Flucloxacillin increased from 25mg/kg (prophylactic dose) to 50mg/kg for 28 days.

(c) Further regrowth within 6 months

– Two oral anti-staphylococcal antibiotics for 4 weeks.

(d) Re-growth after more than 6 months from first growth

– Treat as for first growth.

3.2.3 Infection with Haemophilus influenzae (HI)

(a) First growth

• Well infant

– Oral Augmentin Duo or Co-amoxiclav syrup for 2−4 weeks (based on clinical judgement)

• Unwell infant

– Hospital admission for 2 weeks of intravenous Ceftazidime (50mg/kg three times daily) and intravenous Tobramycin (10mg/kg once daily)

(b) Regrowth less than 6 months from first growth or more than 6 months from first growth

– Oral Augmentin duo or Co-amoxiclav syrup for 2−4 weeks (based on clinical judgement)

(c) Further regrowth within 6 months

– Clarithromycin (7.5mg/kg twice daily) for 2−4 weeks

3.2.4 Viral Upper Tract Infections (otherwise well child)

• Oral Augmentin duo (400/57) 0.3 mls/kg bd for 2 (minimum) to 4 weeks (clinical judgment) or equivalent dose of Co-amoxiclav syrup tds <1 year 0.25ml/kg TDS Augmentin 250/62; >1 - 2 years 5ml TDS Augmentin 250/62 for 2 (minimum) to 4 weeks (clinical judgment)

3.2.5 Respiratory exacerbation with unknown organism, unwell child (clinical judgment)

Depending on severity of exacerbation:

• Oral Augmentin duo (400/57) for 2 to 4 weeks or equivalent dose of Co-amoxiclav

syrup tds (as above) OR

• Intravenous Tobramycin 10 mg/kg once daily and Intravenous Ceftazidime 50mg/kg three times a day for 2 weeks

NOTE: choice of antibiotic may vary from the protocol depending on culture sensitivities

A3: Study visit reward chart

A4: Consent forms and carer test day questionnaire

a) Consent and questionnaire for carer(s) of CF subjects

CF TESTING DAY FORMS: 12/LO/1668 SN:_____

Great Ormond Street Hospital for Children NHS Foundation Trust

Great Ormond Street Hospital for Children NHS Trust and Institute of Child Health Research Ethics Committee REC Number: 12/LO/1668

Consent Form for PARENTS OR GUARDIANS of Children Participating in Research Studies

TITLE OF PROJECT: Maintaining the momentum: will newborn screened cystic fibrosis babies still have normal lung function when they go to school?
Chief Investigator: Dr Paul Aurora

NOTES FOR PARENTS OR GUARDIANS

1. Your child has been asked to take part in a research study. The person organising that study is responsible for explaining the project to you before you give consent.
2. Please ask the researcher any questions you may have about this project, before you decide whether you wish to participate.
3. If you decide, now or at any other stage, that you do not wish your child to participate in the research project, that is entirely your right, and if your child is a patient it will not in any way prejudice any present or future treatment.
4. You will be given an information sheet which describes the research project. This information sheet is for you to keep and refer to. ***Please read it carefully.***
5. If you have any complaints about the way in which this research project has been or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in any other way please contact PALS at GOSH for more information.

		Please initial boxes
1.	I confirm that I have read and understand the information sheet, Version 2 dated 07 February 2013 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily	
2.	I understand that my/my child's participation is voluntary and that I am free to withdraw my child at any time without giving any reason, without his/her medical care or legal rights being affected	
3.	I understand that relevant sections of my child's medical notes and data collected during the study, may be looked at by individuals from the sponsor (GOSH), regulatory authorities or from the NHS Trust (GOSH NHS Foundation Trust), where it is relevant to taking part in this research. I give permission for these individuals to have access to my child's records, and to use relevant information in subsequent scientific publications in a way that ensures neither I nor my child can be identified.	
4.	I agree to my child's GP being informed of my child's participation in the study and for the results of the tests to be notified to my child's GP.	
5.	I agree for my child to take part in the above study.	
6.	I give permission to be contacted in the future regarding further related research studies.	

_____	_____	_____
Name of Parent/Guardian	Date	Signature

Relationship to child

_____	_____	_____
Name of Person taking consent	Date	Signature

When completed, 1 copy for family; 1 copy for researcher site file; 1 (original copy) to be kept in medical notes

NOTES FOR THE RESEARCHER

It is your responsibility to ensure that the parents/guardians and child (if mature enough) understand what the research project involves, both theoretically and practically. **You must allow sufficient time to do this.** You must make the judgement of whether or not the child can understand the project. Age alone is not important. Make sure that the relatives or child can contact you if they have additional questions.

A copy of this completed form must be placed in the patient's clinical records and a copy must be kept by you with the research records.

If there are any unforeseen ethical problems with this study you must inform [a representative of the sponsor] and follow this up in writing.

Does your child have any atopic disorders?	*Yes*	*No*	
If yes what?	*Allergy* *Details_____*	*Hayfever*	*Eczema*
Respiratory problems other than CF?	*Yes*	*No*	
If Yes, please give details:			
Non-respiratory medical problems?	*Yes*	*No*	
If Yes, please give details:			

Note: all symptoms of cough or wheeze should be considered CF related and should not be recorded here.

Intermittent Antibiotic Therapy

Does your child have regular IV antibiotics?	*Yes / No*	**If yes what is the interval?**	*Number of months*

Courses of antibiotics (IV or Oral) **since Last LCFC Lung Function Test;**

Date of course MM/YY	**Duration of course (weeks)**	**Location** (Home/Hosp/ Both, no weeks)	**Reason for course** (e.g. respiratory)	**Route** (Oral / IV / Neb)	**Comment** (eg first isolation of pseudo, ventilation required)
	2 / 3 /Other				
	2 / 3 /Other				
	2 / 3 /Other				
	2 / 3 /Other				
	2 / 3 /Other				
	2 / 3 /Other				
	2 / 3 /Other				
	2 / 3 /Other				

Additional Hospital admissions since last LCFC LFT,

Date Admitted / Discharged	**Reason and hospital name**	**Comment** (e.g. Ventilation)

Any operations since last LCFC visit (date):	

Has the child has ever needed mechanical ventilation since birth?		Yes	No
Date ventilation started:		**No. of days ventilated**	

Current Medications

Pulmonary	
Antibiotics – oral : (not quinolones; please specify)	Yes/ No
Antibiotics – inhaled : (please specify)	Yes/ No
Corticosteroids : (please specify)	Yes/ No
Mucolytics :	DNAse / Hypertonic Saline / No
Oxygen :	Yes/ No
Ivacaftor : (Kalydeco)	Yes/ No
Bronchodilators (Inhalers) :	Long Acting / Short Acting / No* * Long Acting Eg; Salmeterol/formoterol/ aminiphyline etc Short Acting Eg; Salbutamol/ ipratropium bromide etc.
Nutritional	
Pancreatic enzymes	Yes/ No
H_2 Blockers	Yes/ No
Proton Pump Inhibitors	Yes/ No
Motility agents	Yes/ No
Vitamin supplements	Yes/ No

Additional Hospital admissions since last LCFC LFT,

Date Admitted / Discharged	**Reason and hospital name**	**Comment** (e.g. Ventilation)

Any operations since last LCFC visit (date):	

Has the child has ever needed mechanical ventilation since birth?		Yes	No
Date ventilation started:		**No. of days ventilated**	

Current Medications

Pulmonary	
Antibiotics – oral : (not quinolones; please specify)	Yes/ No
Antibiotics – inhaled : (please specify)	Yes/ No
Corticosteroids : (please specify)	Yes/ No
Mucolytics :	DNAse / Hypertonic Saline / No
Oxygen :	Yes/ No
Ivacaftor : (Kalydeco)	Yes/ No
Bronchodilators (Inhalers) :	Long Acting / Short Acting / No* * Long Acting Eg; Salmeterol/formoterol/ aminiphyline etc. Short Acting Eg; Salbutamol/ ipratropium bromide etc.
Nutritional	
Pancreatic enzymes	Yes/ No
H_2 Blockers	Yes/ No
Proton Pump Inhibitors	Yes/ No
Motility agents	Yes/ No
Vitamin supplements	Yes/ No

Other	
Anything	

Has your child ever been prescribed a bronchodilator?	Yes/ No	**If Yes, When was inhaler last taken**	DD:MM:YY HH:MM
If Yes details	Long / Short acting		
Anything additional (details):			

Respiratory Symptoms

Has your child had a cold or URTI in the last 3 weeks? (circle appropriate)	**No**	**Yes, but asymptomatic for _____ days**	**Yes and still symptomatic**

How often has your child coughed in the last 7 days?				
None	**With physio only**	**Not just with physio, but not daily**	**Daily, but not at night**	**Daily and nightly**
X	X	X	X	X
Cough Productive with Sputum		**Cough sounds 'wet', but no sputum**	**Cough sounds 'dry'**	
X		X	X	

Has your child wheezed in the last 7 days?	Yes / No / Not Known

Physiotherapy given? (circle appropriate)	**Not at all**	**Once a Day**	**____Times a day**
No of hours since last Physiotherapy session?	hours		

Is your child enrolled on any interventional clinical trials?	Yes/No	**If Yes, Name of trial**	

Smoking Exposure

Does Mother smoke now?	Yes / No	**If Yes, how many a day?**	
Does Mother's partner smoke now?	Yes / No	**If Yes, how many a day?**	
No of Smokers living in the same house as the child:			
Is your child regularly exposed to non-household smoking?	Yes / No		
Has your child had exposure to any other cigarette smoke in the past 24 hours?	Yes / No	**If Yes, Who?**	

End of Questionnaire

Cough Swab

Cough Swab taken on day of research test?	Yes / No/ In Clinic	**If No, Date of last cough swab**	dd/mm/yyyy
Date Result Checked and Initials	dd/mm/yyyy AA		

Microbiology from previous cough swabs *(note all cultures identified)*		
Pseudomonas aeruginosa NMuc	**E. coli**	**Burkholderia cepacia**
Pseudomonas aeruginosa Muc	**Aspergillus**	**Streptococcus pneumoniae**
Staphylococcus aureus	**S. maltophilia**	**Grp A Strep**
Enterobacter	**Serratia Marescens**	
Haemophilus influenzae	**MRSA**	**No growth / Normal flora**
Candida	**Klebsiella**	

Date and result of CXR:							

b) Consent and questionnaire for carer(s) of healthy controls

HEALTHY TESTING DAY FORMS: 12/LO/1668 SN:_____

Great Ormond Street Hospital for Children NHS Foundation Trust

Great Ormond Street Hospital for Children NHS Trust and Institute of Child Health Research Ethics Committee REC Number: 12/LO/1668

Consent Form for PARENTS OR GUARDIANS of Children Participating in Research Studies

TITLE OF PROJECT: Maintaining the momentum: will newborn screened cystic fibrosis babies still have normal lung function when they go to school?
Chief Investigator: Dr Paul Aurora

NOTES FOR PARENTS OR GUARDIANS

1. Your child has been asked to take part in a research study. The person organising that study is responsible for explaining the project to you before you give consent.

2. Please ask the researcher any questions you may have about this project, before you decide whether you wish to participate.

3. If you decide, now or at any other stage, that you do not wish your child to participate in the research project, that is entirely your right, and if your child is a patient it will not in any way prejudice any present or future treatment.

4. You will be given an information sheet which describes the research project. This information sheet is for you to keep and refer to. ***Please read it carefully.***

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4.	I agree to my child's GP being informed of my child's participation in the study and for the results of the tests to be notified to my child's GP.	
5.	I agree for my child to take part in the above study.	
6.	I give permission to be contacted in the future regarding further related research studies.	

_____	_____	_____
Name of Parent/Guardian	Date	Signature

Relationship to child

_____	_____	_____
Name of Person taking consent	Date	Signature

When completed, 1 copy for family; 1 copy for researcher site file; 1 (original copy) to be kept in medical notes

NOTES FOR THE RESEARCHER

It is your responsibility to ensure that the parents/guardians and child (if mature enough) understand what the research project involves, both theoretically and practically. **You must allow sufficient time to do this.** You must make the judgement of whether or not the child can understand the project. Age alone is not important. Make sure that the relatives or child can contact you if they have additional questions.

A copy of this completed form must be placed in the patient's clinical records and a copy must be kept by you with the research records.

If there are any unforeseen ethical problems with this study you must inform [a representative of the sponsor] and follow this up in writing.

Questionnaire for GOSH Healthy Control

Information From Parent

Study Number: **Date of Birth**

Time of arrival at test site			•			
Time of test commencement			•			
Time of leaving test site			•			

Barometric Pressure	mbar	**Face mask: type /size**	
Temperature	**C**	Male / Female	
Humidity	**%**	Ethnicity	

Operators	

Test	**MBW**	**Pleth**	**Spiro**					**Other**
Order								
Data acceptable?								

Physical examination at time of test:	**Performed by:**

Wheezes	**Yes / No**	**Crackles**	**Yes / No**

Pre test

Respiratory rate			bpm	**SaO_2**			%	**Mean HR**			

Post test

Respiratory rate			bpm	**SaO_2**			%	**Mean HR**			

Remainder of clinical examination normal:	**Yes**	**No – comment:**

Anthropometry

Weight			•			**kg**
Height				•		**cm**
Sitting Height				•		**cm**
Stool Height			•			**cm**

Does your child have any atopic disorders?	*Yes*	*No*	
If yes what?	*Allergy Details_____ _*	*Hayfever*	*Eczema*
Respiratory problems?	*Yes*	*No*	
If Yes, please give details:			
Non-respiratory medical problems?	*Yes*	*No*	
If Yes, please give details:			

Hospital admissions since last LCFC LFT

Date Admitted / Discharged		Reason and hospital name	Ventilation (Date/duration) (Mode/Modes used)	IV/Inhaled Antibiotics (for chest)

Intermittent antibiotic therapy since last LCFC LFT

For each parameter record number of courses and name of drug (if applicable) received since last LCFC LFT:

Date	Reason for course (respiratory/nonrespiratory)	Location (Home/Hosp/Both)	Route (Oral/IV/Inhaled)	Total (Number)

Any operations since birth:	

Has the child has ever needed mechanical ventilation since birth?		***Yes***	***No***
Date ventilation started:		***No. of days ventilated***	

Current Medications:Tick all current medications

Medications

Has your child ever been prescribed a bronchodilator?	Yes/ No	**If Yes, When was inhaler last taken**	DD:MM:YY HH:MM
If Yes details	Long / Short acting		
Anything additional (details):			

Respiratory Symptoms

Has your child had a cold or URTI in the last 3 weeks? (circle appropriate)	**No**	**Yes, but asymptomatic for _____ days**	**Yes and still symptomatic**

How often has your child coughed and has he/she wheezed in the last 7 days?

Cough	**None**	**Yes, but not daily**	**Daily**

Wheeze	**Yes**	**No**	**Don't Know**

Smoking History

Does mother smoke now? ☐ **No** ☐ ***Yes*** ☐☐ cigarettes a day

Does mother's partner smoke now? ☐ **No** ☐ ***Yes*** ☐☐ cigarettes a day

Number of smokers living in the same house as the infant (including mother) ☐ smoker(s)

Child Regularly exposed to non-household smoking? ☐ **No** ☐ **Yes**

Exposure to any other cigarette smoke in the past 24hrs? ☐ **No** ☐ **Yes**

If Yes - Who? _____

A5: Clinician questionnaire

STUDY NO: CENTRE: DOB:	**LCFC NBS PRESCHOOL FOLLOW UP STUDY** **CLINICAL QUESTIONNAIRE**	DATE LAST IPFT: DATE PS PFT:

DATE OF 1st ENCOUNTER/DIAGNOSIS: _____Weight: _____kg; Height:_____cm

				YR1	YR 2	YR 3	YR 4	YR 5	YR 6	YR 7
DATE OF ANNUAL REVIEW/ DATABASE ENTRY(DD/MM/YY)										
NUTRITION										
Weight at encounter (kg)										
Height at encounter (cm)										
Supplemental feeding	PEG/JEJ/NG/TPN									
	Oral feeding supplements									
Pancreatic enzymes	Creon									
Acid blocker / treatment for gastro-oesophageal reflux disease	H_2 blocker (ranitidine)									
	PPI	Omeprazole								
		Lansoprazole								
	Motility agent (domperidone)									
	Other:									
PULMONARY THERAPY										
Ivacaftor (kalydeco)										
Mucolytics/osmotics	DNase									
	Hypertonic saline									
	Other:									
Inhaled dry power antibiotics	Colistimethate dry powder									
	Tobramycin dry powder									
Long term antibiotic nebulisers (>3months that year)	Tobramycin solution									
	Colistin solution									
	Promixin solution									
	Other(s) (please specify):									
Short term antibiotic nebs-list which & length (& reason if available)	Abx	Length	Reason							
Prophylactic/chronic oral antibiotics	Flucloxacillin									
	Augmentin									
	Other:									
Long-term azithromycin	(> 3 months)									
Inhaled bronchodilators	Short acting beta agonist									
	Long acting beta agonist									
	Other (please specify):									
Inhaled corticosteroids										
Oral corticosteroid										
Combined inhaled steroid/bronchodilator										
Leukotriene modifier (montelukast)										
Antifungal	Name:									
OTHER PULMONARY TREATMENT (list any not included and year)										
CF COMPLICATIONS (list & tick year – eg APBA, CFRD, liver disease, port, PEG etc)										
MEDICAL CONDITION NOT CF										

LCFC NBS PRESCHOOL FOLLOW UP STUDY

STUDY NO:

CLINICAL QUESTIONNAIRE

	YR1	YR 2	YR 3	YR 4	YR 5	YR 6	YR 7
YEARLY STATUS							
Shared care?							
Other research trial?							
ADDITIONAL ORAL ANTIBIOTICS							
Days of oral antibiotics							
Courses of oral antibiotics							

IV ANTIBIOTICS

Start date IV course	Duration (days)	IV days in hospital	IV days home	Reason/notes

MICROBIOLOGY

ORGANISM	DATE	ORGANISM	DATE

A6: Acceptability criteria and software protocol for multiple breath washout analysis

The following table is adapted from the ERS/ATS Consensus statement for MBW testing (2013)[71], the most recent guidelines available for MBW acceptability criteria at the time of testing.

Multiple breath washout acceptability criteria

Wash in phase
Stable tidal volume and end-expiratory lung volume over the preceding 30 seconds
• Deviation in end-expiratory lung volume at start of test within 10% of mean tidal volume of preceding five breaths
• No irregular small volume breaths immediately prior to starting the washout (may lead to error in end tidal estimate of starting alveolar concentration)
Equilibration of exogenous wash-in gas within the breath cycle (i.e. inspiratory versus expiratory end tidal concentration)
• Variability 1% relative to mean inspired concentration (i.e. 0.04% for inspired SF_6 concentration of 4%)
Adequate starting end-tidal inert gas concentration, stable over 30 s (i.e. equal to inspired gas concentration)
Washout phase
Regular breathing pattern
No evidence of significant trapped gas release with larger breaths; release of trapped gas
No coughing
Criteria for test termination
• At least three consecutive breaths with end tidal inert gas concentration values below 1/40th of starting inert gas concentration
No evidence of leak occurring during the test
• Failure of equilibration between inspiratory and expiratory inert gas concentrations during wash-in (consistent with pre- or post-gas sampling point leak)
• Sudden drop in inspiratory inert gas concentration during wash-in (consistent with post-gas sampling point leak)
Investigate for artefact
• Marked volume drift during testing or sudden changes in volume (without other evidence of leak)
• FRC or LCI variability 10%, measured as the difference between maximum and minimum values
If FRC differed by 25% from the median FRC value across the three tests were rejected

Legend: Acceptability criteria for multiple breath washout testing adapted from Robinson et al[71]. Abbreviations: FRC=functional residual capacity

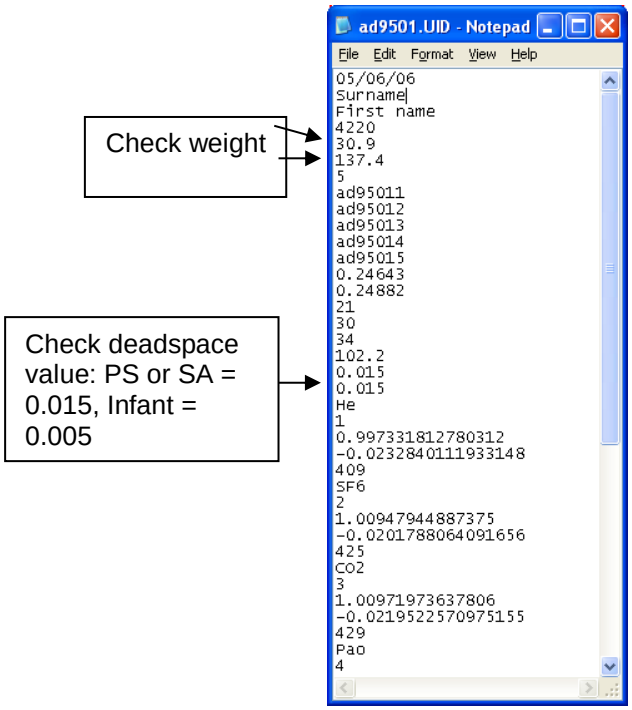
The relevant sections of a local protocol for MBW analysis from the Great Ormond Street lung function laboratory for calculating lung clearance index is also detailed below.

Analysis of MBW Data

Once the data has been backed up, it is then possible to analyse the data using the backup copy of the data.

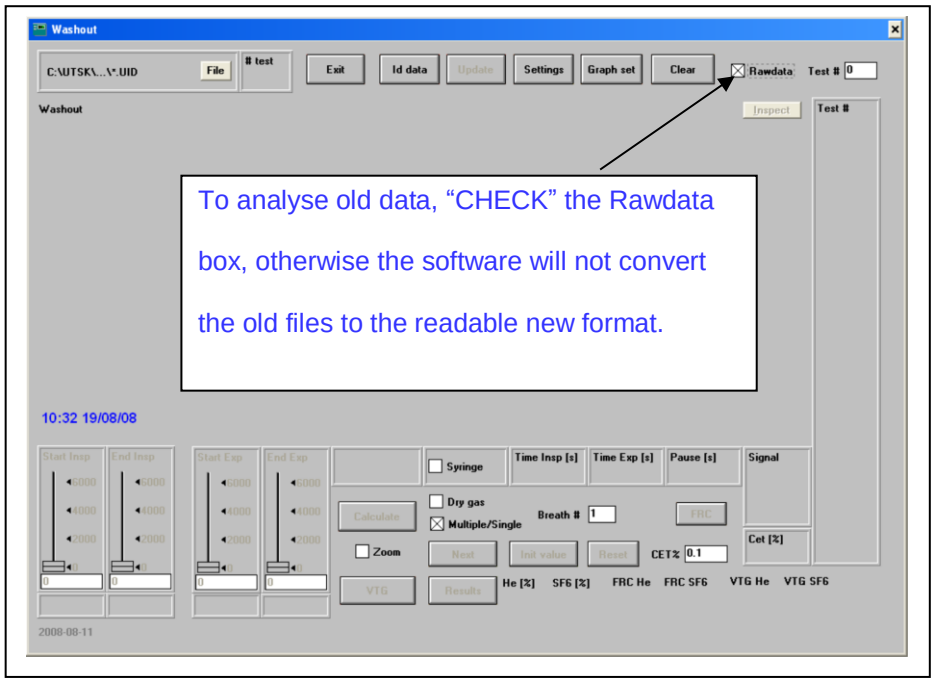
1. Open c:/UTSK
2. Open the folder of data for analysis
3. Open the UID file, check the wt, height and deadspace (see Figure 1).

Fig. 1: Data contained in UID file



4. Copy the UID file to IDData in UTSK (c:/Utsk/Iddata)
5. Copy all 'UPR' files (with data in them i.e. not the 1st and last file) to PROV (c:/Utsk/Prov)
6. Minimise Window explorer.
7. Open 'Off Line Calc' (see Figure 2).

Fig 2: Screen shot of opening page of analysis software



8. Before starting analysis, please check that your settings for the various aspects of the MBW and slope analysis (e.g. breath detection and slope analysis) are correct (especially important for those analysing data from all ages i.e. infants; preschool and school age).

<u>Breath detection settings</u>

The default settings for breath detection and flow levels are more appropriate for the older child (Figure 3a). However, these settings may not be appropriate for analysis of infant data as the tidal flow rate is much lower. Suggested settings for infants are given in Figure 3b.

To amend settings, go to Washout Analyse program, and click on “Settings” button at the top of window.

Settings for breath detection of Flow levels

Figure 3a

Older child (e.g. Preschool)

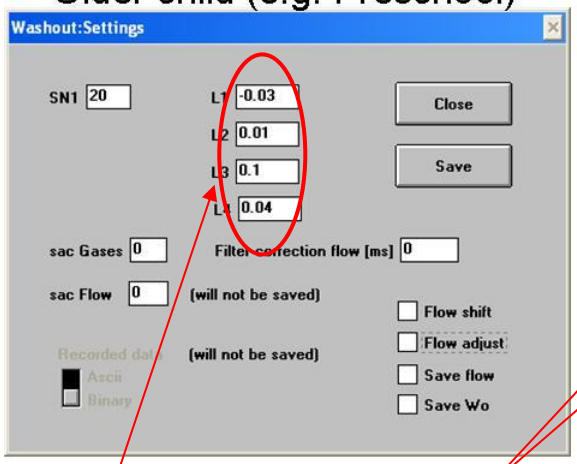
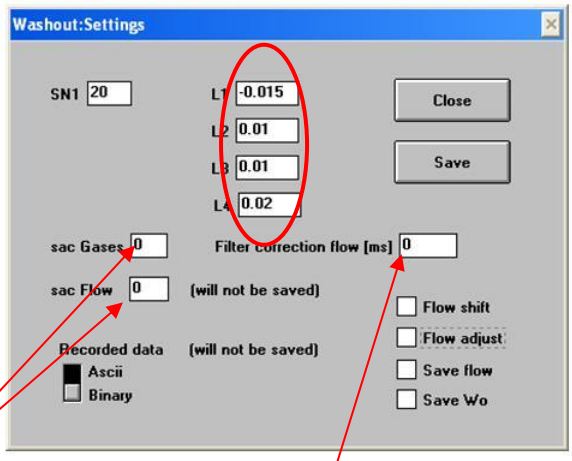


Figure 3b

Infant



Analysis without any filtering

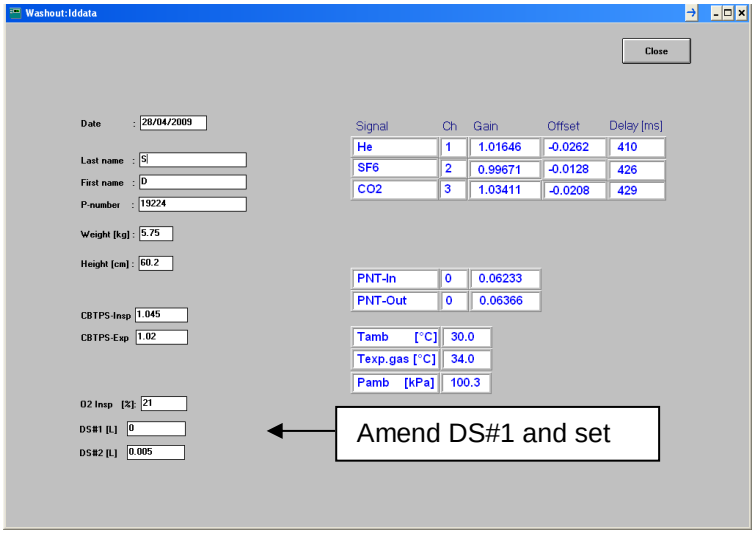
Correction for delay of flow signal with the new amplifier for PNT (not yet installed at GOSH). This new amplifier has a 20Hz electronic low

- L1 (start inspiration)
- L2 (end inspiration)
- L3 (start expiraion)
- L4 (end expiration).

Settings for Phase III slope analysis (see below)

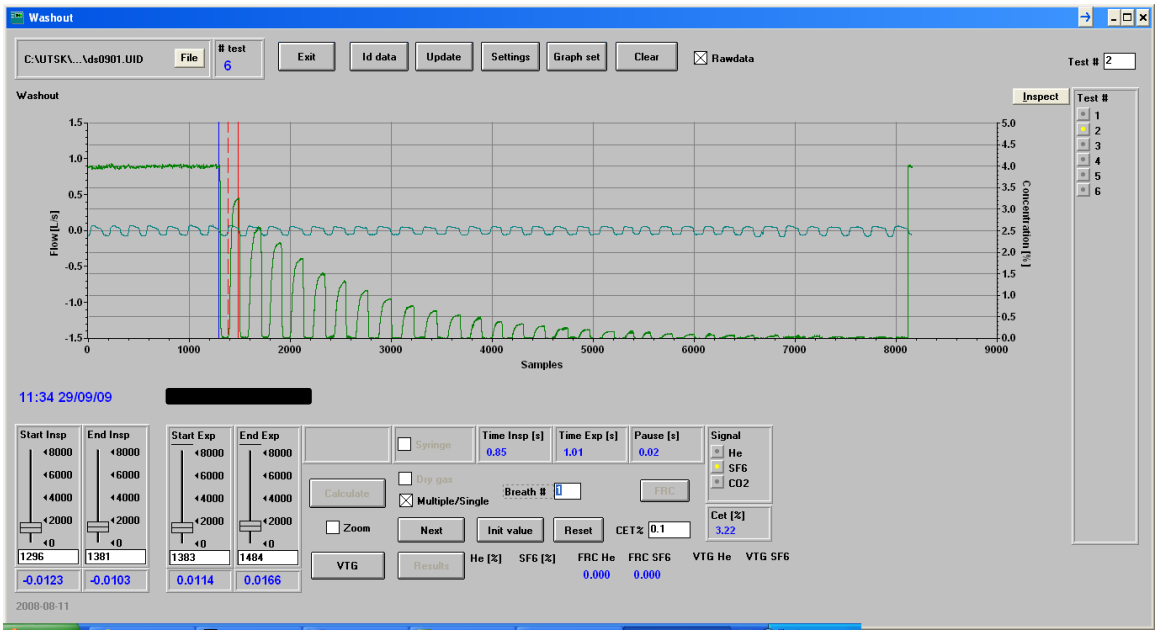
9. Click on ‘file’, ‘load’ IDData file i.e. open it.
10. Go to ‘IDData’, amend DS#1[L] to 0 (zero), check to ensure the weight and height have been corrected when the data was backed up (see Figure 4).

Fig 4: Screen shot of an example of the information contained in an ID Data file



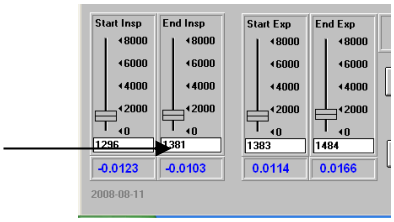
11. Write relevant details on the 'MBW Analysis' summary sheet
12. Close using 'CLOSE' button, i.e. not X in the corner.
13. Click on each 'TEST#' on right hand side and have a brief look at each to make sure the data is acceptable before analysing (i.e. good equilibration of the gas concentration signal and no obvious leaks or sighs immediately before or after washout is commenced)
14. Once you are satisfied with the data, open the first acceptable dataset and the data will appear on the screen. Click on 'NEXT' until the first breath of the washout is highlighted between the red and blue lines (indicating start and end of inspiration and expiration) (See Figure 5)

Fig 5: Screen shot of 'washout' data with the first breath highlighted between the blue(inspiration)/red(expiration) solid and dashed lines, denoting the start and end of that breath.



15. If the whole breath is not exactly within the red and blue lines, it may be necessary to manually amend the placement of the blue or red solid/dashed lines on the relevant portion of the breath to correctly identify the start and end of inspiration and expiration. This can be achieved by placing your cursor in the relevant white box (start/end inspiration or expiration) as indicated by the arrow in Fig 6) and then using the up and down keys to move the line indicating the start or end of inspiration/expiration. It may be necessary to repeat this process during the rest of the analysis if software identification of the whole breath is incorrect.

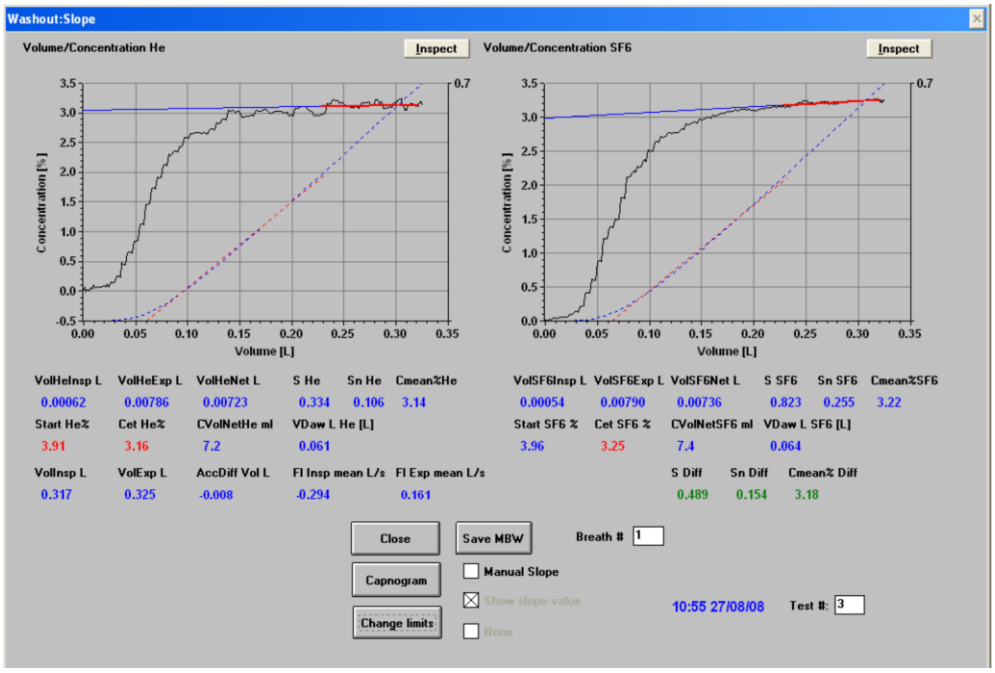
Fig 6: Altering the start and end of inspiration to capture a whole breath.



16. Once you are satisfied that you have highlighted the first breath, click on 'INIT VALUE'
17. Record the starting SF_6 and He concentrations on the 'MBW Analysis' summary sheet.

18. Click on ‘CALCULATE’; a new Window “ Washout:Slope” will appear (Fig 7)

Fig 7: Washout:Slope showing the individual breath data according to He and SF_6 (gas concentration plotted against breath volume) and regression line on PhaseIII slope.



A7: Laboratory protocol for specific airway resistance testing and quality control

Specific Airway Resistance (sRaw) Protocol (Great Ormond Street Lung function laboratory)

This test should be performed after eNO, IOS and prior to spirometry.

Test Procedure

The participant should be seated upright in a closed body plethysmography box (BPB) with their neck slightly extended to reach the mouthpiece and feet flat on the floor. The participant should be seated in the closed BPB for at least 1 minute prior to sRaw testing, allowing the BPB temperature to stabilise. The participant should then be instructed to breathe normally through the mouthpiece at a natural breathing frequency. Throughout testing, the participant should be wearing a nose-clip and supporting their cheeks with their fingers. Once testing has begun and a stable breathing pattern has been established, five or ten flow-pressure loops should be recorded (software version dependent). If the operator judges the loops to be acceptable (see quality control notes below), activate an end expiratory shutter closure. During the occlusion, the participant should be encouraged to continue trying to breathe normally against the closed shutter while at least 3-4 respiratory efforts are recorded (FRC manoeuvre). Following release of the occlusion, the participant should take 1-2 tidal breaths before taking a full inspiration (TLC), followed by a full expiration (ERV) and a further full inspiration (TLC) before returning to normal breathing. The results should then be calculated and the participant invited to rest, i.e. come off the mouthpiece. If a drift is present, the flow transducer should be zeroed. Three technically satisfactory recordings should be obtained.

After the above procedure has satisfactorily been completed three times, the participant should repeat the entire set of measurements while breathing at a frequency of 30-45 breaths per minute followed by an end expiratory occlusion, i.e. FRC (but no TLC/VC maneuver) and then a return to tidal breathing. Jaeger Version 5.0 (Jaeger, Würzburg, Germany) provides a visual stimulus to assist with breathing frequency, which is particularly useful in younger participants. The results should then be calculated and the participant invited to rest. This procedure should be completed three times to a technically satisfactory standard. In participants less than 6 years, a recording of sRaw during spontaneous breathing can be obtained without the need for an airway occlusion (Bisgaard & Nielsen, 2005).

Parameters to report should include breathing frequency, peak inspiratory and peak expiratory flow, tidal volume, specific effective resistance (and possibly specific total resistance) and FRC.

** The mean of the medians from three technically satisfactory recordings of 5 (10) breaths each should be reported.

Acceptability Criteria

Flow-pressure loops should have a similar appearance and be reasonably closed at zero flow. See Figures 1a & b for examples of acceptable and unacceptable flow-pressure loops, respectively. Loops should only be excluded on the basis of shape/pattern, i.e. leak, glottic closure, irregular breathing, not measured values of resistance – although measured values can be examined to verify that a sensible selection has been made. No manual amendments to loops should be made. ** Three technically satisfactory recordings should be the minimum number of trials to report from.

Figure 1a Acceptable Flow-Pressure Loops

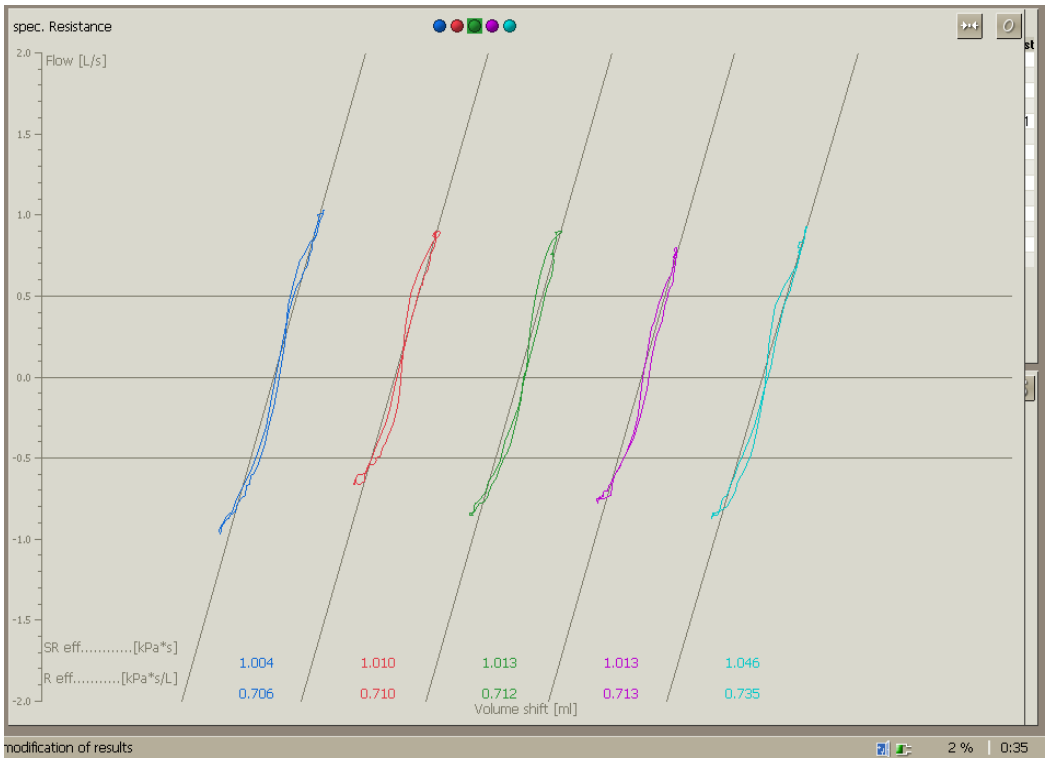
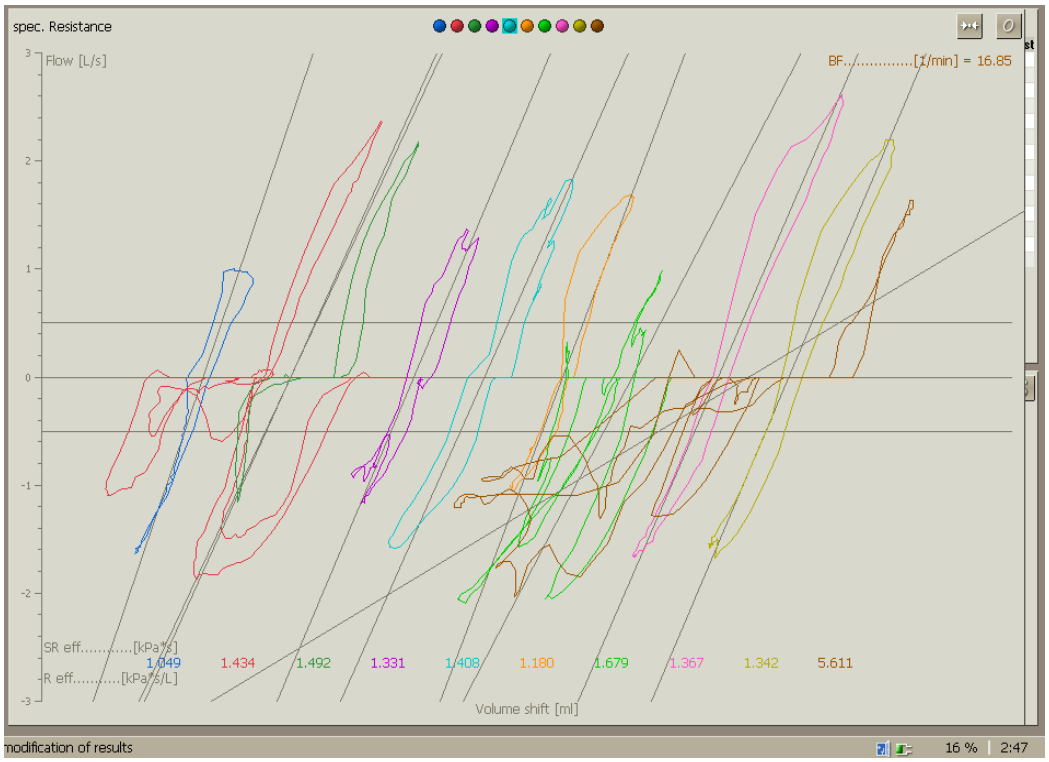


Figure 1b Unacceptable Flow-Pressure Loops



Performing the tests

* * Please read the entire procedure before beginning a test

- After the seat level, mouthpiece height and nose clip have been organised, and the participant has been given an explanation of the test, close the BPB door.
- From the Lab Manager menu select the Body Plethysmography icon.
- Ask the participant to sit at rest for 1 minute before commencing the test.
- When ready to commence testing, either press the F1 key or click on icon 1 on left-hand side of the screen.
- Ask the participant to make a tight seal around the mouthpiece with their lips, ensuring that their tongue and/or teeth are not causing an obstruction.
- Then, ask the participant to fit the nose-clip and support their cheeks with their fingers.

- Encourage the child to breathe at a natural frequency. NB: Where required, animation control can be employed by pressing the F6 key or clicking icon 6 (this must be activated prior to pressing F1).
- After five or ten acceptable loops (software version dependent) are visible in the top left box of the screen, inform the participant that the shutter will soon be activated and encourage them to try breathing against it. (In young children who are unable to tolerate shutter closure, simply record sRaw data during tidal breathing).
- Press the F2 key or click on icon 2 to activate the shutter occlusion.
- Following 1-2 tidal breaths post shutter occlusion, instruct the participant to inspire maximally, then exhale maximally, then inspire maximally again before returning to tidal breathing.
- Press the F7 key or click icon 7 to cease the test and view results.
- Invite the child to rest (come off the mouthpiece) between measurements.
- Press the F9 key or click on icon 9 to save the results and begin a new test.
- When ready to collect further data press F1 or click on icon 1 and repeat as above.
- If a drift on the tidal volume signal is present press the F8 key or click icon 8 for flow/volume zero adjustment.
- Press the F12 key (F10 for version 4.65) or click on icon 12 (icon 10 for version 4.65) to save the results and exit the program.

Reported Value	**How calculated**
FRC	Mean of the technically satisfactory FRC measurements
RV	FRC minus the mean of the technically acceptable ERV measurements
TLC	The reported value for RV plus the largest of the technically acceptable IVC's

Wanger J, Clausen JL, Coates A *et al.* (2005).