# Lung Function and Structure in Cystic Fibrosis Infants One Year after Diagnosis by Newborn Screening

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**This thesis is submitted for the degree**
**Doctor of Medicine MD (Res)**

## DECLARATION

I, Lena Priscilla Lee-Nah Thia, 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 within the thesis.

# ABSTRACT

**Title: Lung Function and Structure in Cystic Fibrosis Infants One Year after Diagnosis by Newborn Screening**

It is challenging to identify lung disease in asymptomatic infants with Cystic Fibrosis (CF) diagnosed by newborn screening (NBS). Since very little is known about the origin and progression of lung disease in these infants, there is uncertainty on how best to design intervention trials to protect these infants from pulmonary functional and structural decline.

This London CF collaborative (LCFC) observational study aimed to assess lung function and structure in NBS CF infants. Infant lung function tests (ILFT) which measured airway calibre (forced expiratory manoeuvres), hyperinflation (plethysmographic lung volumes) and ventilation inhomogeneity (multiple breath washout) were performed in contemporaneous healthy controls and CF infants at 3 months and 1 year of age. In addition, CF infants underwent chest computed tomography (CT) at 1 year under general anaesthesia (GA).

At age 1-year, NBS CF infants (n=72) had impaired lung function compared to controls (n=44). There was significant improvement in forced expired flows and volumes and no deterioration with respect to hyperinflation or ventilation inhomogeneity since 3-months. Fewer NBS CF infants had abnormal lung function by a year. Observed impairment was much less than reported in previous studies.

The challenges of performing multicentre standardised volume-controlled chest CTs under GA became evident. The lack of agreement between two experienced radiologists in scoring the CTs was unexpected, suggesting that the current established CT-CF scoring system may not be sensitive enough to describe mild CF lung disease. Contrary to published data, fewer and milder CT abnormalities were detected and there was poor correlation between lung function and CT changes in our NBS infant cohort.

This LCFC longitudinal study is the only study of NBS CF infants to include healthy controls that has shown no progressive decline in lung function during the first year of life and that structural changes are mild. Adequately powered intervention studies that use objective measures of lung function and structure during infancy will therefore need to be much larger than hitherto believed.

# ACKNOWLEDGEMENTS

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The many long hours spent agonising over the results; making sense of complex statistics and tackling challenges that arose were made easier through the friendship and support of my friends in the research fellow room. Thank you Rachel Bonner, Sarah Rand, Jane Kirkby and Harriet Shannon for all the cups of tea and coffee; and certainly without our weekly supplies of Graze boxes, life would have been dull! Thank you also to Dr Angie Wade and Vasiliki Bountziouka for their statistical support and advice. I am grateful to Julie Duncan, Ah-Fong Hoo, Harriet Shannon and Gwyneth Davies who patiently proof read this thesis.

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# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AREST-CF | Australian Respiratory Early Surveillance Tests for Cystic Fibrosis |
| ATS: | American Thoracic Society |
| BAL: | Broncho-alveolar lavage |
| CF: | Cystic Fibrosis |
| CFTR: | Cystic Fibrosis Transmembrane Receptor |
| CRF: | Clinical Record Forms |
| CT: | Computed Tomography/s |
| CXR: | Chest radiograph/s |
| ERS: | European Respiratory Society |
| $FEV_{0.4}$: | Forced Expired Volume in 0.4 second (mL) |
| $FEV_{0.5}$: | Forced Expiratory Volume in 0.5 second (mL) |
| $FEV_1$: | Forced Expiratory Volume in one second (mL) |
| $FEF_{75}$: | Forced Expired Flow when 75% of FVC has been expired ($mL \cdot s^{-1}$) |
| $FEF_{25-75}$: | Forced Expiratory Flow between 25% - 75% of expired FVC ($mL \cdot s^{-1}$) |
| FEFV | Forced Expiratory Flows and Volumes |
| FRC-$N_{2:}$ | Functional Residual Capacity (mL) obtained using nitrogen MBW technique |
| $FRC_{MBW}$: | Functional Residual Capacity (mL) obtained using $SF_6$ MBW technique |

$FRC_{pleth}$: Functional Residual Capacity (mL) obtained using body plethysmography

FVC: Forced Vital Capacity (mL)

GA: General Anaesthesia

Gas trapping/ Trapped gas: Difference in FRC measured using plethysmography and MBW techniques (i.e., $FRC_{pleth}$-$FRC_{MBW;}$ $\Delta FRC$)

GORD: Gastro-oesophageal reflux disease

HC Healthy control

*HI:* *Haemophilus influenza*

HRCT: High Resolution Computed Tomography

IL-8: Interleukin 8

ILFT: Infant Lung Function Test/s

IRT: Immunoreactive trypsinogen

LCFC: London Cystic Fibrosis Collaboration

LCI: Lung Clearance Index

LFT: Lung Function Test/s

MBW: Multiple Breath Inert Gas Washout

MI: Meconium Ileus

mSv: milliSievert; a unit of measure for effective radiation exposure

NBS: Newborn Screening

NE: Neutrophil elastase

NPV: Negative Predictive Value

*PsA:* *Pseudomonas aeruginosa*

PEEP: Peak End Expiratory Pressure

PIP: Peak Inspiratory Pressure

PNT: Pneumotachometer

$P_j$: Jacket Compression Pressure

PPV: Positive Predictive Value

rhDNase: Recombinant DNase

RV: Residual volume

RVRTC: Raised Volume Rapid Thoraco-abdominal Compression

*SA:* *Staphylococcus aureus*

SD: Standard deviation

$SF_6$: *Sulphur Hexafluoride*

$sR_{aw}$: Specific airway resistance

$sR_{eff}$: Specific effective airway resistance as a measure of airway patency

TLC: Total Lung Capacity

$V'_{max\ FRC}$: Maximal flow at Functional Residual Capacity (mL)

3m: 3 months of age

1yr: 1 year of age

# SUMMARY OF PUBLICATIONS AND AWARDS RELATED TO THE THESIS

## *Peer reviewed publications (Appendix A1)*

- **Is Chest Computed Tomography Useful in Newborn Screened Infants with Cystic Fibrosis at One Year of Age?**
  **Thia LP**/ Calder A, Stocks J, Bush A, Owens CM, Wallis C, Young C, Sullivan Y, Wade A, McEwan A and Brody AS on behalf of the London Cystic Fibrosis Collaboration (LCFC)
  Thorax 2013 online first doi: 10.1136/thoraxjnl-2013-204023

- **Evolution of Lung Function during the First Year of Life in Newborn Screened Cystic Fibrosis Infants**
  Nguyen TTD/ **Thia LP**, Hoo AF, Bush A, Aurora P, Wade A, Chudleigh J, Lum S, and Stocks J, on behalf of the London Cystic Fibrosis Collaboration (LCFC)
  Thorax 2013 online first doi: 10.1136/thoraxjnl-2013-204176

- **Recruiting infants with CF and controls to an observational study**
  Chudleigh J, Hoo AF, Ahmed D, Prasad A, Sheehan D, Francis J, Buckingham S, Cowlard J, Lambert C, **Thia LP**, Nguyen TTD, Stocks J, on behalf of the London Cystic Fibrosis Collaboration (LCFC)
  J Cystic Fibrosis 2013; 12(3): 234-240.

- **Lung Function is abnormal in 3-month-old infants with cystic fibrosis diagnosed by newborn screening**
  Hoo AF, **Thia LP**, Nguyen TTD, Bush A, Chudleigh J, Lum S, Ahmed D,Balfour Lynn I, Carr SB, Chavasse RJ, Costeloe KL, Price J, Shankar A, Wallis C, Wyatt H, Wade A, Stocks J, on behalf of the London Cystic Fibrosis Collaboration (LCFC)
  Thorax 2012; 67: 874-881.

- **Evaluation and use of childhood lung function tests in cystic fibrosis**
  Stocks J, **Thia LP**, Sonnappa S
  Curr Opin Pulm Med 2012;18(6):602-608. (Review)

- **New reference equations to improve interpretation of infant lung function**
  Nguyen TTD, Hoo AF, Lum S, Wade A,**Thia LP**,Stocks J
  Pediatr Pulmonol 2012;48(4):370-380.

## *Abstracts accepted for oral and/or poster presentations (Appendix A1)*

- **Stable lung function is maintained over 2 years in newborn screened (NBS) CF infants**
  **Thia LP**, Hoo AF, Brennan L, Nguyen TTD, Chudleigh J, Wade A, Wallis C, Bush A, Ruiz G, Pao C, Stocks J on behalf of London Cystic Fibrosis Collaboration (LCFC) ERJ 2013; Vol 42; Suppl 57, 1072s. (oral presentation at the Annual ERS Congress, Barcelona, Spain, Sept 2013).

- **Significant bacterial infection missed using cough swabs compared to bronchoalveolar lavage in 1-year old newborn screened CF infants.**
  **Thia LP**, Rand S, Hill L, Prasad SA, Bush A, Balfour-Lynn IM, Pao C, Ruiz G, Stocks J, Wallis C, Suri R on behalf of London Cystic Fibrosis Collaboration (LCFC)
  J Cystic Fibrosis 2013; Vol 12; Suppl 1: p.S1 (oral presentation at the 36th ECFS Conference, Lisbon, Portugal June 2013).

- **High Resolution Computed Tomography (HRCT) in One Year Old CF Newborn Screened (NBS) Infants: Not a Useful Outcome Measure**
  **Thia LP**, Calder A, Owens C, Stocks J, Bush A, Wallis C, Brody A, on behalf of the London CF Collaboration
  Ped Pulm 2012 Supplement 35, Pg 350 (oral and poster presentations at the 26th Annual NACFC, Florida, Oct 2012).

- **Lung Function and Structure in CF Infants diagnosed through Newborn Screening**
  **Thia LP**, Calder A, Owens C, Brody A, Hoo AF, Nguyen TTD, Chudleigh JC, Carr SB, Wallis C, Bush A, Stocks J, on behalf of the London CF Collaboration
  Journal of Cystic Fibrosis June 2012, Vol 11 Suppl. 1, S15 (oral presentation at the 35th ECFS Conference, Dublin, Ireland June 2012).

- **Improvement in lung function during the first year of life in infants diagnosed with cystic fibrosis through newborn screening**
  **Thia LP**, Hoo AF, Nguyen TTD, Ahmed D, Lum S, Chudleigh J, Wallis C, Bush A, Wade A, Stocks J on behalf of the London CF Collaboration
  Eur Respir J 2011; 38: 848s (oral presentation at the Annual ERS Congress, Amsterdam, The Netherlands Sept 2011).

- **Early detection of lung disease in infants with cystic fibrosis diagnosed by newborn screening**
  **Thia LP**, Stocks J, Hoo AF, Chudleigh J, Prasad SA, Lum S, Bush A, and Wallis C on behalf of the London CF Collaboration
  Ped Pulm 2010 Supplement33, Pg 390 (oral and poster presentations at the 24th Annual NACFC, Baltimore Oct 2010).

## *Awards related to the thesis*

- **UCL Institute of Child Health Poster Competition Nov 2012**
  specially commended in the Year 2 category.

- **UCL Deans Travel and Graduate School Conference Fund 2012**
  awarded for attending and giving an oral presentation at the North American Cystic Fibrosis Conference in Orlando, USA in Oct 2012. *Abstract submitted was one of the finalists in the Junior Investigator Best Abstract in Clinical Research Award.*

- **British Lung Foundation (BLF) Travel Fellowship 2011**
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# 1 INTRODUCTION

## 1.1 BACKGROUND ON CYSTIC FIBROSIS

Cystic Fibrosis (CF) is the most common life shortening autosomal recessive condition in the white population; with an incidence of 1:2000-3000 live births.[1] Currently there are 9,000 CF patients in the UK (http://www.cftrust.org.uk/) and 30,000 in the USA (http://www.cff.org/). Although it is a multi-system disease, lung disease leads to most of the increased morbidity and reduced lifespan.[2,3]

CF may present at any time from antenatal period to extreme old age, although it usually presents within the first two years of life with recurrent respiratory symptoms (cough, wheeze or respiratory failure) and failure to thrive. Less common presentations are meconium ileus, rectal prolapse or electrolyte imbalance. Increasingly, CF has been diagnosed through newborn screening (NBS).

CF is caused by mutations in a single gene on the long arm of chromosome 7, which encodes the cystic fibrosis transmembrane receptor (CFTR) protein. The absence or reduced function of CFTR protein is the fundamental defect in CF. The most common mutation is a three base pair deletion that codes for phenylalanine at position 508 of the CFTR protein which accounts for 70% of CF alleles in the white population.[4] Advances in genetic and molecular biology have provided increased understanding into the functions of the CFTR protein and the pathophysiology of CF. This has led to a paradigm shift in therapy for CF, from targeting the downstream consequences of *CFTR* dysfunction, such as bronchial infection, inflammation and mucus retention to the development of therapies correcting the basic defect, including gene therapy and genotype class-specific therapies, such as $PTC_{124}$ to over-ride premature stop codons [5,6] and VX-770 for the class 3 gating mutation G551D.[7,8] It would seem likely that these novel therapies would be most effective in early stage disease, before irreversible airway damage has developed.

### 1.1.1 Pathophysiology and natural history of CF lung disease

CF primarily affects the airways and submucosal glands with sparing of the interstitium until late in the disease. CFTR is highly expressed in the serous epithelial cells of submucosal glands where it regulates chloride secretion and water transport across the cell membrane in all exocrine glands of the body. The precise mechanism by which *CFTR* dysfunction produces CF lung disease is unclear.

In normal airway epithelia, there is an airway surface liquid layer (ASL) which consists of two layers above the epithelial surface - a mucus layer and a periciliary liquid layer (PCL); the latter has a thickness similar to the length of the cilium. The PCL is highly controlled by an adenosine-regulated pathway to provide a low viscosity solution for mucociliary clearance. The current hypothesis is that as a result of absent or dysfunctional CFTR protein, there is defective chloride and water transport due to over-activity of the sodium channel ENaC, leading to abnormally elevated isotonic fluid absorption.[9,10] This depletes the adenosine regulated PCL, reduces mucociliary clearance and encourages mucus stasis and chronic infection due to reduced clearance of micro-organisms (low-volume hypothesis). The PCL is vulnerable to CF microbial insults especially viral infections such as respiratory syncytial virus which diminish motion-dependent ATP regulation of the CF PCL height and volume.[11] Increased ASL salt concentrations have also been reported in CF which would inactivate salt-sensitive antimicrobial peptides (high-salt hypothesis) and predispose CF patients to bacterial infections; however, evidence for this hypothesis is less compelling. It has been suggested from the newborn CF porcine model that lack of CFTR results in lower pH in the ASL i,e a more acidic environment which impairs the antimicrobial activity of ASL. This group hypothesised that *CFTR* dysfunction also affects bicarbonate ($HCO_3^-$) transport leading to defective secretion of $HCO_3^-$, lower ASL pH and hence inhibits antimicrobial function and impairs bacterial killing.[12] Although the 'low volume' hypothesis is favoured, these hypothesised mechanisms on the ASL in CF could lead to defective airway clearance and defence, thus promoting endobronchial infection in young children with CF.[13,14]

Pulmonary involvement is present early, with some CF infants having evidence of inflammation in the bronchoalveolar lavage fluid (BALF) as early as 4 weeks of

age.[15,16] Two main components of CF airway disease are chronic infection and an exuberant host inflammatory response. The most characteristic feature of inflammation within the lung in CF is the infiltration of enormous numbers of neutrophils into the airway lumen.[17] This massive excess of neutrophils is harmful to the lung and is at the centre of a vicious cycle of increased inflammation.[18] Neutrophils undergo necrosis in the airway lumen and these necrotic neutrophils are the major source of the DNA that makes CF sputum so tenacious.[17] These neutrophils release an array of tissue damaging mediators, oxidants and proteases, including neutrophil elastase (NE). Free neutrophil elastase causes uncontrolled proteolysis and chondrolysis of airway support tissue, resulting in damaged airways which eventually become dilated and then bronchiectatic.[19]

The relationship between infection and inflammation in the pathogenesis of CF lung disease is unclear, but the ultimate result of this cycle of infection and inflammation is damaged airways (bronchiectasis), progressive airway obstruction and impaired gas exchange. As airways disease worsens, there is an increased likelihood of serious respiratory complications, including pneumothorax, haemoptysis, and respiratory failure and death.

### 1.1.2 Life expectancy in CF

CF was formerly known as a 'killer disease' of childhood. However, current increased life expectancy means that more than 50% of CF patients in the UK are adults. It has been estimated that those born in the current decade will have a median life expectancy at greater than 50 years.[20] With increasing global uptake of NBS for CF and advanced CF therapies to optimise nutrition and pulmonary health, current CF babies may have an even longer life expectancy. Prognosis for those born with CF diagnosed through NBS has improved dramatically over the years.[21]

## 1.2 NEONATAL SCREENING FOR CF

### 1.2.1 Worldwide and national experience

The first European experience in CF NBS started in the early 1970s with screening programmes that examined the albumin content of meconium.[22] Elevation of blood

immunoreactive trypsin (IRT) in the blood spots of neonates with CF was first described in 1979[22] which led to screening programmes being developed and introduced in Australasia and parts of Europe. Identification of *CFTR* gene mutations in the 1990s with subsequent incorporation of DNA testing into screening protocols led to further improvements in screening programmes, and an increase in the number of countries implementing neonatal screening. There are now eight countries in the European Union,[23] the entire USA (www.cff.org), Australia[24] and New Zealand (www.cfnz.org.nz) who have adopted universal NBS for CF. In Canada, there is currently regional NBS (five Canadian provinces and two territories) available (www.cysticfibrosis.ca). Since October 2007, universal NBS for CF has been implemented in the UK.

There are several different screening protocols currently in use but in general they involve a combination of measurement of IRT and *CFTR* mutation analysis followed by confirmatory sweat testing. In the UK, screening for CF is incorporated into the routine neonatal screening using the blood spot obtained from a heel prick test on the Guthrie card taken 6-7 days after birth. The screening protocol used in the UK consists of IRT-DNA analysis. If measured IRT level is elevated to levels >60 ng/ml (>99% centile), the next step of the screening protocol will include *CFTR* gene mutation analysis for the 4 most common mutations on the same sample. Infants found to be homozygous (or compound heterozygous) for known disease producing CF mutations are immediately referred for a confirmatory sweat test. Those with only one CF disease producing gene undergo a second IRT 4 weeks later in addition to extended *CFTR* DNA analysis (29 or 31 mutations). If the second IRT is still raised, they have a sweat test.[25] Currently, the median age of diagnosis in the UK through CF NBS is 1 month[2,26] while in the US, it is 2.3 weeks.[27,28]

### 1.2.2 Benefits versus disadvantages of newborn screening

The effect of CF NBS has been extensively studied and debated. Evidence from the Wisconsin Cystic Fibrosis Neonatal Screening Project [29-32] (the only randomised controlled trial of NBS) and observational studies from other countries[33-35] is overall strongly suggestive of benefits, especially in terms of nutritional status. With CF NBS, a normal growth pattern can be achieved and maintained. Better nutrition and fat

soluble vitamin levels were associated with better cognitive abilities in children with CF diagnosed through NBS. [31,32]

Evidence that NBS results in improvement with pulmonary health in CF is less clear. The Wisconsin group did not demonstrate better chest radiographic scores or lung function parameters likely due to confounding factors (see below). They showed that at the time of CF diagnosis for either screened or clinically diagnosed groups, quantitative radiographic scores were better for those who were diagnosed early through screening. However when a CXR was repeated later (mean age of 10 years), the mean Brasfield and Wisconsin CXR scores in the screened group were significantly worse than the clinically diagnosed group. Scores were worse in those who had *Pseudomoas aeruginosa* (*PsA)* infection, which accelerated radiographic deterioration.[36] The result of this study was confounded by the fact that one of the older CF centres did not have robust infection control policies which resulted in cross infection within the NBS CF population attending routine outpatient clinics. A British study also failed to demonstrate any pulmonary benefits with screening, although patients in this study were not managed by specialist CF centres using standardised treatment protocols.[37] In contrast, a French[34] study compared a screened CF cohort in one city to that in a neighbouring city in which CF was only diagnosed clinically and found that Brasfield CXR scores in the screened cohort were better than the non-screened CF cohort at all ages to 10 years of age despite similar treatment protocols. In a cross sectional Dutch study, significantly better radiographic scores were seen in a screened CF cohort than non-screened at diagnosis and at 9 years of age. [38,39] Both these studies also showed stable lung function and less progressive decline in the NBS CF cohorts compared to the clinically diagnosed CF cohort.[34,40]

Those with CF diagnosed through NBS have better quality of life, less morbidity and better survival.[21,27,29,30,41] A recent long term longitudinal study in Australia provided the first evidence that spirometric outcomes were superior in NBS CF children at transfer to adult care compared to those who were diagnosed clinically. In the few years just prior to the introduction of screening, the NBS CF cohort also showed improved nutrition during childhood and improved survival at age 25 years.[21] Compared to diagnosis made clinically, NBS is associated with reduced treatment

costs due to the improved clinical status hence NBS for CF might have not only clinical and social benefits, but also economic benefits to society.[42]

NBS for CF has potential disadvantages. In the Wisconsin study, prior to the enforcement of segregation, screened children who were cared for in a specialist centre acquired *PsA* at an earlier age which may explain why no pulmonary benefit was detected in the screened population.[36] With strict infection control measures and segregation clinics, this should no longer be of concern. In fact, from US[27] and UK[2] databases, screened CF children have less chronic infection with *PsA* than those diagnosed clinically. There were concerns that parental bonding may be affected by the early diagnosis through NBS of a child having a life-limiting condition. Breaking bad news to the parents must be handled sensitively to reduce the inevitable shock and anxiety. However some parents may gain consolation that the condition has been identified before any significant lung disease or poor nutrition had occurred. NBS could result in earlier diagnosis of mild and atypical CF at an age when they may never have been diagnosed clinically. It could also detect significant numbers of CF carriers which may increase parental anxiety and have wider implications for the extended family. As with all NBS screening, some cases will be missed (i.e. false negative cases).[43,44] There are concerns that doctors might assume that a child cannot have CF if they have tested negative after the introduction of universal NBS, which could lead to a delay in diagnosis.[45]

Nonetheless, on balance, the evidence in favour of CF NBS outweighs the potential disadvantages,[26,46] such that universal CF NBS is increasingly implemented worldwide.

## 1.3 EARLY DETECTION OF LUNG DISEASE AND OUTCOME MEASURES

The first two years of postnatal life is a particularly important period for normal lung development when the lung is undergoing rapid alveolarisation.[47] During this period the lung is particularly sensitive to noxious insults, which may have profound long-term consequences.[48,49] The airways of CF infants are probably virtually normal at birth and it is crucial to institute treatment early to try and prevent the onset of

pulmonary infection and inflammation. However, it is during this same period that the developing lungs are most susceptible to iatrogenic damage; and when objective monitoring of response to treatment is most difficult.

In the past, clinical therapeutic trials in infants and young children with CF have been hindered by a lack of sensitive and reproducible outcome measures. However in the last decade, significant progress has led to physiologic, structural, bronchoscopic and clinical measures that may serve as reliable and feasible endpoints for future intervention trials in young children with CF.[50] These techniques include infant lung function tests (ILFT)[51] which provide physiological measures, structural markers of disease through various imaging techniques (primarily computed tomography (CT) of chest)[52] and markers of infection and inflammation through BAL.[53,54] For all these outcome measures, attempts have been made to standardise data collection and analysis which, in some cases such as ILFT, have been achieved. One of the main limiting factors for either clinical or research applications of these outcome measures has been the lack of appropriate reference values or contemporaneous healthy control data,[55] together with limited information regarding short or long term repeatability and a paucity of longitudinal data using these tests. In addition, these tests are time consuming, expensive and highly specialised, usually requiring the expertise of tertiary respiratory centres.

The research presented in this thesis addresses gaps in current knowledge regarding the evolution of early lung disease in NBS CF infants, using physiological and structural measures. In the remainder of this chapter, I summarise the background methodology for the various ILFT used for this thesis, together with that for chest CT and the rationale for their use in the early detection of CF lung disease. This will be followed by a literature review of current knowledge until December 2011 about ILFT and chest CT in infants with CF diagnosed clinically or through newborn screening.

## 1.4 INFANT LUNG FUNCTION TESTS

### 1.4.1 Background

Preservation of lung function will likely reduce morbidity and mortality in infants and children with CF. Regular monitoring of lung function has long been considered an essential part of the clinical management of older children and adults, whereas in infants and pre-schoolers, this remains a challenge and ILFT is considered a specialised test conducted only in a few laboratories.

During the past decade, commercial equipment for assessing a wide range of lung function tests in infants has become available together with international guidelines and improved reference equations with which to interpret results.[56-61] Early physiologic abnormalities have been detected in infants with CF using a variety of ILFT techniques,[60,62-67] including plethysmography, forced expiratory flow-volume manoeuvres and multiple breath washout/ gas dilution techniques. The commonest lung function abnormalities described in CF lung disease have been airway obstruction, [60] hyperinflation[62] indicated by elevated resting lung volumes, increased ventilation inhomogeneity and gas trapping.[63,64]

### 1.4.2 Methodologies of different infant lung function tests

For each of the different ILFT [plethysmography, raised volume rapid thoraco-abdominal compression (RVRTC) and multiple breath washout (MBW)], a brief background into the physiology of the test and the rationale for conducting these tests in this research study will be described. A detailed account on how these lung function tests are performed will be reported in chapter 2.

#### 1.4.2.1 Plethysmography

Plethysmography was one of the first techniques used to assess lung function abnormalities in sedated infants and young children with CF. [62,68-73] Historically, only custom-made equipment was available. There is now however commercial equipment available with appropriate reference equations[59] based on healthy control infants measured using the same equipment (Jaeger MasterScreen Body Plethysmograph). International ATS/ERS guidelines are also available to ensure that plethysmographic

measurements of FRC are collected in a standardised manner and as accurately as possible.[61,74] (**section 2.3.2**)

***Theoretical background***

Plethysmographic Functional Residual Capacity ($FRC_{pleth}$), i.e. the resting lung volume at end expiration, is the only static lung volume that can be measured routinely in infants. It measures the total thoracic gas volume, including areas of trapped gas during occluded breathing efforts against a closed shutter, based on Boyle's law. Boyle's law states that for any given mass of gas at a fixed temperature, pressure multiplied by volume remains constant. Assessments of $FRC_{pleth}$ are made while the sleeping infant lies within the closed plethysmograph and breathes through a facemask attached to a pneumotachometer (PNT) which records air flow (and hence volume). A shutter is used to occlude the airways for 6-8 seconds during tidal breathing, retaining a fixed mass of gas in the lungs. During this period, the infant continues to breathe against the occlusion, which causes cyclic expansion and compression of this gas volume. Such changes in lung volume are measured as changes in box pressure while, in the absence of any airflow, the accompanying changes in alveolar pressure are assumed to be the same as pressure changes at the airway opening. By knowing the initial pressure in the lungs (which is atmospheric at end expiration) and the associated changes in alveolar pressure and volume, it is possible to calculate the only unknown variable i.e. the initial lung volume. When performing this technique in infants, the occlusion is usually performed at the end of tidal inspiration, rather than at end expiration as in adults, since for infants this is less disturbing, causes less glottal closure and facilitates improved equilibrium of pressures throughout the respiratory system when compared with end expiratory occlusions.[75] $FRC_{pleth}$ is then obtained by subtracting the tidal volume above the end-expiratory level from the measured thoracic gas volume.[61]

There are several advantages of infant plethysmography which include the following:

- Measurements of $FRC_{pleth}$ can be obtained rapidly and reproducibly.
- Although subject to potential errors which will be discussed later, the difference between paired measurements of FRC obtained by plethysmography and gas dilution technique (see **section 1.4.2.3**) may be a useful reflection of gas trapping.[76]

The major limitations of infant plethysmography are:

- Equipment is expensive and relatively bulky, and therefore cannot be used as a bedside test.
- Overestimation of FRC may occur in the presence of severe airway obstruction, due to poor equilibration of alveolar pressure changes with those occurring at the airway opening during airway occlusions.

Early CF lung disease manifests itself as airway obstruction in the smaller and distal peripheral airways during the initial stages of pulmonary involvement, followed later by obstruction and destruction of more proximal larger airways.[77] An increase in $FRC_{pleth}$ may indicate either dynamic hyperinflation or gas trapping. In the presence of increased airways resistance due to reduced airway calibre, and hence a prolonged expiratory time constant, there may be insufficient time during expiration to empty the lungs to the relaxed elastic equilibrium volume that determines FRC in health. This is particularly likely to occur in the presence of a rapid respiratory rate and hence short expiratory time and results in dynamic hyperinflation. This phenomenon can also occur in healthy infants during the first months of life due to the high compliance of the chest wall and rapid respiratory rates. By contrast, there may be true 'gas trapping' secondary to virtually complete obstruction of some of the smaller airways with secretions, leading to very poorly ventilated areas of the lung. While either phenomenon will result in an increase in $FRC_{pleth}$, when using gas washout methods, measured FRC is likely to be increased in the presence of dynamic hyperinflation, but decreased in the presence of gas trapped behind virtually closed airways, such that assessment of the difference between the two techniques (plethysmography−gas mixing) may be very informative. However, such data do require careful interpretation as if the obstruction is too severe, plethysmographic lung volumes will be erroneously over-estimated due to the poor equilibr ation of alveolar pressure with those at the airway opening.[74] If both dynamic hyperinflation and gas trapping occur, FRC by gas washout may appear relatively normal, but ventilation will be very uneven, further complicating the interpretation of these tests. Nevertheless, plethysmographic lung volumes have been recognised as a potentially sensitive marker of early CF lung disease[62,71,72] and were therefore performed as part of this research protocol.

#### 1.4.2.2 Raised Volume Rapid Thoraco-abdominal Compression Technique

Spirometry is an accepted monitoring tool for school-age children and adults with CF. Modification of this technique for sedated infants has made it possible to obtain forced expiratory flow-volume measurements during either tidal breathing (tidal (or partial) Rapid Thoraco-abdominal Compression (RTC) or 'tidal squeeze' technique), where the main outcome is maximal flow at FRC ($V'_{maxFRC}$) or in the form of the Raised Volume Rapid Thoraco-abdominal Compression (RVRTC) technique which allows full expiratory manoeuvres to be obtained after inflating the infant's lungs towards total lung capacity (TLC).[78]

***Theoretical background***

Since the introduction of the RVRTC technique, the 'tidal squeeze' method to detect airway obstruction has been largely superseded. This is due to limitations related to the variability of FRC in infants and potential overestimation of $V'_{maxFRC}$ in the presence of any gas trapping or hyperinflation which reduces the sensitivity of this outcome.[79,80] Flow limitation may also be more difficult to achieve in healthy controls when using the 'tidal squeeze' method.[80] In contrast, the raised volume technique assesses flow from a reproducible lung volume and flow limitation can usually be achieved, resulting in more reliable and reproducible results [78]

When using the RVRTC technique to produce full forced expiratory manoeuvres (Section: 2.3.3), relaxation of the respiratory muscles and a respiratory pause is induced by inflating the lungs of sleeping infants several times towards TLC. Once relaxed, the infant's lungs are inflated to 30 $cmH_2O$ pressure and a forced expiratory manoeuvre is then produced by applying rapid thoraco-abdominal compression with an inflatable jacket. The forced involuntary expiratory manoeuvres undertaken after the lungs have been inflated towards TLC during the RVRTC technique are similar to the voluntary expiratory manoeuvres undertaken by older children and adults in spirometry. Hence it is possible to obtain 'adult-type' flow-volume curves through this method which can be repeated and monitored long term.

In contrast to the relative insensitivity of conventional spirometry in children with CF,[63,64,81] the RVRTC has been found to discriminate clearly between infants with CF and healthy controls.[60,62,82,83] RVRTC has shown that CF infants have airway obstruction, and this method was found to be as sensitive as the lung clearance index

(LCI), measured through MBW.[83](see **section 2.3.1**). Possible explanations for this age-related discrepancy in relative sensitivity include differences in measurement conditions and developmental changes. The chest wall is highly compliant during infancy which results in more airway closure and early flow limitation in the presence of milder airway disease than in older individuals.[75,83] Infants have relatively large airways compared to lung volume at birth and therefore have a shorter expiratory time constant than older individuals, with relatively rapid lung emptying in less than a second during forced expiration. Consequently, it is not always possible to obtain forced expiratory volume in one second ($FEV_1$) in very young children, which is commonly substituted by the measurement of forced expiratory volume in 0.5 second ($FEV_{0.5}$) or forced expiratory volume in 0.75 second ($FEV_{75}$) in infants and pre-schoolers respectively. Further work is required to assess the relationship between these different outcomes during early life.[84]

Potential advantages of RVRTC include:

- Forced expiratory flow volume (FEFV) outcomes that can be measured from a reproducible lung volume.
- Forced expiratory flows and volumes can be assessed over an extended volume range from near TLC to Residual Volume (RV).
- Easier to obtain flow limitation with RVRTC.
- Longitudinal assessments of similar outcomes are possible from infancy to adulthood.

There are several limitations associated with RVRTC which include:

- Although extensive training of specialised dedicated staff can ensure precision with respect to timing and inflation pressures, it is a more demanding technique compared to tidal flow-volume manoeuvres.
- Leaks are more likely to occur around the face during positive pressure inflations.
- Children with severe airway disease may not relax sufficiently or may consistently inhale before RV is reached.
- Repeated inflations may result in the accumulation of gas in the stomach which may be uncomfortable for the infant and invalidate the results.

- Considerable caution is required in infants who are oxygen-dependent, in whom repeated lung inflations, with associated reductions in $pCO_2$ might lead to prolonged apnoea and hypoxia.

Commercial equipment is internationally available with which to perform the RVRTC in sedated infants. With recent publication of reference data[57,59] and international guidelines[56] it is now possible to ensure standardised performance of tests for data collection and analysis, provided adequate training is received. Forced expiratory volumes and flows can be obtained through these methods.

#### 1.4.2.3 Multiple Breath Inert Gas Washout Technique

MBW is a tidal breathing test that is potentially beneficial in assessing early lung disease in infants and young children as it requires only passive cooperation.[85] In children from 3 years of age, there has been increasing evidence that the MBW technique is more sensitive than conventional spirometry in detecting early pulmonary changes, and there has been considerable interest in the use of MBW to detect early CF lung disease in infants.[83,86]

Conventional spirometry, the most commonly used lung function test (LFT) records forced expiratory flows and volumes which are primarily influenced by changes in airway resistance during linear gas flow in the conducting airways. Gas transport and mixing by convection predominate in the conducting airway zone (defined as respiratory generation 0-16) where linear gas flow velocity is relatively high. When airways further divide into generations 17-23 (intra-acinar regions) there is a markedly increased total cross sectional area for gas exchange such that the linear gas flow velocity in the peripheral airways is very low. The resistance of the peripheral airways therefore makes a relatively small contribution to any expiratory flow limitation measured during spirometry. Despite representing 95% of the total airway volume, the peripheral or 'small' airways (arbitrarily defined as those with a luminal diameter of less than 2mm and corresponding to airway generations 8-23 which would include the gas exchanging units or 'alveoli') account for only 10–20% of the total airway flow-resistance in healthy adult lungs.[87] In addition, heterogeneous changes in distal airway function may be masked by increased flow through non-flow-limited distal airways. For these reasons, spirometry primarily reflects large rather than small airway

function, and is relatively insensitive to early small airway impairment which is where early CF lung disease generally starts.

***Theoretical Background***

Convective gas flow is the predominant transport mechanism in the conducting airways. Within the acinus, contribution of convective gas flow is minimal and the predominant transport mechanism is diffusion. A combination of these two mechanisms occurs in the region known as the convection-diffusion front located around the entry into the acini. Unevenness of ventilation distribution is present even in healthy lungs and is known as ventilation inhomogeneity. This is due to several mechanisms. Inhomogeneity in conducting airways arises due to differences in specific ventilation between large lung regions or between smaller lung units with differing mechanical properties such as decreased airway calibre leading to increased resistance and hence differences in time constants (lung filling and emptying). More distally within acinar airways, inhomogeneity may arise due to marked asymmetry of the lung with respect to the cross-sectional area at branch points and subtended lung volumes at branch points.[88] With the MBW technique, it is possible to measure ventilation inhomogeneity[89] including that occurring due to changes in small airway function when pathological processes affect the distribution of ventilation between different parallel pathways.

Although inert gas washout techniques have been available for the last 60 years, their use has been restricted mainly to research due to lack of commercially available equipment. In recent years, development of fast responding gas analysers and advancing computer technology, have facilitated intensive scientific work in this field. The development of commercially available devices based on photoacoustic/ infrared gas analysers[90,91] and ultrasonic transducer technology[86,92-95] could facilitate both clinical applications of this technique and its use as an outcome measure in multicentre trials, although reference data are currently limited.

***Tracer gas***

MBW can be performed using an inert tracer gas such a sulphur hexafluoride ($SF_6$), helium (He) or Argon (Ar) or by using 100% oxygen ($O_2$) to washout the resident nitrogen ($N_2$) within the lung. In infants due to the risk of apnoea or alteration in tidal

breathing by inhaling 100% oxygen for $N_2$-MBW,[96] the inert gas MBW technique has been more commonly performed using a tracer gas mixture (4% $SF_6$ and/or He, 21% $O_2$ and balanced $N_2$).[83,86,89] $SF_6$ is a synthetic colourless, odourless and tasteless inert gas. At concentrations used in clinical settings, it is non-toxic and has no known side-effects in humans. However it is a potential greenhouse gas and in several countries $SF_6$ mixture is not licenced for medical use, thus preventing it from being used as a routine clinical test. The use of 100% $O_2$ for $N_2$-washout is therefore an attractive alternative to inert gas MBW. $O_2$ is readily available for medical use, relatively cheap and reduces test duration as $N_2$-MBW does not require a wash-in phase and $O_2$ has no adverse effect on the environment.[95] However the potential effect on breathing patterns in infants using 100% $O_2$ is not clear. With young children beyond infancy, there is renewed interest in using $N_2$-MBW technique using one of the commercially available systems.

***Equipment***

There are currently two commercial systems which use ultrasonic technology, which vary according to where the ultrasonic transducer is located to measure changes in molar mass of gases ($N_2$, $SF_6$ or He). Mainstream ultrasonic equipment (Exhalyzer D, Eco Medics AG, Switzerland) using $SF_6$ has been validated in infants.[97,98] In this equipment, the sensor containing two transducers is mounted on opposite sides of the flow tube that transmit pulses crossing the subject's airflow. The lack of validated correction algorithms for the temperature and humidity fluctuations that influence assessment of molar mass may limit its utility beyond infancy.[95] The EasyOne Pro, ndd, Medical Technologies, Switzerland device has overcome some of these problems by limiting the use of the mainstream ultrasonic sensor to measuring flow, while using a side-stream ultrasonic transducer to measure molar mass, but this system has only been validated for use in older children[94] due to the larger equipment deadspace that precludes its use in infants. Although both these devices can potentially be used with $SF_6$ or $N_2$ as a tracer gas, to date, work in infants has only involved use of $SF_6$ with the mainstream Exhalyzer D, Ecomedics system, for reasons discussed previously.

A modified photoacoustic gas analyser (Innocor, Innovision, Odense, Denmark) uses a PNT and a highly sensitive side-stream infra-red gas analyser that is very sensitive to $SF_6$ and has been validated for use in adults and older children.[90] Due to a high gas sample flow of 120ml/min and a longer analyser response time, this device is not

currently suitable for measurements in infants and young children.[90] This system can also be used for nitrogen washout using infrared nitrogen analysers.

***Measurement outcomes***

Although numerous indices can be calculated from MBW, the simplest and most commonly used outcome variable that is sensitive and robust to changes seen in early CF lung disease in children and adults is the LCI. LCI reflects the ventilation or the number of FRC turnovers required to clear an inert tracer gas from the lungs, corrected for lung size. An increase in LCI would signify increased ventilation inhomogeneity (unevenness) or inefficient gas mixing which may occur even in the presence of mild peripheral lung disease.[77,81,89,99,100]

Until recently, LCI (which is internally adjusted for lung volume) was considered to be constant across all age groups in health but with increasing availability of data from young children and infants, it became apparent that values of LCI are higher in the first few years of life even when adjusted for both airway and equipment deadspace.[58,101] This is probably due to developmental physiological changes seen with rapid lung growth in this age group.[58,94] Although variability of LCI in healthy children and adolescents is low over a wide age range,[102] with minimal within- and between-test variability,[81,94] variability in infants may be greater. When using MBW procedures in studies of early lung disease or treatment effects, it is important that reported changes detected over time do not merely reflect alterations in respiratory pattern. Longitudinal data for ventilation inhomogeneity indices during normal lung development with age are needed.[103] Therefore although a potentially useful and sensitive outcome measure for longitudinal studies, results of LCI in infants with lung disease will need to be interpreted in light of early lung development by testing contemporaneous healthy controls.

Functional Residual Capacity ($FRC_{MBW}$) can also be measured using MBW techniques. Lung volume that readily communicates with central airways during tidal breathing can be measured through this method. MBW is unable to assess any volume of thoracic gas that is not contributing to ventilation i.e. any trapped gas and may therefore underestimate any hyperinflation or poorly ventilated areas of the lung secondary to airway narrowing.[96] By measuring the difference between FRC obtained

through plethysmography and MBW, it is possible to obtain a broad estimate of the amount of gas trapping present.[104] However, due to the relatively large variability in the measurement of lung volumes using MBW, the measurement of trapped gas may have limited use on an individual basis.

Potential advantages of the MBW technique include:

- It is useful for bedside measurements.
- It can be undertaken in all ages including unsedated preterm and fullterm infants during the first few months of life.[105,106]
- Provides an assessment of ventilation inhomogeneity.

Disadvantages of the MBW technique include:

- Only readily ventilated gas volume rather than any gas trapped behind closed or non-ventilated airways can be measured. This may lead to underestimation of lung volume.
- A prolonged duration of washout may be necessary in subjects with marked airway obstruction.
- Lack of commercially available and well validated equipment for MBW measurements; although this situation is currently being rectified.

LCI has been shown to be correlated closely with structural chest CT changes in older children.[64,107] CT has itself been advocated to be a sensitive surrogate marker of early CF lung disease even during infancy and early childhood.[108-111] The relationship between LCI and CT structural lung disease has not been established in infants diagnosed through NBS. Hence in this study, LCI measured through MBW with $SF_6$ as the inert tracer gas was performed to investigate this relationship and to establish its role as a sensitive surrogate marker of structural CF lung disease.

## 1.5 LITERATURE REVIEW OF LUNG FUNCTION TESTS IN CF INFANTS AND YOUNG CHILDREN

In recent years, an increasing number of studies have explored the potential role of ILFT in the clinical management of CF infants or as an objective clinical trial endpoint

or outcome measure for research.[60,62,67,82,83,112-115] Despite a wide range of tests shown to be well tolerated and feasible in infants, with plethysmography, RVRTC and MBW techniques being able to detect early CF lung disease, their application as clinical tools for routine assessments is limited by the need for sedation, highly specialised equipment and staff, inability to repeat frequently enough and lack of appropriate reference equations and information regarding between-test variability.[50] On the other hand, there is convincing evidence for the use of ILFT to provide objective research outcome measures involving CF infants.

**Sections 1.5.1** and **1.5.2** describe the current literature about early lung function results in CF infants and young children at the inception of this thesis in December 2011. Salient information from important research studies have been summarised and presented in tables which can be found in **Appendix A1** due to word constraints in the main thesis. I concentrated mainly on studies published in the last decade to the end of 2011 involving infants (≤2 years) and only briefly mention studies involving young children (≤5 years) or older children (≥6 years). In the **Appendix A1-a** summarises studies performed during the past decade which utilised ILFT to identify early functional change in either cross-sectional or longitudinal observational studies of CF infants (0-2 years). A review of earlier studies investigating lung function in infants with CF has been published by Gappa et al.[70] **Appendix A1-b** summarises studies during this period involving children (≥3years) with CF limited to studies where LCI was measured, otherwise the literature would be massive if all lung function studies on children were included. **Appendix A1-c** summarises interventional studies using lung function parameters to assess response to interventions in infants, younger and older children. Studies published from January 2012 will be discussed in the final discussion chapter. Finally, this is followed by **section 1.5.3** which describes the lung function results from the current London Cystic Fibrosis Collaboration (LCFC) cohort of NBS CF infants at 3 months of age. A brief summary of the use of LCI as an outcome measure in older children with CF during the past decade is presented in **section 1.6**.

### 1.5.1 Lung function changes in clinically diagnosed infants and young children with CF

The LCFC has shown that clinically diagnosed infants, including those without respiratory symptoms, have impaired airway function shortly after diagnosis[79,116] and that this persists into school age with no improvement despite specialist treatment in CF centres.[60,65,102] Lung function impairment was greater in those with previous *PsA* infections (even if apparently eradicated) with further reductions seen in the presence of wheeze on auscultation or recent cough.[65] Cross sectional studies, including those from the LCFC have reported that abnormal LCI was evident even when no deterioration was observed in parameters derived using standard tests such as spirometry[117] in preschoolers and older children and RVRTC in infants.[83] Furthermore, an observational longitudinal LCFC study showed that a normal LCI at age 3-5 years in clinically diagnosed CF children was strongly predictive of normal lung function at age 6-10 years.[102] In another longitudinal study involving LCI performed during infancy at time of clinical diagnosis of CF, infants were found to have elevated LCI. Tracking of LCI was present from infancy to school age, especially in those with the most severe disease. This study also showed that it was feasible to perform MBW tests in unsedated infants during their natural sleep.[105]

$FRC_{pleth}$ has also been demonstrated to be significantly higher in CF compared to healthy infants indicating hyperinflation due to small airway obstruction.[62,114] In a recent US multicentre study of infants with CF comprising of those diagnosed clinically or by NBS, $FRC_{pleth}$ was significantly elevated in CF infants by 2 years of age when compared with reference data from historical controls and was more feasibly conducted than RVRTC with respect to obtaining technically acceptable results across different centres with varying experience.[62]

Kraemer *et al* confirmed that LCI predicted abnormalities earlier in life and reflected a more reliable functional progression in 6-20 year olds with clinically diagnosed CF than $FEV_1$. Pulmonary hyperinflation, airway obstruction and ventilation inhomogeneity were associated with chronic *PsA i*nfection and specific CFTR genotypes. There was tracking of lung function from early childhood to adult life.[72,76]

Attempts to use ILFT to assess acute response to treatment in infants with CF have been limited due to the issue of sedation which is relatively contraindicated in acute exacerbations. A study of 11 symptomatic infants (mean age 102 weeks) who had ILFT at the start of a pulmonary exacerbation and 3 weeks later after treatment, unsurprisingly demonstrated significant improvements in $FEV_{0.5}$ and $FRC_{pleth}$ after a course of intravenous antibiotics for pulmonary exacerbation. Although lung function parameters changed in response to the intervention given, it is not clear how clinically useful this would be in guiding clinical management.[118] In an open label randomised cross over trial involving 9 clinically diagnosed stable CF infants, significant improvements were observed in $V'_{maxFRC}$ when infants were treated with nebulised DNase compared to nebulized normal saline,[113] suggesting that objective assessments might be feasible in CF infants by using ILFT as outcome measures.

LCI was sensitive and responded appropriately to interventional therapy in older CF children (aged 6-18 years) with mild lung disease ($FEV_1 \geq 80\%$ predicted) as seen by a reduction in LCI in those treated with nebulised DNase[119] and hypertonic saline[120] even though no spirometric changes were observed. These studies suggest that LCI may be sensitive to acute changes even when evidence of clinical benefit is lacking.

However, much less is known about the evolution of lung function in NBS CF infants. In my study, a range of lung function outcome variables (LCI, $FRC_{pleth}$, FEFV) were used to detect early lung disease in NBS CF infants. These outcomes were used for longitudinal assessments during infancy to improve understanding of the evolution of lung disease in NBS CF infants. At the inception of this thesis (2011), information with respect to lung function outcomes in NBS CF infants was largely limited to that provided by the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST-CF) group, as discussed in **section 1.5.2.**

### 1.5.2 Early lung function in newborn screened infants with CF

Newborn screening for CF has been in existence in Australia for nearly three decades. AREST-CF study is an early disease surveillance programme conducted at the Royal Children's Hospital, Melbourne and the Princess Margaret Hospital for Children, Perth. All infants diagnosed with CF, whether through positive NBS or clinically

(including meconium ileus) were included in this early surveillance study. Eligible CF infants underwent ILFT when well and stable and the median age at testing was 59 (range: 6-131) weeks in their first cross sectional study.[82]

In their first publication, the AREST-CF team reported that FEFV were normal in 68 NBS (50 exclusively diagnosed through NBS) and meconium ileus (8 presenting with meconium ileus) CF infants during the first 6 months of life, but declined rapidly thereafter compared to historical healthy controls.[82] The normal lung function may reflect the small number of infants tested at <6 months of age and the fact that the raised volume technique was performed at 20 $cmH_2O$ which was below the recommended inflation pressure of 30 $cmH_2O$ as stated in the ATS/ERS guidelines.[56] This meant that forced expiratory manoeuvres may not have been executed from TLC, reducing the sensitivity of detecting abnormalities in FEFV in very young NBS CF infants.

The same group in a later publication performed RVRTC on 37 CF infants of whom 28 were diagnosed through NBS from one centre using the standard ATS/ ERS guidelines with an inflation pressure of 30 $cmH_2O$.[67] They demonstrated diminished $FEV_{0.5}$ z-scores within the first 6 months of life with continued deterioration over the next two years of life.[67] The mean (SD) $FEV_{0.5}$ z-scores at the first, first year and second year visits were -1.4(1.2), -2.4(1.1) and -4.3(1.6) respectively. In both these studies, no contemporaneous healthy infants were tested and z-scores were derived from published data collected using entirely different equipment.

In a US multicentre evaluation of infant lung function involving ten CF centres between 2003 and 2006, 100 CF infants diagnosed either antenatally, through NBS, meconium ileus or clinically, underwent ILFT at mean (SD) age 14 (6.2) months. Compared to historical healthy controls, CF infants showed significantly diminished $FEF_{75}$ z-score; mean (95% CI): -0.52 (-0.78; -0.25) and elevated lung volumes, $FRC_{pleth}$ z-score; mean (95% CI): 1.92 (1.39; 2.45).[62] No reductions were seen in forced expiratory volumes. In this study, there was great variability in skill-mix and experience of the laboratories and hence measurement acceptability rates in lung function results. RVRTC measures were acceptable in 85% of tests performed in experienced centres compared to only 59% in less experienced centres, whilst FRC

measurements were deemed successful in both experienced (96%) and less experienced centres (85%). The low success rate for RVRTC measures could account for the lack of abnormal results seen in forced expiratory volumes. Once again lung function results were only compared to historical controls. Although these studies provided some information about the evolution of lung disease in NBS CF infants, there were several unanswered questions which led to the development of the current study (see **section 1.6**).

### 1.5.3 Early lung function in newborn screened infants: the LCFC study

The LCFC NBS study (see Chapter Three) took place from January 2009 until July 2011. Results from initial assessments at 3 months have been published recently,[71] and are summarised below.

Seventy nine CF infants and 54 contemporaneous healthy control (HC) infants were recruited and had their first lung function test measured at mean (SD) age of 11.4 (2.3) and 12.2 (2.0) weeks respectively. For clarity during these very early assessments, results from 8 infants with meconium ileus were excluded and data were reported from 71 CF infants diagnosed purely by NBS. However, when analysis was repeated including results from the 8 infants with meconium ileus, results obtained were not significantly different. With the exception of a slightly lower, statistically significant but clinically trivial difference in gestational age [CF-HC: Mean (95%CI) -0.9 (-1.4; -0.5) weeks] and birth weight [CF-HC: Mean difference (95%CI) -0.35 (-0.67;-0.03) z-score] in those with CF, background characteristics including the proportion of boys, those born to white mothers, pre- and postnatal exposure to tobacco smoke and maternal history of asthma were very similar when compared with contemporaneous controls. NBS CF infants were diagnosed by a median (IQR) age of 3.6 weeks (3.0–4.4).

All lung function measurements were expressed as z-scores to adjust for length, age and sex where appropriate.[57-59] Baseline measurements at ~ 3-months of age showed increased ventilation inhomogeneity reflected by significantly elevated LCI z-score (CF-HC: Mean difference [95% CI]: 0.51[0.10; 0.91] z-score; $p \leq 0.05$) and evidence of

hyperinflation (CF-HC: Mean difference [95% CI] $FRC_{pleth}$ z-score: 0.85 [0.43; 1.28]; p <0.001). There was also significant gas trapping observed in NBS CF infants compared to HC (CF-HC: Mean difference [95% CI] $\Delta FRC_{pleth}$-$FRC_{MBW}$ z-score: (0.48 [0.08; 0.88]; p≤0.05). Airway obstruction was seen in NBS CF infants, who had significantly lower $FEV_{0.5}$ z-scores (CF-HC: Mean difference [95%CI] z-score: -0.92[-1.29; -0.56]; p<0.001) and $FEF_{25-75}$ z-scores (-0.66[-1.10; -0.21]; p≤0.01) than controls. Passive lung mechanics (respiratory compliance and resistance; $C_{rs}$ and $R_{rs}$ respectively) had a high failure rate due to infants waking up prior to completion of test protocol or technically unacceptable data. With the exception of tidal volume, which was slightly higher (0.4 z-scores) in those with CF, there were no significant differences for any of the tidal breathing outcomes or passive respiratory mechanics.

To date, this is the largest prospective study of early pulmonary function in CF infants diagnosed by NBS. The study has a number of strengths:

- Contemporaneous control infants were prospectively recruited and tested by the same team using the same equipment (Jaeger BabyBody MasterScreen system, v.4.6) and lung function protocols as used for the NBS CF infants.[55]
- All CF and control infants were studied before 16 weeks of age in one single lung function laboratory by experienced staff and techniques were in accordance with international guidelines[56,74] thus minimising any potential methodological and analytical bias. This is in contrast to the two previous AREST-CF studies when infants and young children with CF had their first test at different ages (6 weeks to 30 months).
- The relatively large sample size of both CF and healthy controls provided 90% power to detect a 0.6 z-score difference in primary lung function parameters (i.e. LCI and $FEV_{0.5}$) at the 5% significant level between infant groups.
- Lung function data were expressed as z-scores to account for sex, age and body size at the time of testing. Reference data collected using the same equipment from healthy control infants provided appropriate reference equations for comparison of lung function in NBS CF infants.[57-59] This allowed accurate identification of the extent to which abnormalities in lung function were present in individual infants.

- A wide range of physiological investigations was undertaken, giving information on lung volumes ($FRC_{MBW}$, $FRC_{pleth}$) and both proximal and peripheral airway function ($FEV_{0.5}$, $FEF_{\%}$ and LCI). The chance of missing early changes in lung function was minimised by performing this variety of LFT.[51]

The results from this study indicated that despite early diagnosis and rapid implementation of therapy, including prophylactic antibiotics, a third of NBS CF infants have abnormalities of lung function within the first three months. The apparent clinical wellness of the cohort should not lead to complacency; prompt and aggressive treatment of any abnormal symptoms or clinical signs is vital.[54]

## 1.6 RATIONALE FOR 1-YEAR LUNG FUNCTION TESTS IN THIS RESEARCH PROJECT

Normal lung development is essential for attaining maximal lung health in adulthood.[49,122] Early life factors have a significant impact on subsequent development of lung disease. Genetic predisposition, antenatal insults such as maternal smoking,[123] preterm delivery,[124,125] intra-uterine growth retardation[126] and neonatal respiratory disorders as well as early environmental insults such as postnatal passive smoking and impaired growth and nutrition,[127] environmental pollution[128] and childhood respiratory infections[129] are all potential causes for preventing the attainment of maximal lung health which could lead to subsequent chronic obstructive pulmonary disease (COPD) in early adult life.

There is considerable evidence which exists to show that diminished airway function in infancy and early years do track through childhood and early adulthood. The longitudinal Tucscon study of wheezy infants revealed that those with wheezing early in life were more likely to have lower forced expiratory flows in the first year of life and continued to have the lowest lung function as adults.[130] A Dunedin cohort who had assessments at school age showed tracking of lung function from 9 to 26 years of age.[131] Tracking of lung function was also demonstrated by the Melbourne asthma cohort,[132] with lower lung function in those with asthma by 7 years of age (many of

whom developed COPD). Hence the monitoring of lung function early in life is important for detecting early lung disease. This may identify those most "at risk" of developing lung disease and minimise potential insults hence allowing lung development to reach its full potential. In the context of a CF child, the detection of early lung disease and the monitoring of lung function are vitally important aspects of CF care. Information obtained through ILFT may aid in the understanding of the evolution of early lung disease in CF infants and young children.

Deterioration in lung function within the first year of life may indicate the need for early aggressive or novel treatments using physiological endpoints to detect benefit. However if lung function improves or remains stable with conventional CF treatment, then novel, molecular- based therapies[5,7] may be deferred to a later date, at an age when it would be easier to monitor lung function and the potential risk of toxicity to the developing lung may be less.

Results from the AREST-CF study suggested that lung function deteriorates from 1-year and beyond, albeit when interpreting results in relation to published data or historical controls. It was therefore important to monitor lung function longitudinally in the current LCFC NBS cohort, in order to ascertain the extent to which the previously reported early changes in lung function persist throughout the first year of life. It is also essential to provide information on the natural history of lung disease in NBS CF infants, in direct comparison with contemporaneous healthy control infants. The ability to undertake identical serial measurements in healthy controls would facilitate interpretation of changes over time particularly with respect to the extent to which any longitudinal changes in lung function in those with CF were due to disease rather than normal growth and development. Such information would inform future study design with respect to calculation of sample sizes required for randomised control trials of treatment.

A wide range of lung function tests were conducted in this study to ensure that as far as possible, no significant lung disease would be missed. During infancy, measures of LCI and forced expiratory parameters appear to be complementary tests which do not necessarily identify the same infants with abnormalities. By including both tests, detection rate for early lung disease may increase.[83]

Early CF lung disease occurring first in the lung periphery may manifest as hyperinflation and gas trapping and/or ventilation inhomogeneity (see **section 1.5**). For these reasons, plethysmography and MBW techniques were used to obtain $FRC_{pleth}$ and $FRC_{MBW}$ results and LCI as described in **section 1.4.2.** The within-subject difference between $FRC_{pleth}$ and $FRC_{MBW}$ gives an indication of 'trapped gas'. Raised volume forced expiratory parameters measured during infancy are comparable to spirometric variables that will be performed throughout life. Passive lung mechanics parameters were not used in this 1 year study due to the high variability of results obtained and their lack of discrimination between CF and normal controls in the 3 month data[71]. Apart from that, all other lung function measurements mentioned above could be useful in the long term monitoring of CF lung disease, hence the decision was to repeat all these lung function tests at a year of age.

With the gap in knowledge on the evolution of lung function in NBS CF infants beyond the first three months of age as alluded to earlier (see **section 1.5**), this study was designed to improve understanding of lung function in these infants at a year of age when compared to contemporaneous healthy controls. The widespread uptake of NBS for CF has led to increased interest and a real need to develop and identify sensitive, appropriate and feasible surrogate markers for detecting early CF lung disease during infancy.[50,62,133-135]

The second section of this introductory chapter pertains to chest computed tomography as a potential sensitive surrogate marker of early CF lung disease.

## 1.7 LITERATURE REVIEW OF CHEST COMPUTED TOMOGRAPHY IN EARLY CF LUNG DISEASE

Imaging techniques that can detect structural changes in CF lung disease have been of great interest for many years. The argument for using chest CT as an outcome surrogate for monitoring progression of lung disease in CF patients is the ability of CT to detect subtle lung changes more readily than can conventional CXR or spirometry. Therefore clinical trials that involve infants and young children with mild CF lung

disease have increasingly advocated the use of chest CT as a clinical trial endpoint.[52,110,136]

### 1.7.1 Structural information from chest CT

Emerging evidence suggests that CF structural changes occur early in life[109,111,137,138] confirming previous autopsy results of clinically diagnosed children with CF showing significant structural changes such as bronchial wall dilatation, bronchial wall thickening and mucous plugging from as early as the first four months of life.[139] These are structural changes that one would hope to prevent with the introduction of NBS for CF.

Changes observed in different stages of CF lung disease are as follows:

- **Bronchiectasis-** defined as the size of the dilated bronchi relative to an accompanying vessel;
- **Peribronchial thickening**- defined as a bronchial wall thickness greater than 2mm in the hilar region, 1mm in the central lung, or 0.5mm in the peripheral lung;
- **Parenchymal changes**- opacities, areas of ground glass opacity, cysts or bullae;
- **Mucous plugging**- defined as the presence of bronchial opacification of the lumen, centrilobular nodules, or peripheral branching structures;
- **Air trapping**- defined as well-circumscribed areas of decreased parenchymal density on expiratory CT images.[140]

Although mucous plugging and air trapping are not necessarily structural airway abnormalities, they are prevalent in CF lung disease and can improve with appropriate treatment.

In established CF lung disease, a significant structural finding is bronchiectasis which is generally considered to be irreversible in the adult population. Although gross bronchiectasis might be detected on plain CXR, mild changes with regards to bronchial wall thickening or bronchial dilatation in young children would be difficult to detect on CXR. Chest CT could reveal more information with regards to lung disease however even if changes were identified, these changes may not necessarily be irreversible as CT images merely provide a snapshot view of lung disease at the time of imaging.[141] It is important to be cautious in giving a radiological diagnosis of

bronchiectasis in children as diagnostic criteria were derived from adult studies that have not been validated in children.[142]

Long *et al* showed that 10-20% of airways measured in 32 asymptomatic infants and toddlers with CF were bronchiectatic and 20% had bronchial wall thickening.[143] In another study by the same group,[137] clinically diagnosed and stable CF infants from 0-5 years of age compared to infants who had a CT for non- respiratory reasons had significantly thicker airway walls and more dilated airways. Martinez *et al*[138] also measured airway lumen and wall thickness in CF and control infants undergoing chest CT for non-respiratory reasons. Air trapping manifested by a significantly lower lung density at passive FRC was observed in CF infants compared to controls. Her group confirmed airway wall thickening in CF infants, however the airways were narrowed and not dilated as reported by Long *et al.*[137] Possible explanations for this discrepancy included the fact that the average age of infants studied by Long *et al* was higher and therefore lung disease may have been more severe.

Methodological differences with regard to different distending airway pressures during CT acquisition (**section 1.8.1.2**), different software for analysis and whether airway measurements were related to the diameter of the adjacent pulmonary artery must be considered carefully when designing future studies.

Even with early diagnosis through NBS, CF infants and young children had bronchial wall thickening and dilatation, mucous obstruction and air trapping and these changes were more evident in those with greater degree of airway inflammation and infection.[109,111] In the AREST-CF studies, radiologic evidence of structural lung disease on chest CT were common in infants at diagnosis and very young children.[109,111] Sly *et al* reported abnormal CT findings in 80% of infants who had a CT performed at median age of ~3months; ~20% had bronchial dilatation; ~45% had bronchial wall thickening and ~65% had air trapping.[109] Stick *et al* reported an incidence of bronchiectasis in the first year of life as 8.5%; this incidence increasing with advancing age such that a prevalence of 36% was reported by 4 years of age.[111] Both studies demonstrated significant associations between neutrophilic inflammation and structural CT changes. Increased inflammation was observed in the presence of

infection especially those with *PsA* infection and respiratory symptoms although the majority of infants who displayed inflammation and CT changes were clinically well.

Information regarding the evolution of these early structural changes in infant and young children remains limited. The only longitudinal study investigating structural lung disease of CF infants diagnosed through NBS is from the AREST-CF team.[108] In this study, 301 chest CT scans were performed in 143 NBS CF infants and young children aged 0.2-5.4 years. Median age of first scan was ~2 years (interquartile range, IQR: 1.2-3.3y) while the repeat scan was undertaken a year later at ~3 years (IQR:1.9-4.0y). Bronchiectasis was detected on the first scan in 44% of scans performed with bronchiectasis extent score reported as median 0 (IQR= 0-2) out of a maximum extent score of 12 per scan. With repeated scans a year later, bronchiectasis persisted in 74% while 26% resolved. Of those who had no evidence of bronchiectasis the previous year, it was present in 50% a year later. Air trapping was present in 73% on the first scan with a median extent score of 2 (IQR: 0-5). With repeated scans a year later, air trapping persisted in 80% while 20% resolved. However of those who did not demonstrate air trapping on first scans, ~50% developed the abnormality on subsequent scans. Radiological progression of bronchiectasis and air trapping was associated with severe CFTR genotype, worsening neutrophilic inflammation and pulmonary infection.

Results from these AREST-CF studies have provided useful insight into the early development of CF lung disease in NBS CF infants and the factors associated with persistence and progression of structural lung disease. The main limitation in these studies is the scoring system used. Only one radiologist scored the scans and the scoring system was not validated. As the authors stated, since there is currently no scoring system validated specifically for use in infants with CF they decided to use one based on a modification of the Brody-II scoring system.[108] However, their binary scoring system may have oversimplified the detection and quantification process by allocating a score simply based on the presence or absence of change and, when change was present, simply dichotomising the abnormality as occupying either less or more than half of the lobe assessed. In young CF infants where milder abnormalities would be expected, this binary scoring system could potentially over-estimate the abnormalities detected, leading to an over-estimation of the incidence of

bronchiectasis and air trapping by the authors. In addition, it is important to note that of those classified as having bronchiectasis on first scan, classically defined as irreversible lung damage, 25% showed complete resolution in the subsequent scans. This suggests that CT changes detected were probably so subtle in the first instance that it was difficult to score changes consistently when using this binary scoring system. Besides, when mild and subtle changes were detected as in these young CF infants, bronchial dilatation seen during the first scan may have resolved with time, reflecting the paucity of knowledge that currently exists about the natural history of these so-called structural changes. Furthermore, there is no evidence regarding the clinical significance, if any, of these mild changes in NBS CF infants and young children.

### 1.7.2 Chest CT and its relationship with clinical measures

Chest CT and CT scores have been used to evaluate and quantify pulmonary disease progression, response to treatment, prediction of future respiratory exacerbations, acquisition of *PsA* infection and future respiratory outcome in CF.[144-149] The different scoring systems will be discussed in **section 1.8.2**.

Improvements were reflected by Brody II CT scores in 17 young CF patients < 4 years of age treated with IV antibiotics for respiratory tract exacerbations. CT was able to identify pulmonary lobes with varying severity of disease. Regional differences in airway inflammation were closely correlated with Brody II scores, neutrophil count and Interleukin 8 (IL-8) in BAL taken from different lobes as identified through the CT scans. After IV antibiotics and intensified airway clearance therapy, improvement in total Brody-II and sub-scores for bronchial dilatation and hyperinflation were seen.[144] Similar findings of improved CT scores were identified in older children and adults (mean age 17 years old; range 9-33) following treatment for acute respiratory exacerbations. Mucous plugging improved significantly after treatment suggestive of it being a reversible CT abnormality. Air trapping, bronchiectasis and bronchial wall thickening (BWT) did not significantly change after treatment, though there was a trend towards a decrease in BWT.[146]

Findings from a 1-year double blind placebo-controlled interventional trial of recombinant human deoxyribonuclease (rhDNase) in CF children with mild lung disease reported improvement in mucous plugging sub-score and total CT scores in the treatment group compared to the placebo group after 12 months of treatment.[147] Hence it is necessary to understand which CT features of CF lung disease are reversible or irreversible with time so that outcome measures for trials using CT as an endpoint can be appropriately chosen. For example if the intervention involved a mucolytic, it may be more appropriate to monitor mucous plugging sub-score rather than bronchial dilatation sub-score.

Total CT score and bronchiectasis sub-score were significantly associated with mucoid *PsA* infection in chest CT undertaken at mean age 11 years from the Wisconsin national screening programme.[150] Bronchiectasis sub-score was the strongest predictor for increased respiratory tract exacerbation rate 2 years later in clinically diagnosed CF children and adults tested at median age of 12 years (range: 5-20 years); the more severe the bronchiectasis was, the higher the rate of respiratory tract exacerbations in subsequent years.[145]

Chest CT severity showed good association with future lung disease in CF children who took part in the Wisconsin Neonatal Screening project. Every additional point accumulated in the Brody II score was associated with a reduction of about 3% in $FEV_1$ when measured 2-10 years later.[149] Bronchiectasis sub-score from chest CT two years previously was most strongly associated with current spirometry. This is the first study to suggest the potential of using CT in early childhood to predict longer term outcome in NBS CF cohort.

### 1.7.3 Chest CT and its relationship with physiological measures

Chest CT has been shown to be a sensitive measure of CF lung disease in older subjects and the clinical relevance of these structural abnormalities has been established through its relationship with other surrogate markers such as lung function tests,[64,107,117,121,144,151-154] patient outcomes measures such as pulmonary exacerbations[144-147,150,155] and long- term clinical outcomes such as morbidity and mortality.[149]

Despite many different scoring systems being available, most studies used either the original or modified Brody scoring system for quantification of CT-CF lung changes. The different CT scoring systems will be discussed later (**section 1.8.2**), while the rationale for using the Brody-II system in this research study will be discussed in **section 1.9**. Irrespective of which scoring system was used, chest CT has been shown to be more sensitive in detecting CF lung disease than conventional spirometry which displays limited ability to monitor progression of lung disease, especially in the early stages.[152] In contrast, a higher CT score indicated worse lung function.[156] De Jong *et al* reported progressive structural abnormalities detected on CT despite stable and normal spirometry in 48 children with first high resolution CT (HRCT) scan undertaken at mean age of 11 years and a repeat scan a mean age of 13 years.[154] Brody *et al* demonstrated that chest CT using Brody II scoring system was more sensitive in detecting abnormalities than spirometry in 60 clinically diagnosed CF children aged 6-10 years taking part in the Pulmozyme interventional trial.[151]

However when compared with newer, more sensitive measures of lung function such as MBW, chest CT has been shown to be closely correlated with LCI with a similar number of abnormalities being detected by both techniques in clinically diagnosed CF children.[64,107,117] In a retrospective study, LCI was shown to be strongly correlated with all structural abnormalities coded according to Brody II scoring system.[64] Gustafsson et al suggested that LCI may provide an alternative, safer measure than HRCT for detecting early pulmonary abnormalities in CF. These findings were confirmed by a prospective study in school age children showing good concordance between LCI and CT scores calculated according to the Bhalla scoring system.[117] A prospective cross-sectional study by the LCFC in clinically diagnosed CF school age children demonstrated that LCI was abnormal as frequently as HRCT, and was abnormal more frequently than any other lung function indices derived from spirometry or plethysmography.[107] Total CT scores correlated more closely with LCI than with spirometry. The close correlation between LCI and CT changes, both hailed as sensitive measures of early lung disease and potential clinical trial outcome measures, could enable LCI to be a screening tool for structural changes (i.e. only performing CT in those with normal LCI), hence reducing radiation burden associated with chest CT and its repeated use.[107,157] Thus, even though considerable evidence

exists for the close relationship between LCI and CT changes detected on imaging in older children and adults,[64,107] this relationship is less well understood in young NBS CF infants and children with mild disease. Two recent studies from Australia showed no correlation between LCI and CXR changes[86] or between LCI and bronchiectasis or air trapping from CT scans[86,121] when using a commercially available ultrasonic flow sensor (USFM) device for MBW with $SF_6$ in NBS CF infants and very young CF children.

Despite promising results on the use of chest CT as a surrogate measure of lung disease and hence a potential outcome in clinical trials involving older children, there are still several unanswered questions about the use of chest CT at a very young age. Firstly, although the AREST-CF group has provided some information about the evolution of CF lung disease in early childhood, this knowledge is still limited, particularly with respect to the early changes (bronchial wall thickening, dilatation and air trapping) detected in infants at a year of age and whether these lung changes were permanent or improve with time. Secondly, it remains unclear how these changes are related to functional abnormalities during infancy, or the long term clinical implications of these CT changes. Finally, the use of CT involves a significant amount of ionising radiation [158] which limits its routine use.[159]

The second part of my research thesis will investigate if there was any association between CT changes and sensitive functional markers such as LCI in NBS CF infants at a year.

## 1.8 CONSIDERATION OF CHEST CT AS AN OUTCOME IN MULTICENTRE TRIALS

The literature indicates that significant airways disease is detectable by chest CT in infants and children with CF[109,111] and that some of these changes may be reversible.[108] Chest CT may have a role in future clinical trial as it has been shown to be a sensitive, reproducible and feasible outcome measure, albeit in studies involving older children.

Techniques for chest CT must be standardised if it is to be used as a multicentre trial outcome. This includes recommendations regarding the different types of scanner and settings recommended for use in infants and young children, types of images acquired (volumetric or limited slices; volume controlled or quiet breathing), the different scoring systems available to quantify CT specific CF lung disease and finally doses of radiation exposure with different CT techniques. Each of these criteria will be dealt with in **sections 1.8.1**, **1.8.2** and **1.8.3**.

## 1.8.1 CT technique

### 1.8.1.1 Scanner settings and parameters

Chest CT is considered the gold standard for detecting bronchiectasis. However diagnostic ability is very much dependent on the images acquired.

CT image quality depends on several factors:

- Thin beam collimation (slice thickness) which in infants and young children is typically 0.5-1.0mm to obtain good resolution images of the smaller airways found in children of this size.
- Cathode ray tube settings of beam energy (measured in kilovolt potential, kVp) to reduce degradation of image quality due to background quantum noise. Background 'noise' can also be reduced further by increasing tube current (measured in milliamperes, mA) and scan time. When scanning infants and small children, a lower tube voltage of 80-100 kVp and lower tube current of 10-20 mA has been recommended and according to these settings, radiation exposure could be reduced by 75%.[160]

The young child must be as little exposed to radiation as possible with CT scanning, because of their greatly increased radiosensitivity. This may be achieved by adjusting scanners such that adequate quality images are obtained at a much lower radiation dose.[157,161,162] If infants and young children are to be sedated or anaesthetised for volume-controlled ventilation images (see **section: 1.8.1.2**), this could allow scanning parameters and radiation exposure to be reduced further without degradation of image quality. This is possible due to the inherent contrast seen in the lung parenchymal tissue at higher lung volumes and the lack of movement artefact. The scanner settings

that were used in this research study were established by the Great Ormond Street Hospital radiology department and will be discussed in Chapter 4 (**section: 4.2.1**).

#### 1.8.1.2 Controlled lung volume imaging

It is impossible for infants and young children either to lie still or sustain lung volumes near TLC and FRC voluntarily for inspiratory and expiratory scans. Hence in infants, lung volume controlled chest CT is usually acquired during GA or deep sedation with mask ventilation.[160,163] In older children, controlled lung volume scan images can be achieved using a spirometer.[164]

The volume at which the lung is scanned has a significant effect on what is detected.[160,165] Long *et al* demonstrated the importance of detecting bronchiectasis at TLC (obtained by inflating the lungs to 25 $cmH_2O$ via a facemask during deep sedation) and air trapping at FRC (controlled ventilation at end expiratory pressure of 0 $cmH_2O$) in 16 infants and young children with mild CF. Bronchiectasis was detected in only 6% of the scans at FRC, compared with 30% when images were obtained near TLC. Data from previous studies suggested that early bronchial dilatation observed at a Peak Inspiratory Pressure (PIP) of 25 $cmH_2O$ cannot simply be iatrogenic due to effects of high inflation pressures during imaging.[160] An inflation pressure of 25 $cmH_2O$ in a sedated infant is within the physiologic range and is equivalent to the transmural pressure that occurs during a voluntary inspiration near TLC.[160] In a study by Brown *et al*, effects of lung inflation on airway diameters showed that normal airways reach a maximal size with no further distension up to an airway pressure of 30 $cmH_2O$. Further evidence for this was demonstrated in a study involving asymptomatic CF infants and young children which showed early bronchiectasis on CT imaging using inflation pressures up to 25 $cmH_2O$, whilst no bronchiectasis was seen in normal healthy controls.[137]

In contrast to bronchiectasis, air trapping, as an indirect measure of small airway disease, was predominantly detected on expiratory scans at FRC rather than at TLC or during quiet breathing. This was demonstrated in a study of older CF children and healthy controls where inspiratory images were obtained at 25 $cmH_2O$ and expiratory scans at 0 $cmH_2O$ using a spirometer to guide breathing patterns. No difference in lung attenuation (i.e. air trapping) was seen between the CF and healthy children from the

inspiratory images whereas those with CF had significantly lower lung attenuation than the controls on the expiratory scans.[165]

The Fleischner society has published criteria for specific CT features in lung disease hence enabling objective assessments of these abnormalities. Although for bronchiectasis, the guidelines state that it should include 'bronchial dilatation with respect to the accompanying pulmonary artery, lack of tapering of bronchi and identification of bronchi within 1cm of pleural surface',[166] these guidelines do not stipulate what broncho-arterial ratio (BAR) should be to define bronchiectasis. Although generally, it has been accepted that bronchial dilatation is usually interpreted as a BAR >1, there is lack of international consensus on how to define bronchial dilation in infants and young children. It has been suggested that a BAR threshold of 0.76, rather than 1, should be applied in children[167] and higher BAR with increasing age.[137] However measuring changes in small bronchial luminal size to define bronchial dilatation may be beyond current CT spatial resolution. The accuracy of assessing BARs, especially in health, is also critically dependent on reliably achieving full lung inflations.[160] If images are obtained at varying lung volumes within-or between-subjects at different times, this would alter the size of distending bronchi in relationship to accompanying vessels which could confound interpretation and lead to non-standardised classification of bronchiectasis in research studies. Hence obtaining CT images at similar lung volumes is vitally important for multicentre or longitudinal studies.

#### 1.8.1.3 Scanning protocol: non-contiguous (limited slice) vs contiguous (volumetric) imaging

Multislice (multidetector) scanners with 16 or more channels can provide contiguous (volumetric) imaging where very thin slices of the entire lung are imaged, or non-contiguous imaging (so called high resolution CT, HRCT) whereby the lung is sampled with thin sections obtained at regular intervals.

HRCT uses less radiation and can be performed quickly making it an adequate investigation to assess the presence or severity of CF lung disease. In an effort to reduce radiation further, limited slice protocols have been developed and used in research studies which typically involve three to six slices at anatomically designated

positions on inspiration and expiration. Using this approach, differences in airway dimensions and air trapping between CF and control children, as well as the response to different treatments,[137,138,146,147] have been demonstrated. The main limitation of using limited slice HRCT protocol would be the danger of missing small lung abnormalities in areas that were not being sampled i.e. in between the levels that were sampled. For heterogeneous lung disease like CF, there is a danger of missing abnormalities using HRCT reduced slice imaging protocol. In one study, the ability of CT to detect and track bronchiectasis over time was lost with reduced slice frequency.[168] The same group also reported a reduction of air trapping score based on Brody II scoring system when expiratory scans were reduced from volumetric to three slices expiratory scans, grossly underestimating the degree of air trapping with no progression in air trapping observed over time.[169] Limited slice imaging protocols were therefore not recommended for quantifying CF lung disease in children especially in the context of multicentre trials.

With the development of modern multidetector CT scanners, thin-slice volumetric images can be produced with rapid scanning times. The use of such scanners in research has been revolutionary. Contiguous imaging with dedicated software allows three-dimensional reconstructions to be generated. Bronchial tree and sub-segmental bronchial generations can be mapped out with accurate estimation of airway lumen and airway wall thickness without the need to resort to a comparison with the accompanying vessel. Quantitative assessment of air trapping is also feasible.[140,170] For longitudinal research studies whereby monitoring of changes in airways over time is paramount, volumetric CT contiguous imaging has the ability to better match airways and regional air trapping to allow accurate comparisons over time.

The main limitation of contiguous imaging is the increased radiation dose which has potentially serious implications for growing children. In view of the literature available on the sensitivity of detecting abnormalities based on the different scanning protocols (i.e. non-contiguous or contiguous volumetric) and the radiation risk associated with scanning, most research groups involved in the structural evaluation of CF lung disease or the use of CT as trial endpoint have performed a volumetric inspiratory scan with a 3-section expiratory scan. This approach is a compromise

between the need for detailed and accurate structural evaluation and the importance of reducing radiation dose.[107,117,121,149]

In this research study, the initial plan was to perform a volumetric inspiratory CT scan at 25 $cmH_2O$ during GA and a 3-section limited expiratory scan at 0 $cmH_2O$. A low dose scanning protocol was utilised as stated in chapter 4 (**section: 4.2.1**).

## 1.8.2 CT scoring systems

### 1.8.2.1 Types of CT scoring systems

The first CT scoring system was described by Bhalla and colleagues in 1991.[171] Since then several other scoring systems have been published based largely on the Bhalla scoring system with modifications and have been validated for use in CF lung disease in older children and adults.[146,171-174]

In all these scores, abnormalities were identified and the severity graded. Important abnormalities included in most of these scoring systems were bronchiectasis, airway wall thickening, mucous plugging and parenchyma opacities. Other abnormalities such as mosaic attenuations, small nodules, sacculations and air trapping on expiratory images were only included in some of the scoring systems.[173] More recent CT studies have included expiratory images to document air trapping.[146,172]

In the Bhalla scoring system, mucous plugging and peribronchial wall thickening were combined which limited the evaluation of individual findings. No anatomic localisation was identified so lobar distribution of abnormalities could not be assessed. There was no air trapping sub-score as no expiratory image was scored. Robinson *et al* modified the Bhalla scoring system; an abnormality that occupied less than 25% of the volume of the lobe received the same score as a lobe with no abnormality, hence reducing the discriminatory power of detecting mild abnormality and probably underestimating the extent of lung disease using this scoring system.[146]

The system published by Nathanson *et al* evaluated only bronchiectasis and mucous plugging, with no evaluation of peribronchial thickening or the lung parenchyma.[174] To address these limitations, Brody *et al* developed the Brody scoring system which

was modified in 2006 in response to the need for better evaluation of structural lung disease in young CF subjects who were part of the Wisconsin Neonatal CF Screening project.[140] This modified scoring system focussed on younger patients (6-10 years old) with milder lung disease even though this scoring system was first designed to describe a wide spectrum of CF lung disease; to encompass mild lung disease on one end of the scale to the most severe lung disease requiring lung transplant on the other end of the spectrum. In particular with the Brody-II scoring system, lobar location and extent of abnormality can be differentiated further into thirds of each lobe (i.e. one third of lobe affected vs 2/3 vs > 2/3) and severity of bronchiectasis, peri-bronchial wall thickening, mucous plugging and quantification of air trapping were emphasised.[172] A detailed description of the Brody-II scoring system will be discussed in the methodology chapter for chest CT (**chapter 4, section 4.4.2**).

To date, the most widely used validated CT scoring system that allows the most comprehensive assessment of CF lung changes is the Brody-II. The AREST-CF study devised a modification of the Brody scoring system to simplify scoring of abnormalities into a binary fashion as discussed earlier (**section 1.7.1**). No studies have established the use of any of these existing scoring systems in detecting and quantifying lung disease in NBS CF infants during the first year of life when changes may be presumed to be milder.

#### 1.8.2.2 Reproducibility of CT scoring systems

If a CT scoring system is to be a sensitive outcome measure for trials, the variability or reproducibility of the scoring within and between observers must be known.
A cross sectional study comparing five scoring systems revealed similar results for each. Twenty-five CT scans from subjects with CF aged 5 to 18 years with wide ranging lung function ($FEV_1$ 36-118% predicted) were scored and rescored after an interval of 1 to 2 weeks and again after 1 to 2 months by three observers. Between- and within-observer agreement was good with intra-class correlation coefficients generally greater than 0.8.[175] There was no difference in the ability of the 5 scoring systems to detect disease reflecting the contribution of the features common to these different systems.

The Brody-II scoring system has good inter-rater and intra-rater reproducibility of 95% for the total score and reproducibility that are better within than between raters over time. The authors suggested that for scoring CT in longitudinal studies, the same individual should score the scans over time.[172] de Jong *et al* employed the Brody-II system in a large clinical follow up study of children and adults with CF. The study demonstrated that CT scores were sensitive to lung changes with worsening of CT scores to document progression of abnormalities over time despite stable spiromtery.[152]

The inter-observer and intra-observer variability of component CT scores (sub-scores) were poorer for some components than for the total scores. In the Brody-II validation study,[172] Brody *et al* reported inter-observer agreement of 74% for bronchiectasis, 89% for mucous plugging and 61% for air trapping. De Jong *et al* using the Brody-II scoring system showed different intra-class correlations (r value) between two observers for total scores and CT components; total score (r=0.92), bronchiectasis (r=0.88), opacities (r=0.80), mucous plugging (r=0.72), airway wall thickening (r=0.67), bulla and cysts (r=0.53) and air trapping (r=0.27).[152] Owen *et al* reported structural CT changes in LCFC clinically diagnosed school-aged children (mean age: 7.8 years) using the Brody-II scoring system.[107] Inter-observer agreement for total scores and some sub-scores were reported as excellent using Kendall's Tau statistics. Coefficient of agreement for different component scores were: Total CT score (0.76), bronchial dilatation (0.77), peribronchial wall thickening (0.74), mucous plugging (0.74), air trapping (0.59) and parenchyma change (0.40).

Although generally total CT scores have good inter- and intra-observer variability, reproducibility was lower for certain components or sub-scores. This limited the ability of the scores to track changes in subcomponent features of CF over time. A possible reason for high variability in some sub-scores may be related to a lack of unambiguous definitions and reference images for defining an abnormality.[173] There was also significant increase in variability between scores when the total scores were low, as in early lung disease when changes are mild. Early lung changes were more subtle making it potentially difficult for a subjective observer to assign broad ordinal scores in a consistent manner. This may be the case when scoring systems are used in

young infants.[170] To date, no scoring systems have been developed and validated appropriately for scoring CT changes in young CF infants.

### 1.8.3 Radiation risk

Radiation exposure is the main concern for considering the use of chest CT as an outcome measure in trials or as a monitoring tool. It is measured in terms of the quantity of radiation energy 'absorbed' by the body tissue and is expressed in millisieverts (mSv). This is a measure of radiation dose which accounts for the fact that ionising radiation can affect different parts of the body to a different extent. This then allows different sources of radiation to be compared. For example in an adult, a CXR has a radiation dose of 0.02 mSv, transatlantic flight: 0.07 mSv, total body CT scan: 10 mSv, level at which changes in blood cells can occur: 100 mSv, acute effects of radiation leading to cell death and organ failure: >1000 mSv and a dose which could be fatal: 5000 mSv (http://www.hpa.org.uk/topics/radiation/).

With low dose ionising radiation associated with medical investigations, the principal concern is with respect to genomic damage leading to an increased lifetime risk of cancer. This risk is greatest in children < 10 years old.[158] This is explained by the fact that young children face a larger lifetime background risk of cancer mortality which magnifies their relative risk per unit dose of radiation and that young children absorb a greater fraction of any given radiation dose.[158] The natural risk of childhood cancer is 1 in 5000 and the average annual background radiation in UK is ~2.5 mSv whilst in the United States of America, it is between 3.5-6.2 mSv (http://www.hpa.org.uk). The lifetime risk of subsequent malignancy with the use of a chest CT in a young child (<2 years) will increase that risk of childhood cancer by 1 per 5000 cases.[176]

It is therefore crucial that there must be judicious use of radiation associated imaging in CF infants and young children. If chest CT is required then there is a responsibility to ensure that images are obtained with the lowest possible radiation dose. Consequently, there is a concerted effort internationally to develop standardised low dose CT protocols.

## 1.9 RATIONALE FOR CHEST CT IN THIS RESEARCH PROJECT

The second part of this research study was conducted to increase the understanding of structural lung changes in NBS CF infants at a very young age, specifically at 1 year. Although previous studies may have demonstrated structural lung changes in infants and young children through the use of chest CT, very few were in NBS CF infants at 1 year.

In addition, there are no established CT scoring systems available for scoring abnormalities during infancy. We opted to use the Brody-II scoring system to validate its use in infants through this study. The Brody-II scoring system was chosen due to its comprehensive assessment of structural lung changes which takes into account not just the presence of an abnormality but the extent and severity of the abnormalities with accurate lobar identification. In addition if a 'simpler' and hence quicker scoring system was required in the future, this could potentially be derived from the complex array of information documented in the Brody-II scoring system.

As NBS CF infants had a flexible bronchoscopy and BAL as part of their first year annual review (details of which are beyond the scope of this thesis), a decision was made to perform the CT scan under the same GA just before the bronchoscopy. Volumetric inspiratory image was obtained at PIP of 25 $cmH_2O$ whilst 3-slice limited expiratory image was obtained at PEEP of 0 $cmH_2O$ for each CF infant. Since there is also a need for more information regarding the relationship between lung function and structure in NBS CF infants, efforts were made to ensure that ILFT and chest CT were performed within 1-2 weeks of each other and while the child remained in a clinically stable condition (see Chapter 4).

## 1.10 HYPOTHESES, AIMS AND OBJECTIVES

There is evidence, both from lung function testing and chest CT that there is early and progressive functional and structural airway disease in CF, despite diagnosis by NBS and the institution of modern therapy early in life. The main source of this evidence is AREST-CF. However, as described above, there are flaws and limitations in some of

these studies which mandate further investigation. This forms the basis of this research study. Limitations of previous studies were addressed so that results would provide more robust evidence for lung function and structure in NBS CF infants at a year of age. Without this accurate information, outcomes for future interventional trials cannot be devised.

### 1.10.1 Primary hypothesis, aims and objectives

The primary hypothesis of this study pertained to the one year lung function of NBS CF infants. In view of impaired lung function with continual deterioration in the first year of life demonstrated in clinically diagnosed LCFC as well as in NBS CF infants from the AREST-CF studies, the **primary hypothesis** of this study was:

1) Despite early diagnosis and specialist treatment, NBS CF infants have further loss of lung function from first diagnosis until a year of age.

The **primary aims** were to:

1) Compare lung function in NBS CF to that in healthy control infants at a year of age.
2) Compare the changes in lung function during the first year between the two infant groups.

The **primary objectives** were:

1) To assess LCI, $FRC_{pleth}$, gas trapping ($\Delta$ $FRC_{pleth}$ - $FRC_{MBW}$), $FEV_{0.5}$ and $FEF_{75}$ z- scores measured using MBW, plethysmography and RVRTC in NBS CF infants and healthy controls at a year of age, and compare these results between the two groups.
2) To measure and compare the rate of change of lung function between 3 months to 1 year in NBS CF and healthy infants.
3) To determine whether lung function at 3-months of age is predictive of 1-year lung function.

### 1.10.2 Secondary hypotheses, aims and objectives

The **secondary hypotheses** of this research study were:

1) At one year of age, NBS CF infants have evidence of significant lung disease detected on chest CT scan using the Brody-II scoring system.

2) In NBS CF infants, there are significant associations between lung abnormalities detected on chest CT and the various lung function outcomes described above when assessed at ~ 1 year of age.

The **secondary aims** to address the hypotheses were:

1) To perform chest CT at a year of age in NBS CF infants using standardised protocols across different study centres.
2) To explore the relationship between pulmonary changes detected on CT and functional abnormalities detected using MBW, plethysmography and RVRTC in NBS CF infants at a year of age.

The **secondary objectives** were:

1) To assess the feasibility and adherence to standardised CT and GA protocols in using chest CT as an outcome measure in this multicentre study.
2) To assess inter and intra-observer agreement of Brody-II total CT and CT sub-scores allocated to NBS CF infants at a year of age.
3) To detect and quantify CF lung disease according to total and component CT scores (bronchial dilatation, airway wall thickening, parenchymal change, mucous plugging and air trapping) using the Brody-II scoring system by two experienced radiologists
4) In NBS CF infants at a year of age, to assess whether LCI, $FRC_{pleth,}$ $FEV_{0.5}$ and $FEF_{75}$ were as sensitive as CT in identifying pulmonary abnormalities and which, if any of these lung function outcomes were best correlated to total CT scores and sub-scores for bronchial dilatation, airway wall thickening, parenchymal opacities, mucous plugging and/or air trapping.
5) To determine potential clinical associations with physiological and structural lung outcomes of NBS CF infants at a year of age.

## 1.11 SUMMARY

Early detection and specialist treatment of CF lung disease is beneficial and can be achieved through NBS. It is vital to have a greater understanding of the evolution of lung disease in CF infants diagnosed through NBS so that novel interventions can be targeted appropriately, and randomised control trials can be adequately powered. In recent years, potentially sensitive, reproducible and feasible outcome measures for

quantifying lung disease in infants and young children have been identified which may prove to be important and crucial endpoints for clinical trials in this CF population.

In this thesis, I will investigate lung function and structure in NBS CF infants at a year of age and assess whether these physiological and structural measures could be suitable endpoints for clinical trials in infants with CF. The next chapter describes the ILFT protocols, subjects and methods in detail.

# 2 LUNG FUNCTION IN NEWBORN SCREENED CF INFANTS: SUBJECTS & METHODS

## 2.1 OVERVIEW OF STUDY

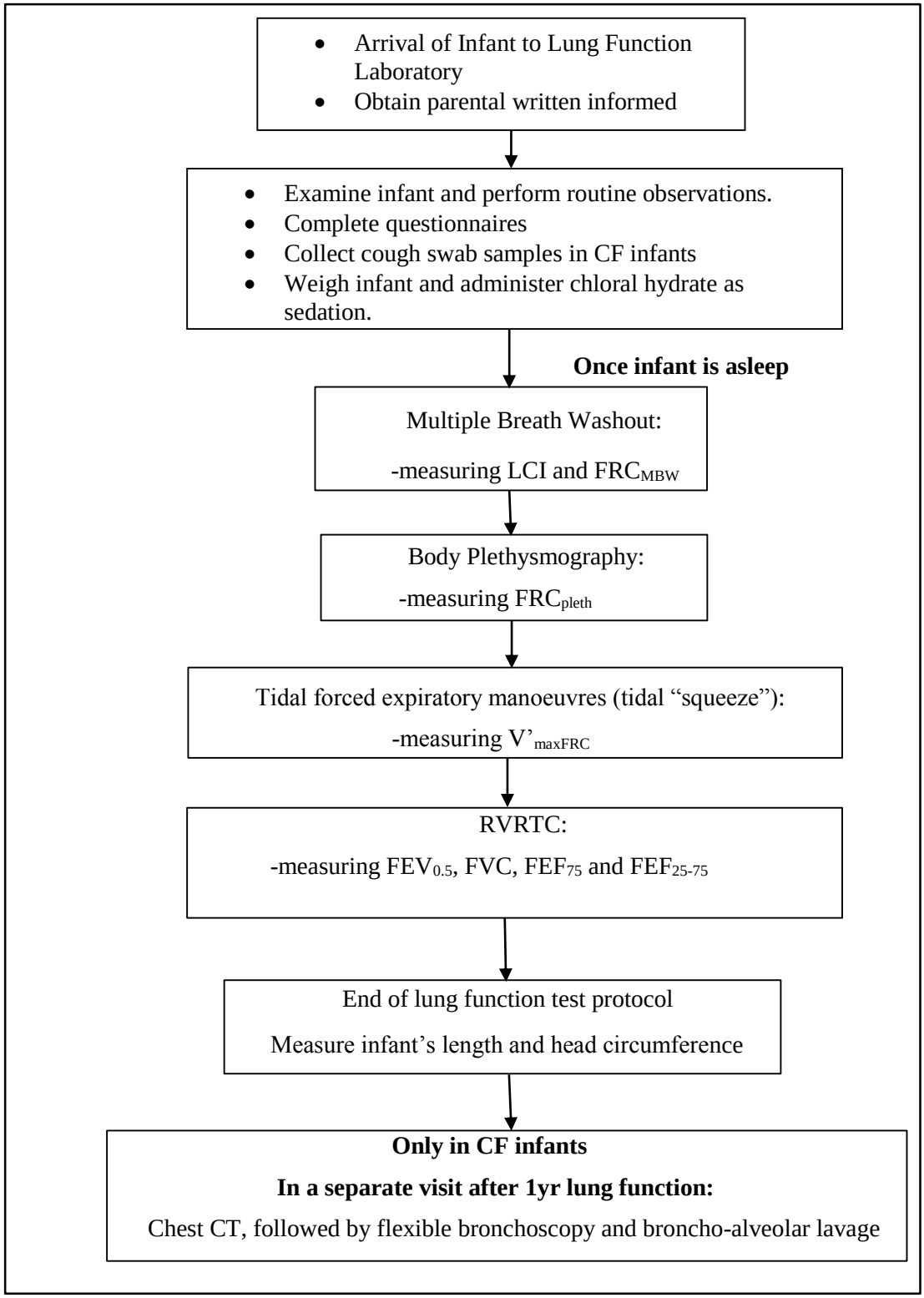
The research described in this thesis was part of a prospective longitudinal cohort study investigating early lung function, structure and inflammation in NBS CF infants. These infants were referred from six paediatric tertiary respiratory centres within the Greater London region. Healthy control infants were also recruited for lung function assessments.

Following informed, written parental consent, ILFT were performed at around 3 months and 1 year of age for all infants. All infants underwent the MBW test to measure LCI and $FRC_{MBW}$, RVRTC to measure forced expiratory volumes and flows, as well as body plethysmography to measure $FRC_{pleth}$. All ILFT were performed at one centre- Great Ormond Street Hospital for Children (GOSH). ILFT were performed in the order as stated in **Figure 2-i**. Protocols for ILFT will be discussed in this chapter with results presented in the next chapter.

CF infants also underwent additional tests at 1 year on a separate hospital visit, namely chest CT and bronchoscopy under GA. These tests were performed at three of the referring hospitals, with infants from the remaining three referring hospitals undergoing CT/ bronchoscopy at GOSH (**Figure 2-i**). Protocols involved in the GA management and acquiring of CT scans, the process and methodology of scoring CT scans from these CF NBS infants will be discussed in Chapters 4 and 5 with the results of CT findings presented in Chapter 6.

Flexible bronchoscopy and BAL were undertaken following the chest CT but the results of these investigations will not be discussed as this is beyond the remit of my thesis.

**Figure 2-i: Schedule of testing in CF and control infants at 3months and 1year: Chest CT, Flexible Bronchoscopy and Broncho-Alveolar Lavage at ~1 year only performed in CF infants**



### 2.1.1 Ethical approval

The study had full ethical approval by the National Research Ethics Service (NRES) Committee London-Bloomsbury REC (Ref No: 09H071314) and the Local Research Ethics Committees of each of the six collaborating centres. For all subjects, informed written consent was obtained. Written consent for photography was sought at the same time to obtain photos for publications and this thesis.

## 2.2 SUBJECTS

Two groups of subjects were recruited for this study, namely:

1. CF infants diagnosed through NBS or presentation with meconium ileus;
2. Healthy full term infants (≥36 weeks gestation)

### 2.2.1 Recruitment of CF infants

Infants born in London and the surrounding areas who screened positive for CF were seen at one of the collaborating hospitals (GOSH, Royal Brompton Hospital, Royal London Hospital, Kings College Hospital, Lewisham University Hospital and East Surrey Teaching Hospital) for diagnostic sweat testing. Once the diagnosis was made, individual infants underwent comprehensive assessments and their families received detailed information and education regarding CF treatment and condition.

Infants with CF were recruited to this study by their consultants when attending follow up approximately 2 weeks after a positive diagnosis of CF. The purpose of the study was explained verbally, and parents were given both the parental information sheet and additional illustrated leaflets (**Appendix: A2 and A3**)**.** They were given time to consider the information before giving written consent for ILFT and CT/bronchoscopy (**Appendix: A4**).

Those who were diagnosed with meconium ileus also underwent screening. Once CF genotype had been identified, they had a confirmatory sweat test. They were subsequently treated in accord with a pre-determined treatment protocol (**Appendix: A7**)

**Inclusion Criteria:**

- Infants diagnosed with CF by NBS within the Greater London catchment area

**Exclusion Criteria:**

- Infants with CF born <36 completed weeks of gestation
- Severe congenital, cardiovascular or neuromuscular disorders that could impact on the development and function of the respiratory system
- Inability of parents to understand and give informed consent
- Recruitment contra-indicated based on psycho-social factors
- History of apnoeic episodes or upper airway pathology
- Family due to move out of area

### 2.2.2 Recruitment of Healthy Control (HC) Infants

Healthy term infants with no congenital abnormalities, born ≥ greater than 36 weeks gestation were recruited from the community by specified research assistants or fellows working specially on this project.

With permission from the Head of the Midwifery Services and the Consultant Paediatrician of the Homerton University Hospital, East London, monthly birth records were transcripted as a password protected Excel spreadsheet and forwarded to the research team. From the birth list, the research team carefully selected healthy infants who were likely to be eligible for recruitment (*see the list of Inclusion and Exclusion criteria below*). Letters were sent to the general practitioners (GPs) of the infants/families to investigate if it was appropriate to approach the families of selected healthy infants. Following replies and confirmation from GPs, detailed information sheet and additional illustrated leaflets were sent to parents of healthy infants (**Appendix: A2**). This was followed by a telephone call 7-10 days later from the research team to further discuss the study, particularly the need for chloral hydrate sedation. If parents were interested in participating in the study, an appointment for LFT was made over the phone with confirmatory letter sent subsequently.

**Inclusion Criteria:**

- Healthy term infants (≥36 weeks gestation) who lived within reasonable travelling distance of London for specialist ILFT, and whose parents consented to these measurements
- Mainly of Caucasian descendance (matching the proportion of ethnic groups of CF cohort)

**Exclusion Criteria:**

- Inability of parents to understand and give informed consent
- Recruitment contra-indicated on psycho-social factors
- History of apnoeic episodes or upper airway pathology
- History of chronic diarrhoea or failure to thrive
- History of neonatal lung disease, or coexistent heart, lung, neuromuscular or renal disease that could impact on the respiratory system
- Previous history and/or hospital admissions for lower respiratory tract infections

Any healthy infant who was recruited into the study but was subsequently admitted to hospital with a respiratory infection, chronic diarrhoea or failure to thrive was also excluded from the control group, whereas any who developed a lower respiratory tract infection or wheezing illness that did not require hospitalisation, and had been tested at ~3m and/or 1yr were retained within the cohort.

### 2.2.3 Preparation of infants for lung function test procedure

Appointments for lung function tests were arranged to coincide with normal periods of day-time sleep. On arrival, parents were talked through the study protocol and encouraged to ask questions. They were reminded of the potential side effects of chloral hydrate, which was the enteral sedation used in the lung function laboratory, and about the bitter taste of the sedative syrup which often upset infants temporarily.

Infants were assessed clinically including auscultation of the chest for wheeze or crackles. If there was evidence of coryza, nasal blockage or cough, the test was delayed for a minimum of 3 weeks. Routine clinical observations such as heart rate

and oxygen saturation were performed using the Masimo pulse oximeter (Masimo Radical-7, Irvine, CA, USA), and such vital signs were monitored continuously throughout the test session.

Parents were advised to fast their infant for 4 hours (2 hours if the infant was breastfed) and to abstain from clear fluids for 2 hours prior to the ILFT in accordance with the NICE guidelines on sedation in children and young people published in December 2010 (http://www.nice.org.uk). An empty stomach from fasting enabled better and quicker absorption of the oral sedation, and reduced the risk of gastric aspiration. As soon as oral sedation was given, parents were encouraged to settle the baby to sleep. On rare occasions when the infant was fretful and unable to settle (more than one hour) post administration of oral sedation and feeding was overdue, a small drink or light feed was given.

### 2.2.4 Study questionnaires and smoking history

Study questionnaires previously designed by our laboratory (**Appendix: A5 and A6**) were used to record background and medical information of CF and healthy infants. Relevant background information including parental and sibling (if any) health, ethnicity, any significant neonatal history, antenatal/postnatal cigarette smoke exposure, and parental occupation (hence socio- economic status) were recorded. Infants with CF also had date of diagnosis, genotype and sweat test results recorded in this initial questionnaire.

**For CF infants:**

Significant medical problems at birth and within the first year were recorded in the questionnaires completed at each test occasion and the clinical record forms (CRF), completed at each CF clinic visit. These included any surgery, the need for ventilatory support, hospital admissions and the use of additional intermittent antibiotics for respiratory causes, development of wheeze or allergy, as well as anthropometric measurements. CF infants were managed by each of the six CF centres through a standardised treatment protocol (**Appendix: A7**). The type, route and number of courses of antibiotic received were clearly documented. Current therapies subdivided into ‘pulmonary’ and ‘nutritional’ treatments, and the frequency and duration of

parental administered physiotherapy were recorded. Parents were asked about the recent occurrence and frequency of coughing. As parental identification of wheezing was more difficult, no quantification of this symptom was asked except for whether the infant had wheezed or not.(**Appendix: A5**)

In addition, clinical information recorded for CF infants at each hospital clinic visit were reported prospectively onto a standardised CRF (**Appendix: A8**).

**For Healthy control infants:**
An adapted questionnaire similar to that used for CF infants was used for the healthy control infants (**Appendix: A5**).

**Smoking history**
Parental smoking history was elicited via the study questionnaire. Current non-exposure to environmental tobacco smoke was validated by collecting a urine sample from infants (or cotinine assay) whose parents reported to be non-smokers. Cotinine is a by-product of nicotine and is a sensitive measure of recent smoke exposure, which allows discrimination between active, passive and non- smokers.[177,178] The urine specimen was obtained by placing cotton wool balls in the nappy which were removed once saturated with urine, using disposable gloves to avoid potential contamination (from the investigator's fingers), at the end of the test occasion. Clear urine was extracted from the cotton wool balls by placing them into a syringe and then using the plunger to squeeze the urine out into a plain specimen bottle (minimum amount: 0.2 mL). Alternatively, if an infant urine sample could not be collected then a maternal salivary sample was obtained by placing a dental roll into the mother's mouth for ~5-10 minutes until it is saturated with saliva. The saturated dental roll was placed in a syringe and a saliva sample collected into a plain specimen bottle by squeezing down on the syringe plunger (minimum amount: 0.2 mL).

Samples were immediately stored at ─20˚C prior to analysis. The frozen urine and salivary samples were sent periodically in batches for cotinine assay to the ABS Laboratories Ltd (BioPark, Broadwater Road, Welwyn Garden City; http://www.abslabs.com/contact.cfm). Urinary cotinine values <49.7ng/ mL and

salivary cotinine values <12ng/ mL are generally considered compatible with that from non-smokers.[179]

### 2.2.5 Microbiology

Cough swabs were taken from infants with CF before the ILFT, unless one had been performed 7-10 days previously. A standard hospital swab (Transswab, Medical Wire and Equipment Co.Ltd., Wiltshire, England) was inserted above the tongue and the tip aimed towards the back of the oropharynx to elicit a cough. The swab was subsequently removed and inserted into a sterile container and was processed at the Camelia Botnar Laboratories, GOSH, for culture and sensitivity of any bacterial growth in accordance to CF Trust guidelines (www.cftrust.org.uk).

Cough swabs were also taken from CF infants, during their regular 2-monthly clinic visit locally and whenever respiratory symptoms were reported by the parents.

### 2.2.6 Sedation

Chloral Hydrate sedation was given at a standard dose of 60-100mg/kg body weight[180] ( maximum of 1 gram regardless of body weight) in accordance to established guidelines. These doses have been administered to well over a thousand babies over the last three decades in this London and other respiratory function laboratories world-wide with no major adverse effects. The majority of the infants were given chloral hydrate syrup orally although parents were able to request chloral hydrate suppositories in older infants who refused to take it orally or were able to spit out the syrup.

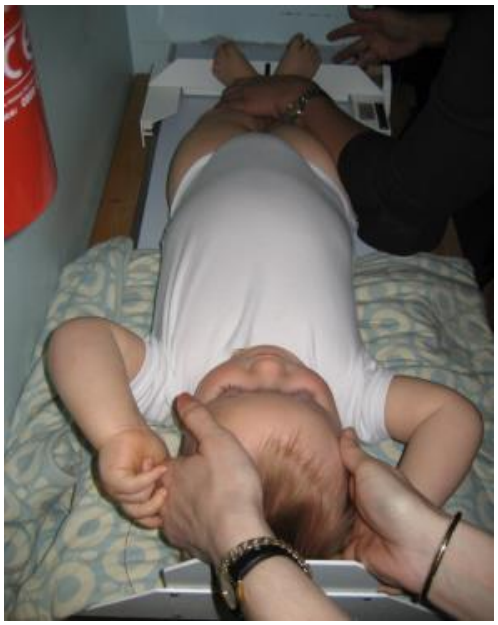
Parents were advised of possible unsteadiness post sedation. Sub-optimal doses can result in hyperactivity and irritation. Although never required to date, an emergency trolley containing equipment for advanced cardiorespiratory resuscitation (including resuscitation bag and mask, tracheal suction apparatus with catheters, piped medical air and oxygen within the room where LFT were conducted) was available at all times and all staff involved were trained in airway management and administration of advanced life support.[181]

### 2.2.7 Anthropometric measurements

Since respiratory parameters are closely related to body size, the infant's weight and length were measured as accurately as possible. The infant's naked weight was measured prior to sedation in order to calculate the dose of Chloral hydrate, using Seca electronic digital scales (Seca Ltd, Birmingham, England). Weight was recorded in kilograms to two decimal places.

All other anthropometric parameters were usually measured after the respiratory function tests. Whilst the infant remained slightly sleepy, the infant's crown-heel length was measured by two members of the research team using a calibrated Harpenden stadiometer/ infantometer (M K Scales LTD, Bletchley, UK http://www.mkscales.co.uk/products_486_34_Harpenden-Infantometer.html). One person gently held the infant's head in a mid-line position, with the crown touching the top of the stadiometer, whilst the other gently depressed the infant's knees to fully extend the legs. The sliding footplate was adjusted to rest firmly against the soles of the feet (**Figure 2-ii**). The measurement was repeated two to three times and the results reported as the mean of two measurements which were within 0.5 cm of each other. Length and weight were expressed as z-scores to adjust for age and sex.[182]

**Figure 2-ii: Measuring crown-heel length of infant**



***Legend**: This photograph shows one investigator gently holding the infant's head in the mid-line position with the crown touching the top of the stadiometer, with the other investigator depressing the infant's knees to fully extend the legs. Written permission was obtained for the scientific use of all photographs presented in this thesis from the infants' parents.*

## 2.3 LUNG FUNCTION PROTOCOL

### 2.3.1 Multiple breath inert gas washout technique (MBW)

The MBW method was used to measure LCI and $FRC_{MBW}$ using a respiratory mass spectrometer and customised software for data collection and analysis.[83]

#### 2.3.1.1 MBW apparatus and calibration

Two computer systems were used for the MBW test. One of these was attached both to the mass spectrometer and to the second PC which, loaded with the customised software, was used to collect respiratory data and subsequent analysis of MBW results.

The gas analyser used in this study was an AMIS 2000 quadropole respiratory mass spectrometer (Innovision, Odense, Denmark). It operates by identifying gases according to their mass-charge ratio. A gas mixture is drawn into a vacuum chamber along a narrow-bore capillary tube. When it is in contact with an electrical filament,

positively charged ions are created which are then accelerated along a voltage gradient towards a receptor. The mass-charge ratio is determined by the molecular mass of the gas, so only gases of one mass-charge ratio (i.e. in most cases, only one gas) can reach the receptor at any one time. For this study, the AMIS 2000 was programmed to recognise helium (He), nitrogen ($N_2$), oxygen ($O_2$), carbon dioxide ($CO_2$) and sulphur hexafluoride ($SF_6$). The gas sampling rate of the AMIS 2000 is 15 mL/ min.

As instructed by the manufacturer, the AMIS 2000 was calibrated prior to each test occasion using a certified concentration gas (alpha-gravimetric standard, British Oxygen Company, Guildford, UK), i.e. the "calibration" gas containing 3.97% He, 3.98% $SF_6$ , 7.04% $CO_2$, 21% $O_2$ and 64% $N_2$. A signal-noise ratio of 100 or greater was deemed acceptable. The calibration procedure included an automatically performed re-optimisation of atomic mass unit peaks. A short one-point calibration check was performed prior to each measurement using the same gas mixture for calibration.

Analogue outputs from the demodulator and from the mass spectrometer were recorded at 100 Hz using a desk-top computer (Dell Computers, Round Rock, TX, USA) through a 16-channel AD-conversion board (Model RS485, Keithley Metrabyte, Taunton, MA, USA), connected to a Laptop computer loaded with custom written software (by Per Gustafsson et al, Sweden) based on a commercially available data acquisition software pack (TestPoint, Capital Equipment Corp., Billerica, MA, USA).

The pneumotachometer (size 0 Fleisch PNT; Lausanne, Switzerland), attached to a differential pressure transducer (Validyne, Model MP 45-14-871, Validyne Corp., Ca, USA) by means of two equal length of firm translucent vinyl tubings (AlteVin, UK) was calibrated at room temperature prior to use with separate calibration constants for inspiratory and expiratory flows using a 100mL precision syringe (Hans Rudolph, Inc; Shawnee, Kansas, USA).

Gas samples and flow signals were aligned in time. Delay to the gas signal was measured using a custom-made delay switch (manufactured by Mr E Bergsten, Swedish Defense Research Agency, Department of Defence Medicine, Linköping, Sweden). This system measured the delay between gas appearing at the capillary inlet

of the mass spectrometer (enabled by opening the switch) and that gas bolus being recorded by the software. During the pre-test calibration process, a series of 20 delay recordings were performed, and the median delay and rise times obtained were used to align flow and gas signals from subsequent recordings. The software corrected the flow signal sample-by-sample for changes in dynamic viscosity caused by the variations in gas composition.

Once the PNT has been calibrated, it was connected to a heating circuit (provided by the Biomedical Engineering department, GOSH: heater model FWS4D, Hugo Sachs Elektronik, Germany) and heated to 37°C prior to LF measurements. A facemask was connected to one end of the PNT and the gas sampling capillary from the respiratory mass spectrometer was inserted between the facemask and PNT via a short connector.

During data collection, inspiratory and expiratory flows and volumes were automatically corrected to body temperature, barometric pressure and saturated with water vapour (BTPS) conditions and data were stored. The mass spectrometer measured the concentrations of the mixture of inert tracer gases contain in the "test" gas (used during the wash-in phase): i.e, $SF_6$ and He, and all other respiratory gases ($N_2$, $O_2$, and $CO_2$) as dry gas concentrations. Only $SF_6$ data were used for analysis in this study.

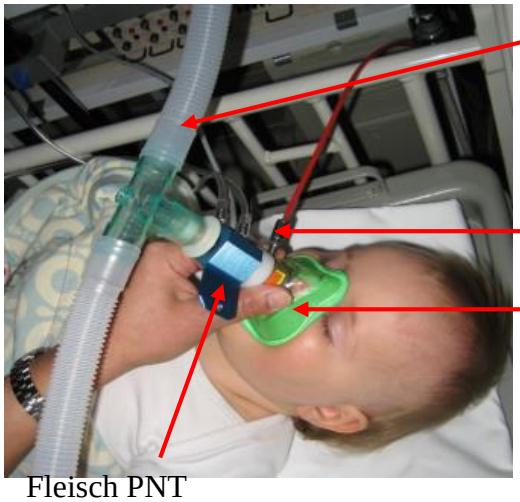
A Rendell-Baker Soucek size 1 or 2 (Rusch UK Ltd, High Wycombe, UK) facemask was placed over the sleeping infant's nose and mouth during data recording (**Figure 2-iii**). The size 1 mask was generally only used for infants ≤ 5kg in weight at time of the 3m test. The majority of infants were tested with the size 2 mask. An air-tight seal was achieved using a rim of therapeutic putty (Patterson Medical, Sutton-in-Ashfield, UK) (**Figure 2-iii**). The facemask was connected to the inspiratory port of the PNT by a custom made connector which had a side port to accommodate the capillary inlet of the mass spectrometer. The expiratory port of the PNT was connected via a second custom-made connector to a T-piece (Intersurgical, Wokingham, UK) to which large bore anaesthetic breathing circuit tubing (elephant tubing) was attached. The afferent limb of the elephant tubing was attached to the cylinder of medical grade gas containing 4% $SF_6$, 4% He, 21% $O_2$ and balance $N_2$. The efferent limb of the elephant

tubing was open to room air. The connector attached to the T-piece and the elephant tubing were collectively known as the bias flow apparatus. Dead space of facemask and different components were measured in previous validation studies using water displacement. The residual effective deadspace volume was 5ml and 7.5ml for the size 1 and 2 facemasks respectively.[183]

#### 2.3.1.2 MBW data collection in infants

The sleeping infant breathed through the PNT via a facemask which was applied to his/her face with a rim of therapeutic putty around it to ensure a tight seal. **Figure 2-iii** shows the set up for the wash-in phase of MBW. The flow and gas signal outputs were monitored in real time on the computer screen. Once the infant was in non-rapid eye movement (REM) or quiet sleep and breathing regularly, the distal end of the PNT was briefly occluded (i.e. a test occlusion) to ensure that there was no evidence of leak around the mask or PNT (**Figure 2-iv**).

**Figure 2-iii: Infant undergoing Multiple Breath Washout**




***Legend**: Sleeping infant with the facemask and Fleisch pneumotachometer(PNT) in situ. A ring of therapeutic putty (green material) is used to create a seal around the mask. A T-piece is used to connect the elephant tubing which enables the bias flow of air mixture to be delivered during the wash-in phase.*

Data collection was performed in two stages:

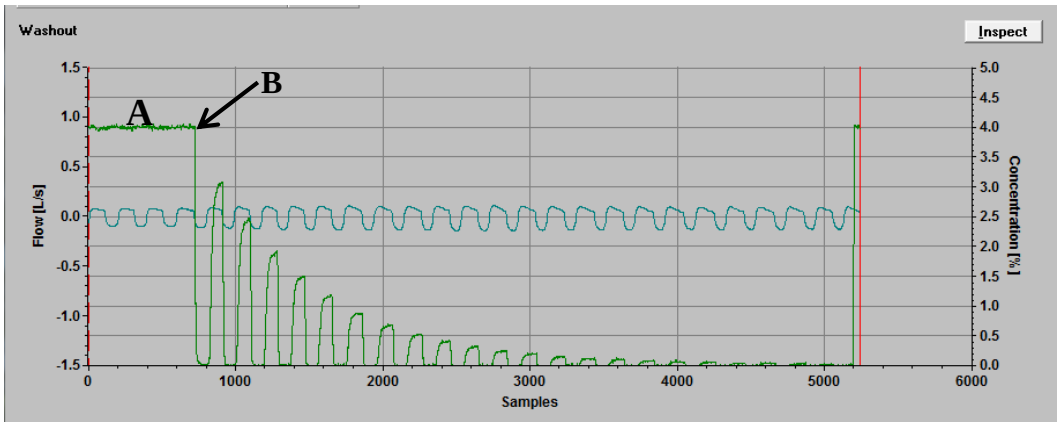
a) the wash-in phase which involved the infant inspiring a bias flow of dry air mixture containing the tracer gas 4% $SF_6$, 21% $O_2$ and balance $N_2$, and continued until inspiratory and expiratory $SF_6$ concentrations were stable and equal to 4% for a

minimum of 5-8 breaths. At this moment, the wash-in was stopped by disconnecting the bias flow assembly during the start of expiration, and
b) the wash-out phase began with the infant inhaling room air and continued until end-tidal $SF_6$ concentration was consistently below 0.1%, i.e., < $^1/_{40}^{th}$ of the starting concentration. The output obtained during the wash-out phase is displayed **in Figure 2-iv, a.**

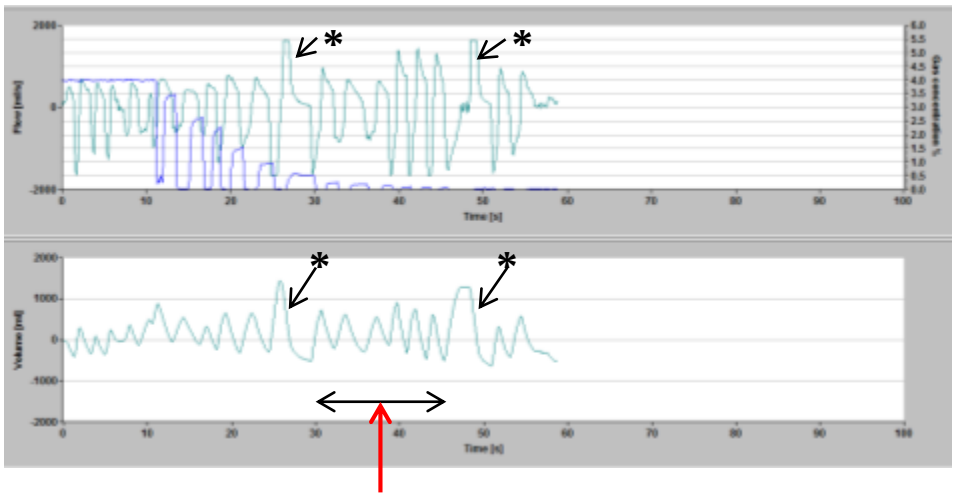
A recording was considered technically acceptable if there was no evidence of leak during the latter part of the wash-in or at any stage during the wash-out. If the infant had a big "sigh", respiratory pauses and irregular breathing pattern during the wash-out, the recording was stopped and the test restarted (**Figure 2-iv, b**). A minimum of three wash-in-wash-out manoeuvres were recorded on each test occasion. MBW data were only acceptable if there was no evidence of mask or PNT leak and were analysed as described previously.[81,89]

**Figure 2-iv: Washout Curve**

**(a) Acceptable trace**




**(b) Unacceptable trace**




***Legend: (a) Acceptable trace****: The sky blue trace represents flow (left axis). The green trace represents $SF_6$ concentration (right axis). Point A indicates the end of wash-in when $SF_6$ is constant at 4% with no 'wobbling' of horizontal line which would be suggestive of a leak. The bias flow is disconnected at point B, i.e., at the end of inspiration or beginning of expiration. From this point onwards, $SF_6$ concentration during inspiration is zero as the child is inhaling room air. The $SF_6$ concentration on expiration falls with each breath, as the resident gas of the lung is progressively diluted by the inspired room air.* ***(b) Unacceptable trace****: The green trace represents flow on the top panel (left axis). The bright blue trace represents $SF_6$ concentration during washout. As can be seen, during the wash-out phase, several deep sighs and irregular breathing pattern took place. These were confirmed on the volume trace (lower panel) showing larger tidal volume with 'sigh' breaths and inconsistent tidal volumes during irregular breathing pattern (denoted by asteriks).*

LCI is defined as the number of lung volume turnovers (or number of FRCs) that are required to clear the lungs of the inert tracer gas to $^{1}/_{40}^{th}$ of the starting concentration of the tracer gas, i.e.,

LCI= [CEV] / [FRC]

where CEV is the cumulative net (after adjustment for equipment dead space) expired volume (i.e., the sum of tidal volumes) during the wash-out phase.

$FRC_{MBW}$ is calculated as follows: -

$FRC_{MBW}$ = [net volume of inert gas exhaled] / [Cet start – Cet end]

where Cet represents the concentration at end-tidal volume of the inert tracer gas, at the start (Cet start) and end (Cet end) of data collection during the wash-out phase.

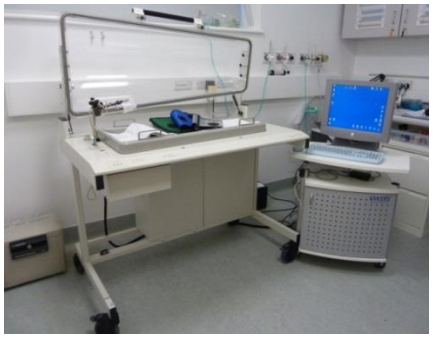
Results were reported as mean (standard deviation, SD) from three technically satisfactory MBW recordings for LCI and $FRC_{MBW;}$ in exceptional cases, a minimum of two recordings were used if results were within 5% of one another.

## 2.3.2 Body plethysmography

### 2.3.2.1 Equipment

The MasterScreen BabyBody System (CareFusion™, USA; v 4.65) was used to measure the $FRC_{pleth}$[184] and to perform the RVRTC manoeuvres (or infant "spirometry").[83] (**Figure 2-v**)

**Figure 2-v: CareFusion™ Masterscreen BabyBody Plethysmograph**



***Legend:*** *BabyBody Plethysmograph with lung function software incorporated into a computer in an infant lung function laboratory.*

#### 2.3.2.2 Masterscreen BabyBody system: calibration

Prior to calibrating the infant plethysmograph (internal volume: 98 L), the Jaeger PNT with a resistive screen was connected to the pressure transducers, followed by the insertion of a shutter (with a latex balloon attached internally) to the distal end of the PNT. The infant system was then switched on for at least 20 minutes to allow for thermal equilibration. The ambient conditions (barometric pressure; room temperature, relative humidity) documented.

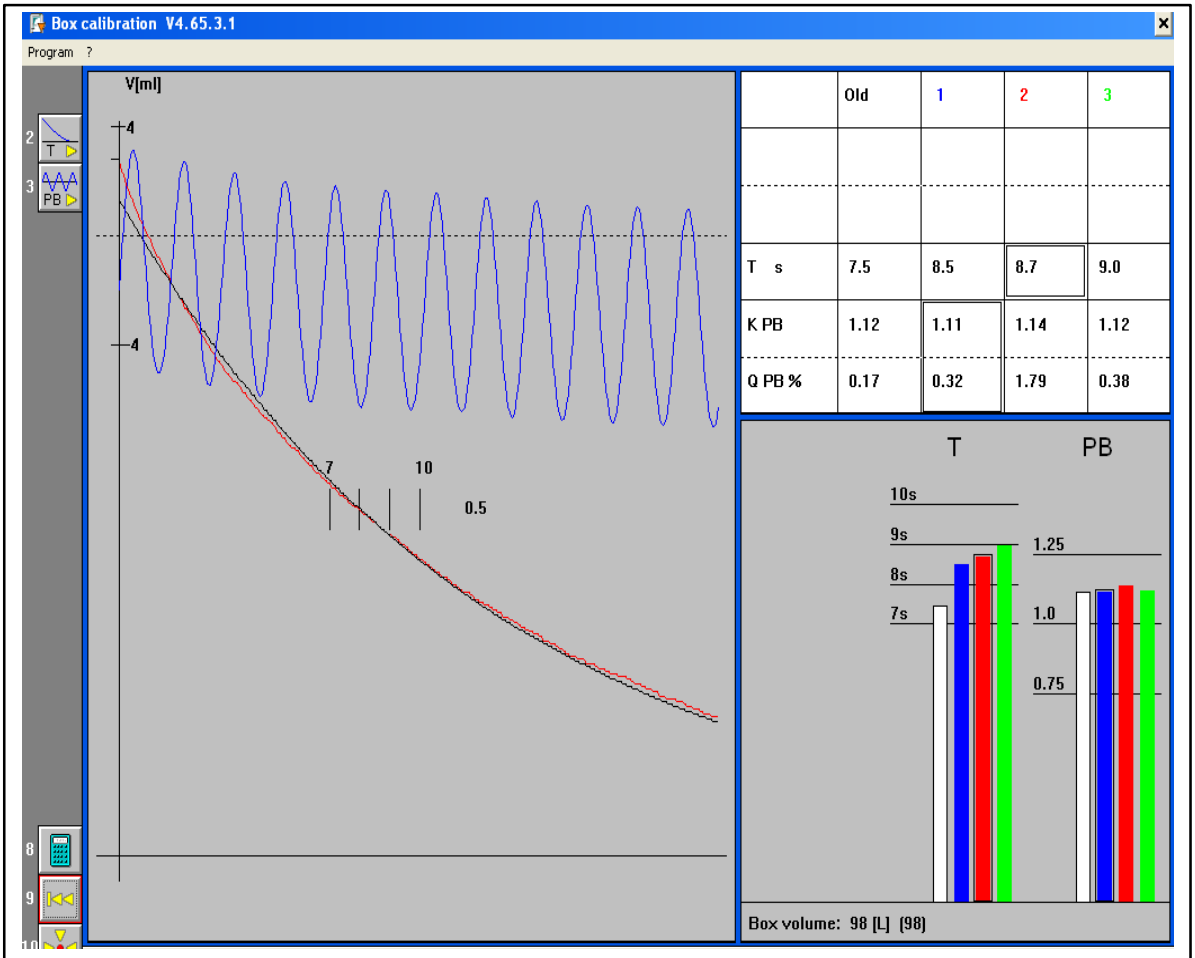
The Babybox system was calibrated with windows and door closed to minimise disturbances and noise. The calibration procedure was carried out in two steps:

a) the low-deadspace PNT was calibrated with a volume signal using a calibrated 100 mL syringe. A pre-set pairs (e.g., 6-8) of complete stroke volumes were delivered and values within 1-2% were acceptable (i.e., recorded signals of 98-102mL);

b) the plethysmograph (also known as the 'box') was calibrated with the hood closed following the activation of the "Box Calibration" software program. Prior to this, the estimated weight of the baby was keyed into the computer [Patient Data] record and the box was calibrated with whatever may potentially be included during the FRC test, e.g., a small towel, pulse oximeter and the RVRTC jacket. This was to ensure that the condition under which the box calibration took place was similar to that during actual testing condition.

The software automatically performed three trials of the half-life time constant (in seconds) of the box by assessing the decay of a square wave signal, and calibration of the change in box pressure in response to a known cyclical volume changes using a built-in sine pump (8 mL at 0.5 Hz frequency). The calibration curve and the half-life time constant (acceptable range: 7s to 10s) were displayed on the computer screen and the 'best' trial (i.e., the median value without any distortions) was accepted and saved (**Figure 2-vi**). BTPS (barometric pressure, temperature and saturated water vapour) correction factors for the tidal volume signal were calculated automatically by the computer software, using the details of ambient conditions saved by the user.

**Figure 2-vi: Screen display of "Box calibration"**




***Legend:*** *Three trials of the half-life constant were within 7-10 seconds during the box calibration and the trial with the median time constant (trial 2) was selected and saved in the calibration data.*

### 2.3.2.3 Data collection and analysis

The sleeping infant is transferred from the cot onto the Jaeger infant system, lying supine with the head supported in the midline, and neck and shoulders slightly extended. The facemask, attached to the PNT, is placed over the mouth and nose with a rim of therapeutic putty to achieve an airtight seal as described above for the MBW technique (**Figure 2-vii**).

**Figure 2-vii: Infant in the body plethysmograph**

***Legend**: A sleeping infant lying supine with head supported in the midline, neck and shoulders slightly extended.*

Data for the calculation of resting lung volume or $FRC_{pleth}$ were recorded with the hood lowered and the box closed (Figure 2-v). The $FRC_{pleth}$ software program enables the user to perform an airway occlusion (by inflating the shutter balloon, thereby occluding the lumen of the shutter situated immediately above the PNT) remotely by activating a specific function key on the keyboard. The default for the release of the airway occlusion was: a) once 3 respiratory efforts made by the infant have been detected, or b) after a duration of 10 seconds, whichever occurred first.

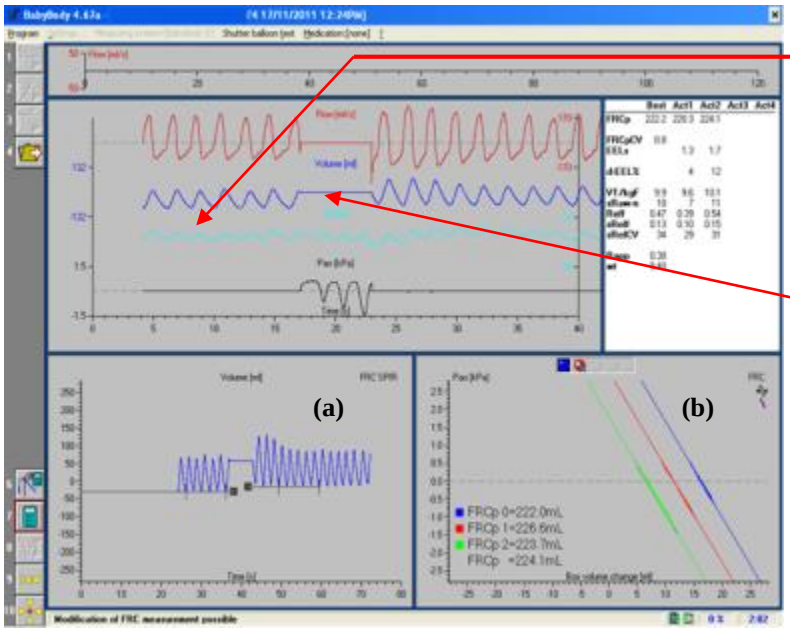
During an airway occlusion, although airflow is occluded, the infant continues to make respiratory efforts such that the alveolar gas volume is alternately rarified and compressed as the chest expands and relaxes. The accompanying changes in chest wall movement result in reciprocal pressure changes within the plethysmograph, which have been previously calibrated in terms of volume change (**section 2.3.2.2**). By relating these changes in alveolar volume to changes in alveolar pressure (measured at the airway opening proximal to the shutter during periods of no flow when pressures equalise throughout the respiratory system, provided there is no significant airflow obstruction) and to the absolute initial pressure within the lung (atmospheric pressure), the total occluded volume of the lungs at end inspiration can be calculated. $FRC_{pleth}$ (volume at end expiration) is automatically derived by the program software by subtracting the inspired tidal volume immediately prior to occlusion and any apparatus deadspace from the total measured volume.

Measurements of $FRC_{pleth}$[74] were undertaken according to the American Thoracic Society (ATS)/ European Respiratory Society (ERS) guidelines. After recording 6-10 regular tidal breaths, a brief airway (test) occlusion was made to ensure that there was an airtight seal around the facemask before closing the plethysmograph. Immediately after closing the lid, the box volume signal tends to drift upwards due to a slight increase in temperature as the infant's body heat and breath warms the interior of the box, indicating that thermal equilibrium has not occurred. It is important to wait for equilibrium to take place, before attempting to collect $FRC_{pleth}$ data.[74]

Once thermal equilibrium has been achieved (i.e., no drift of the box pressure signal) which takes ~2-3 minutes (**Figure 2-viii**), $FRC_{pleth}$ data collection began with 6-10 tidal breaths being recorded prior to triggering the inflation of the balloon shutter to effect an airway occlusion. The occlusion was held for two to three respiratory efforts, during which time simultaneous changes in box pressure and pressure changes at the airway opening were recorded. Since infants tolerate airway occlusions better at a higher lung volume (less likely to make poor respiratory efforts, close their glottis or become restless), airway occlusions were performed at end-inspiration rather than at end-expiration, and $FRC_{pleth}$ calculations subsequently corrected to end-expiratory level. Eight to twelve breaths were recorded after release of the shutter to allow time for the infant to re-establish their end-expiratory level (EEL) to restore lung volume and enable any mask leak to be detected. If the infant did not re-establish EEL (i.e., the likelihood of a mask leak as indicated by a step-up in EEL after release of the airway occlusion), the plethysmograph was opened and the PNT and/or mask re-adjusted to eliminate any leaks **(Figure 2-viii, a**). Data were accepted if the changes in plethysmographic pressure and changes in mouth pressure recorded during the airway occlusion were in phase (**Figure 2-viii, b**).

All tidal breathing based measurements such as MBW and plethysmography were performed before tidal squeeze and RVRTC as thoraco-abdominal compression and lung inflations could alter ventilation distribution and/or extent of gas trapping hence affecting results obtained from MBW and plethysmography.

**Figure 2-viii: Screenshot during an $FRC_{pleth}$ measurement**




Thermal equilibrium reached within the plethysmograph as there is no drift in box volume signal prior to airway occlusion

Balloon shutter activated to perform airway occlusion for 3 respiratory efforts. Note change in box pressure drift during the occlusion when no gas exchange into the box

***Legend:*** *(a) After release of balloon shutter, the infant generally re-established end-expiratory level within a few breaths. If a big 'step up' was seen, this could indicate a leak around the mask. If this was the case, then the plethysmograph was opened to adjust the infant's mask or PNT. (b) This shows good phasing i.e. no 'looping', between changes in box volume and mouth pressure during airway occlusion. The mean (SD) of 3 measures of $FRC_{pleth}$ (minimum 2) that were within 10% were reported.*

## 2.3.3 Raised Volume Rapid Thoraco-abdominal Compression technique (RVRTC)

### 2.3.3.1 Equipment

The RVRTC technique was performed using the Jaeger MasterScreen BabyBody System (CareFusion,v 4.65) and measurements were undertaken according to the ATS/ERS consensus.[56] To acquire data generated from the raised volume forced expiratory manoeuvres, additional items of equipment were required.

**<u>Inflatable bladder and polythene jacket</u>**

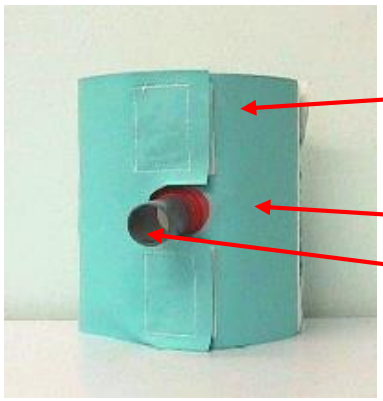
- Clear rectangular polythene expandable bladders with a short wide-bore tube in the centre, which allowed connection to a built-in air compressor tank for inflation; available in three sizes: 17x19 cm, 19x22 cm and 21x22 cm (Medizinische Hochschule Hannover, Germany) (**Figure 2-ix**).

- Width adjustable outer jacket (resembling a cummerbund), made of non-stretchable vinyl held together by Velcro strips at the front and back, which permitted adjustment of the circumference to accommodate infants of various sizes; available in three lengths of 16, 18 and 23 cm (Medizinische Hochschule Hannover, Germany).

**Large bore elephant tubing**

- The encased inflatable bladder was attached to a 55 L built-in air compressor tank by means of a rigid large-bore elephant tubing (3 cm internal diameter). This arrangement enabled a rapid supply of compressed air into the inflatable jacket to force expiration.

**Figure 2-ix: Polythene rectangular jacket bladder and a non-stretchable outer vinyl jacket used during RVRTC**

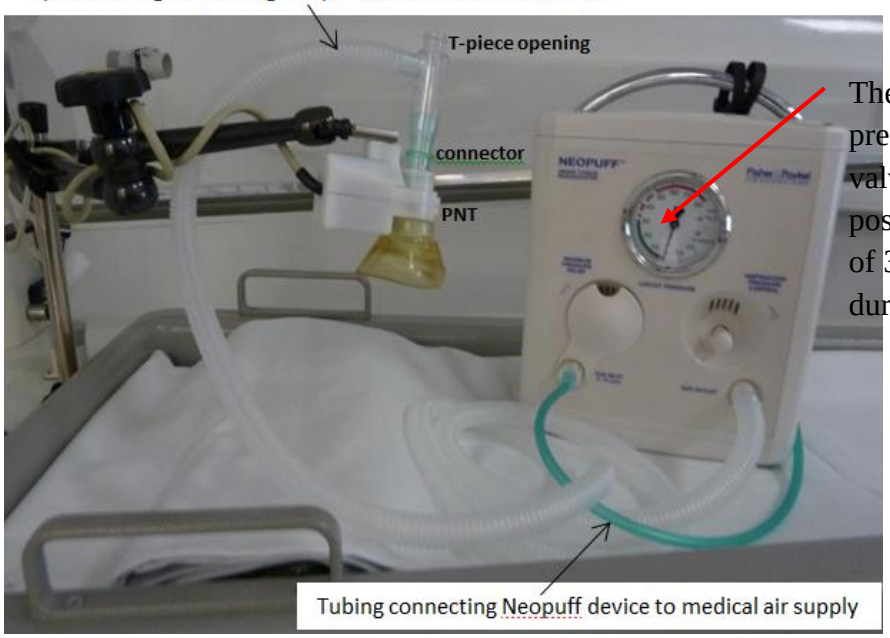



***Legend:*** *Velcro fittings available at the front and back of the vinyl jacket to allow for width adjustment.*

**Neopuff infant resuscitator**

The Neopuff RD1000 system (**Figure 2-x**) allowed the setting of a pre-determined pressure, i.e., a positive inflation pressure of 30 $cmH_2O$ (2.94 kPa), using a flow of 10–12 $L \cdot min^{-1}$ of air, to be delivered to the infant's lungs to augment lung volume towards TLC during the RVRTC test. This was achieved by applying intermittent manual occlusions (i.e. PIP) at the T-piece opening (**Figure 2-x).**.

**Figure 2-x: Neopuff Infant Resuscitator and apparatus set up for the RVRTC manoeuvres**



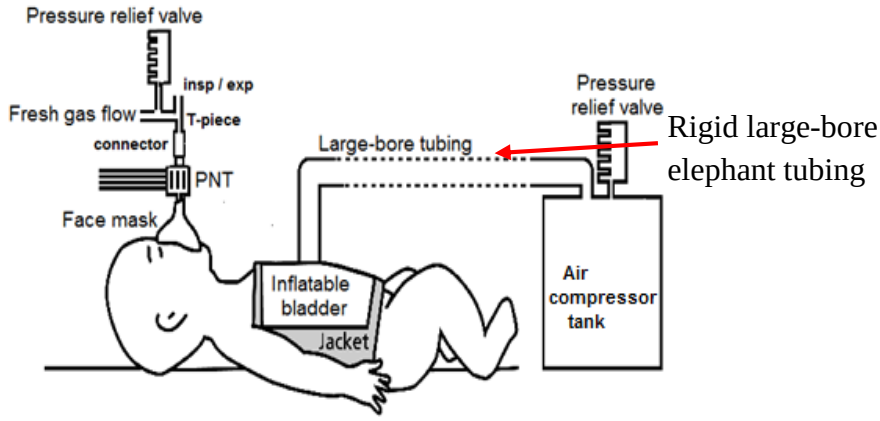

***Legend:*** *This illustration shows the Neopuff Resuscitator connected to a supply of medical air (via the green tubing), and the T-piece tubing connecting the Neopuff device to the PNT and facemask. Infant's lungs inflated using the neopuff infant resuscitator delivering a positive pressure of 30 cm $H_2O$ using 10-12 $L \cdot min^{-1}$ of air.*

### 2.3.3.2 Methods

The inflatable bladder placed over the anterior aspect of the chest and abdomen; the outer jacket (extended from the level of the axillae to the symphysis pubis) was adjusted to fit over the bladder snugly (**Figure 2-xi**), allowing sufficient space between the sternum and chest to accommodate the insertion of three to four adult fingers.[56] The arms remained outside the jacket to avoid splinting of the thorax.

The encased inflatable bladder was attached, via a large-bore elephant tubing, to the pressurised air tank from which compressed air was delivered to inflate the bladder effecting a rapid chest/abdominal compression to force expiration, coinciding with the end of a passive inflation when the lung volume had been augmented towards TLC.

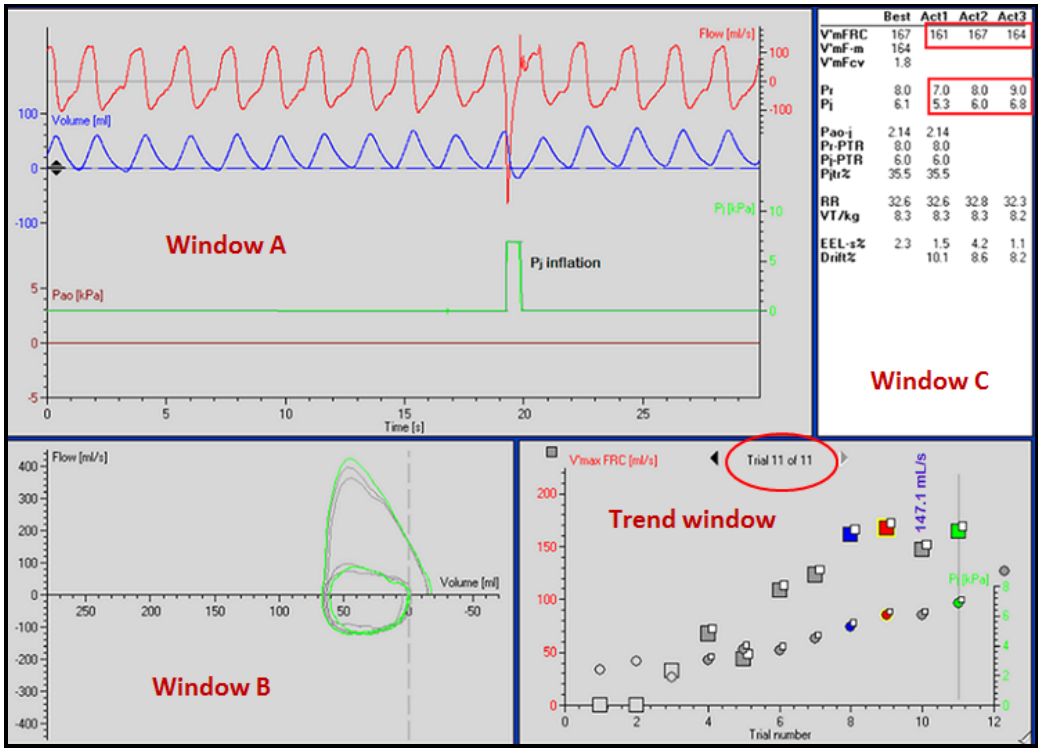
**Figure 2-xi: Schematic diagram showing the apparatus set up for performing the raised volume manoeuvres**




***Legend:*** *Air from the air compressor tank was delivered via the elephant tubing and rapidly inflates the jacket bladder which was secured over the infant's chest and abdomen by the outer jacket.*

Prior to performing the RVRTC, partial or tidal forced expiratory manoeuvres were undertaken to determine the optimal jacket compression pressure ($P_j$) at which flow limitation was achieved, i.e., the point at which no further increase in expiratory flow was observed despite further increases in applied jacket pressure (**Figure 2-xii**).

**Figure 2-xii: Screenshot showing display of V'$_{maxFRC}$ results from an acceptable test**

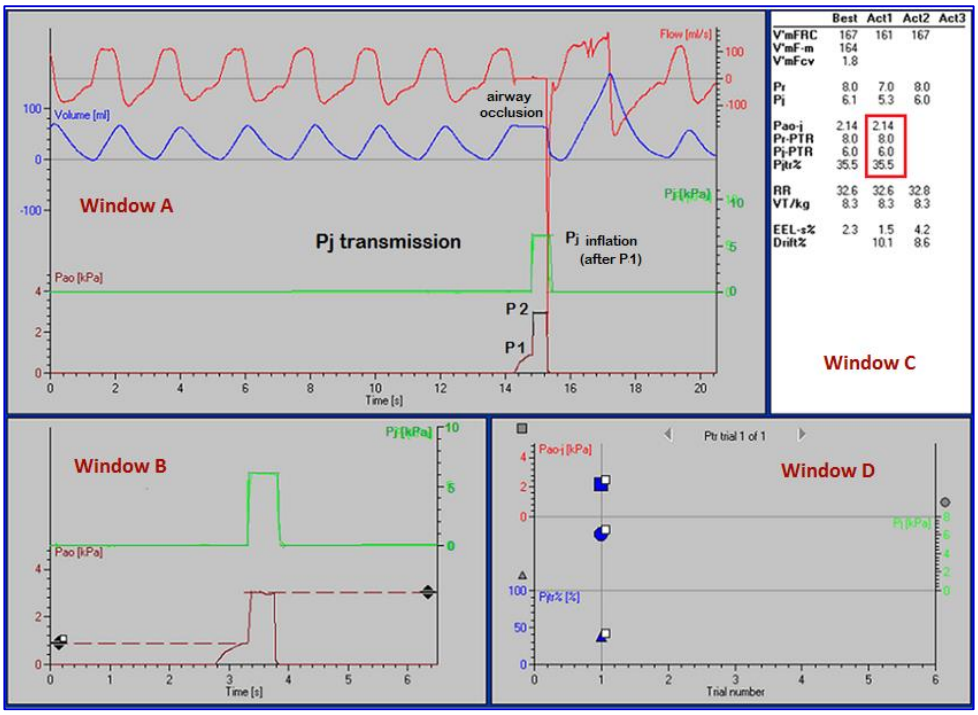



***Legend*** *This screen display shows results of 3 reproducible PEFV curves, with window A showing a real-time trace from the 11$^{th}$ trial, illustrating jacket inflation pressure of 6.8 kPa (achieved using a reservoir pressure [$P_r$] of 9 kPa) which resulted in a V'$_{maxFRC}$ of 164 mL/s, is similar to V'$_{maxFRC}$ achieved when a $P_r$ of 7 and 8 kPa had been used, resulting in $P_j$ of 5.3 and 6.0 kPa respectively (window C and trend window). Since no further increase in V'$_{maxFRC}$ was observed with increasing $P_j$, it was evident that "flow limitation" had been reached in this child at an average $P_j$ of 6.1 kPa (Window C).*

The optimal $P_j$ thus obtained was then used during the raised volume manoeuvres. To further assess the efficiency of chest/abdomen compression with the jacket in situ, the software program was able to estimate the magnitude of the external pressure being transmitted to the intra-thoracic structures (**Figure 2-xiii**). The transmission pressure ($P_{ao-j}$) may be calculated by subtracting the value of $P_{ao}$ during an airway occlusion while breathing tidally [P1], from $P_{ao}$ value when chest/abdominal compression was applied while maintaining the airway occlusion [P2]. Under the condition of no air flow (i.e., during airway occlusion), $P_{ao}$ represents alveolar pressure ($P_{alv}$), hence the change in $P_{alv}$ or $P_{ao}$ during tidal breathing and during chest/abdominal compression (P2 minus P1) provides an indication of the efficiency of jacket compression. In healthy infants, the $P_{ao-j}$ should be at least 2 kPa (*but should not exceed 3 kPa*),

whereas it may be < 2kPa in infants with airway disease in whom flow limitation is achieved at lower intra-thoracic pressures.

**Figure 2-xiii: Assessment of jacket pressure transmission ($P_j$tr) during tidal RTC**



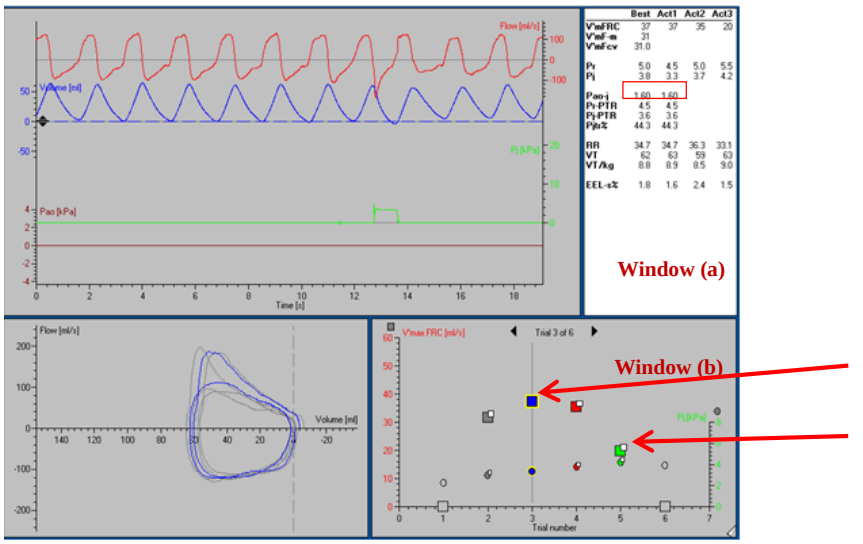

***Legend:*** *During an airway occlusion, i.e., no air flow, pressure equilibrates rapidly within the respiratory system such that the pressure measured at the airway opening ($P_{ao}$) represents the alveolar pressure ($P_{alv}$).*

*The time-based trace in window A illustrates the change in $P_{ao}$ during a brief airway occlusion prior to jacket inflation (indicated as P1), and during jacket inflation (P2). In this example, the difference between the absolute $P_{ao}$ values ($P_{ao}$-j) (i.e., P2–P1) was 2.14 kPa, or when expressed as percentage, jacket pressure transmitted ($P_j$tr) was 35.5% (see red rectangular in window C).*

It is important not to apply inappropriately high jacket pressures to these infants since such manoeuvres are likely to result in glottis closure and/or negative flow dependence.[185,186] (**Figure 2-xiv**)

The $P_j$ at which optimal $P_{ao}$-$_j$ was achieved is used for the RVRTC manoeuvres, provided that the fitting of the jacket remained unchanged.

**Figure 2-xiv: Screenshot showing reduced V'$_{maxFRC}$ in an infant with airway obstruction**



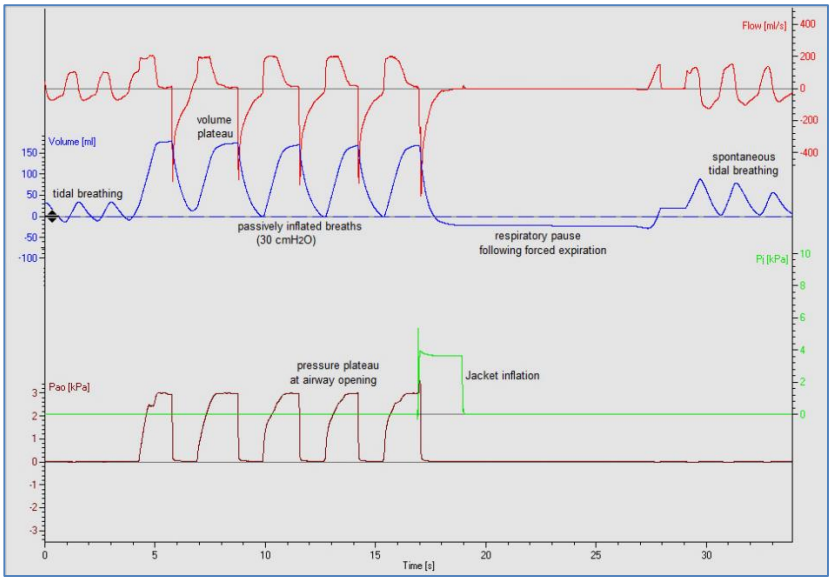

***Legend:*** *In infants with peripheral airway obstruction, it may not always be possible to achieve Pao-j close to 2 kPa if they are severely flow limited (window a). In this case, despite increasing the jacket pressure (Pj) on four occasions, there was no further increase in V'$_{maxFRC}$ (see the last four points starting from the blue, red, green and later grey dots). Negative flow dependence was seen with the green dot where V'$_{maxFRC}$ reduced with increasing jacket pressure (window b).*

To obtain a FEFV curve from raised volume, 3-6 passive lung inflations using a pre-set pressure of 30 cm$H_2O$ (2.94 kPa) via the NeoPuff infant resuscitator device (**Figure 2-xv**) (Fisher & Paykel Healthcare, UK), were administered to induce muscle relaxation (by evoking the Hering-Breuer pulmonary stretch receptors before the jacket was inflated to the previously determined optimal Pj, at the end of full lung inflation of the final augmented breath. To aid relaxation and to ensure that the infant's lungs were fully inflated towards TLC, the individual inflations were held until a pressure plateau was observed on the airway pressure trace (**Figure 2-xvi**). Thoraco-abdominal compressions were performed at end inspiration and held until all volume had been expired or the next inspiration had commenced (**Figure 2-xvii**).

**Figure 2-xv: RVRTC apparatus set up for measurement of RVRTC manoeuvres**


***Legend:*** *The photo shows the Neopuff$^{TM}$ resuscitator (left) connected to the T-piece and straight connector, which are inserted to the PNT to enable intermittent delivery of 3-5 augmented breaths at a positive inflation pressure of 30 cm$H_2O$ to raise or extend lung volume towards TLC prior to forced expiratory manoeuvre.*

**Figure 2-xvi: Screenshot displaying five passive lung inflations to relax the respiratory muscles prior to the jacket compression**

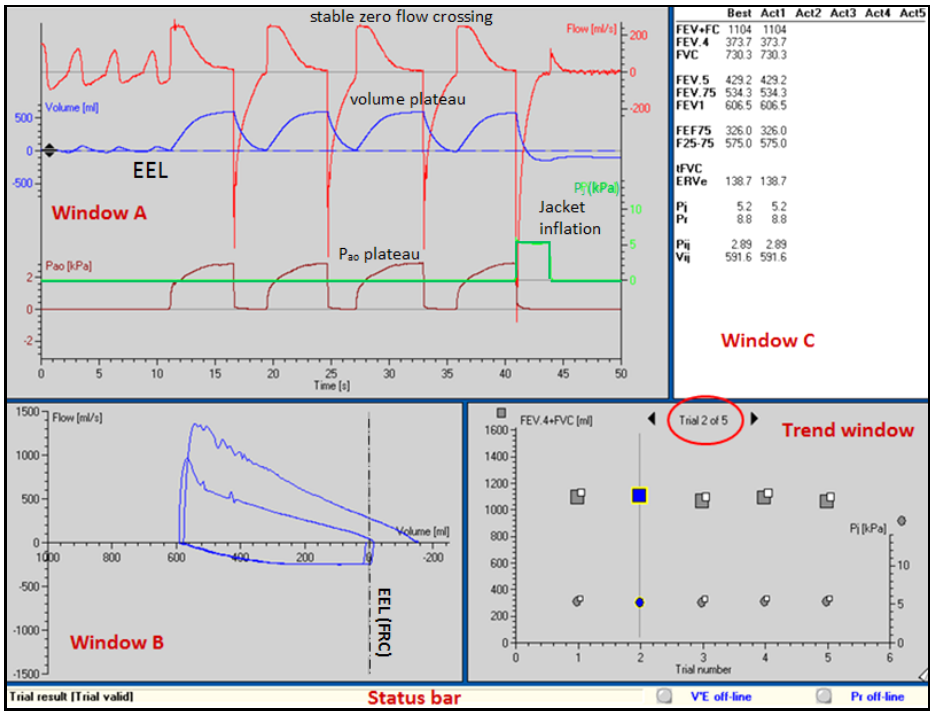



***Legend****: Time-based trace showing five passively inflated breaths and timing of jacket compression during a raised volume squeeze manoeuvre.*

This procedure was repeated until three (a minimum of two) acceptable and reproducible FEFV curves were achieved. The "best" FEFV curve was defined as the

technically satisfactory curve with the highest sum of forced vital capacity (FVC) and forced expired volume at 0.4 second ($FEV_{0.4}$), with at least one other curve within 10% of these values.[56] The reason for using the parameter $FEV_{0.4}$ instead of $FEV_{0.5}$ is because often in the first few weeks of life, lung emptying occurs in less than half a second. Other parameters calculated from the "best" raised volume FEFV curve included forced expired volume at 0.5 second ($FEV_{0.5}$), forced expired flows when 75% of FVC had been expired ($FEF_{75}$) and forced expired flow between 25-75% of FVC ($FEF_{25-75}$). Forced expiratory flow-volume data were analysed according to international consensus statements.[56] The criteria for technically acceptable forced expired flow-volume curves were: no mask or PNT leak, peak expiratory flow achieved prior to 30% of expired volume, complete expiration towards residual volume (RV) (i.e., no evidence of early inspiration), no marked flow transients or glottic closure, and airway inflation pressure within ±5% of the pre-set 30 $cmH_2O$ (i.e., 2.94 kPa; acceptable range: 2.8 to 3.1kPa).

**Figure 2-xvii: Screenshot displaying RVRTC measurement**




***Legend:*** *A screen display showing a technically acceptable RV squeeze manoeuvre, with jacket inflation synchronised with termination of passive lung augmentation (window A).*

## 2.3.4 Post study procedure

### 2.3.4.1 Post sedation and discharge advice

Although infants were not discharged home until they had fully woken up, parents were warned to be extra vigilant for up to 48 hours, since the half-life of trichloro-ethanol, the active metabolite of chloral hydrate, is approximately 10-18 hours.[187] Parents were given an advice note stating the name and the possible effects of the sedative agent, together with the contact telephone numbers of the research team members directly involved with the study, in case of any problem or queries. A telephone call to the parents was made the following day to check on the well-being of the infant and to answer any further queries.

## 2.3.5 Lung function outcomes measured in this thesis

### 2.3.5.1 Expression of results

All results were expressed as z-scores to adjust for body size, sex and age where appropriate using reference equations derived from up to 140 healthy white infants studied in our department over the past decade using identical equipment and protocols.[56-59,74] Reduced $FEV_{0.5,}$ FVC, and $FEF_{75}$ were defined as those falling below the lower limit of normal (<2.5$^{th}$ centile) i.e. < –1.96 z-score.

LCI, $FRC_{MBW}$ and $FRC_{pleth}$ were also expressed as z-scores using reference equations formulated from separate normative datasets obtained using identical equipment and methods.[58,59,74] 'Abnormal' values were those falling above the upper limit of normal (>97.5$^{th}$ centile), i.e., >1.96 z-score. The reason for choosing the more conservative 95% (1.96 z-score), rather than 90% (1.64 z-score) limits of normality as the threshold for determining 'abnormal' results was to reduce the possibility of over-diagnosing lung disease, which may have occurred had we used ±1.64 z-scores as commonly applied in lung function studies in older subjects. This choice took into account the rapid developmental changes that occur in the first year of life, which are associated with increased within and between-subject variability of lung function, as well as the fact that several different lung function outcomes were being examined statistically which may potentially increase the risk of false positives.

The rationale for using the following lung function parameters as outcome measures has been discussed in chapter 1, **section 1.6**.

#### 2.3.5.2 Primary outcome measures

- LCI: a measure of global ventilation inhomogeneity, an indicator of early airway disease including that in the distal airways;
- $FEV_{0.5}$ : a measure of central airway obstruction;
- $FRC_{pleth}$: a measure of total thoracic lung volume at end expiration including any non- ventilated gas " trapped" behind narrowed or obstructed airways.

#### 2.3.5.3 Secondary outcome measures

- ΔFRC: a surrogate measure of 'gas trapping' which was calculated as the difference between $FRC_{pleth} - FRC_{MBW}$ z-scores.

### 2.3.6 Statistical analysis and power of study

Standard software packages were used to inspect data for distribution and calculate descriptive statistics (PASW Statistics v.18, Chicago, IL, US). Student *t* tests or $\chi^2$ analyses as appropriate were used to compare background characteristics and lung function results between CF and healthy controls at a year. Data were summarised using n (%), mean (SD) or ranges if parametric, or median (Inter-quartile range, IQR) if non-parametric; model estimates and differences between groups are presented with 95% confidence intervals (CI).

The proportion of CF infants with positive *Staphylococcus Aureus (SA)* and *PsA* growth in the first year of life was also recorded. Multiple linear regressions (MLR) were used to investigate how the lung function variables at one year varied according to potential determinants including background characteristics, clinical symptoms, antibiotic treatment, microbiological results and lung function at 3m of age.

Taking into account three primary outcomes (LCI, $FEV_{0.5}$ and $FRC_{pleth}$), a sample size of 40 infants in each group would provide 80% power to detect a difference of 0.62 z-scores at 5% significance level between infants with CF and healthy controls.[188-190] Hence we aimed to recruit at least 50 NBS CF babies born in the Greater London

catchment area over an 18 month period, and 50 matched controls, after allowing for attrition of 20%.

### 2.3.7 Database management

In accordance to the Data Protection Act and requisite of the Ethics Committee, CF and healthy infants in the study were each assigned a unique identification number (Study ID) and all paper documentations (including scanned copies) were stored securely. Clinical and background information and lung function data were manually entered into the Re-Base$^{TM}$ customised database (Re-Base Ltd, London, UK) using double-entry method. Once lung function data had been analysed, results were exported in numeral format as Excel worksheet and electronically transferred to the Re-Base$^{TM}$ database to avoid transcription errors. Dataset were double-checked to ensure results were transferred to the correct individual infant folders.

Clinical information documented in CRF pertaining to the CF infants by the participating LCFC centres were analysed and summarised into password protected Excel sheet by the author and another researcher on the team. Cross-checking of clinical data was undertaken to ensure accuracy. The challenges involved in collecting detailed clinical information from the different centres will be discussed in detail in the final chapter (**chapter 7, section 7.2.2**).

## 2.4 SUMMARY OF LUNG FUNCTION TESTING PROTOCOL

Identical lung function protocols according to established international standards were adhered to at each testing occasion (3m and 1yr of age) at one centre (GOSH) for CF and control infants. The order of tests performed was as shown in the flowchart in the beginning of this chapter (**Figure 2-i**) and for reasons discussed in **section 2.3.2.3**. A wide range of tests was selected for this protocol to ensure that different physiological abnormalities due to early lung disease in NBS CF infants would be detected as mentioned in **section 1.6**. Results of these lung function tests will be presented in chapter 3.

# 3 LUNG FUNCTION IN NEWBORN SCREENED CF INFANTS AT ONE YEAR OF AGE: RESULTS

## 3.1 INTRODUCTION

This first results chapter summarises the lung function results at a year of age in CF infants compared to contemporaneous healthy controls, in order to test the primary hypothesis that despite early diagnosis and specialist treatment, NBS CF infants have abnormal lung function at a year of age.

ILFT had previously been undertaken in NBS CF infants and healthy controls at 3m of age as part of this longitudinal observational study[71], and some of these 3-month results will be included here. The predictive value of lung function results at 3 months of age (**section: 3.5.6**) and clinical determinants of lung function at a year of age will also be investigated in this chapter (**section: 3.5.7**).

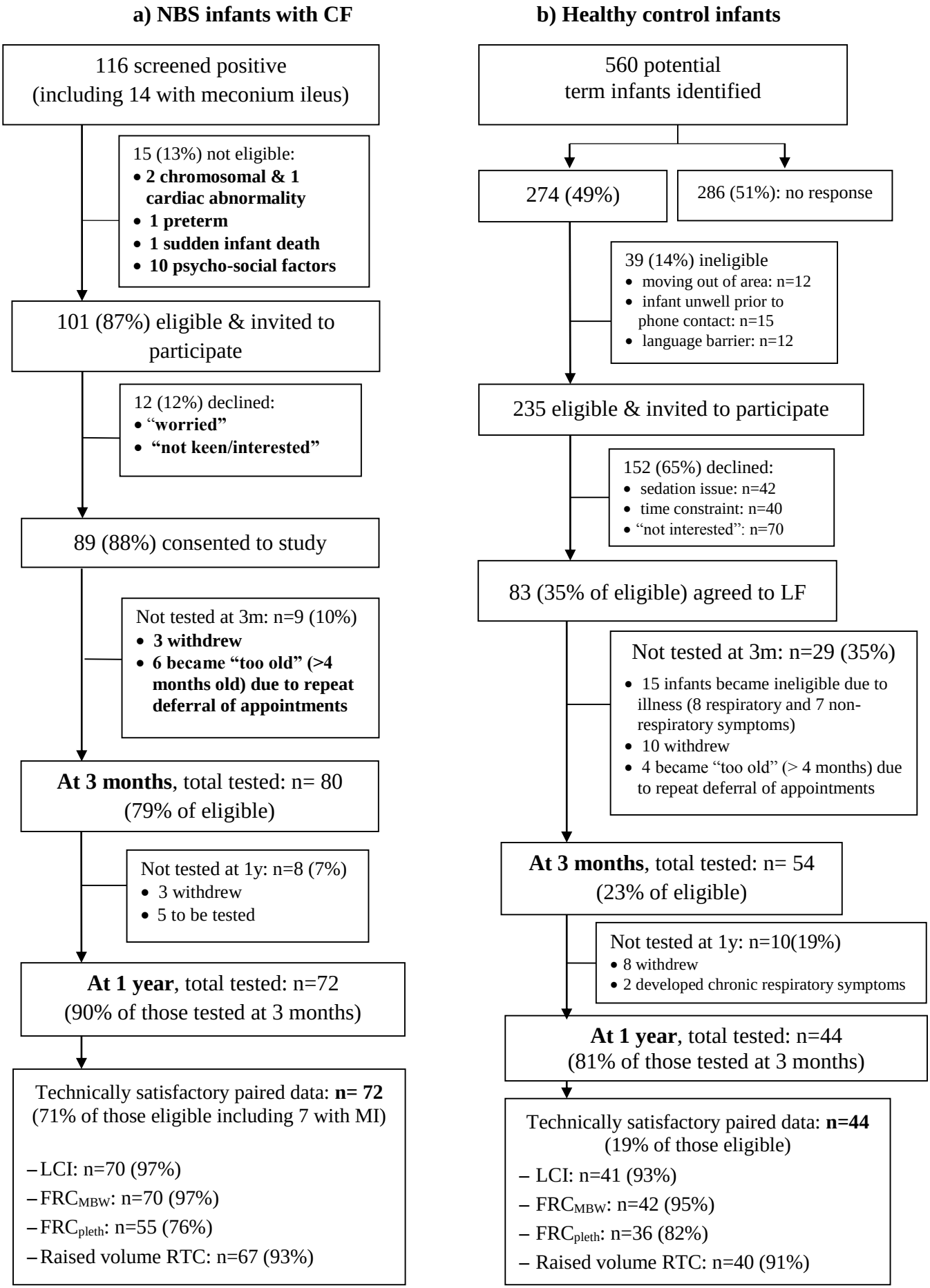
## 3.2 RECRUITMENT DATA

The study was conducted from January 2009 through May 2012. The screening, recruitment and follow up of subjects are shown in **Figure 3-i**.

**CF infants**

Eligible infants were recruited from Jan 2009 until July 2011. A total of 116 infants screened positive for CF during this period, of whom 14 (12.1%) also presented with meconium ileus. Fifteen (13.0%) infants were ineligible for the study: 3 due to serious co-morbidities such as chromosomal abnormalities and significant cardiac defect, 1 was born preterm at 32 weeks gestation, 1 died of sudden infant death and 10 had either complex social issues or were living beyond the catchment area for the study. The social issues included excessive parental anxiety and depression, living beyond the study area of recruitment and other complex psychosocial issues arising from complicated surgery for meconium ileus. Hence 101 (87%) infants were eligible and invited to participate in the study. 12 declined to be in the study and cited reasons of being “worried about the tests” or “not interested” in participating in

**Figure 3-i: Flow diagram showing recruitment and retention of CF and control infants**



the research study.[191] Eventually 89 sets of parents (88% of those eligible) gave consent for their NBS CF infants to participate in the study.

At the 3m ILFT, 9 consented infants did not undertake the test: 3 families withdrew from the entire study after further consideration, while 6 infants became too old due to repeated deferral of appointments as a result of coryzal symptoms. In total, 80 (79% of eligible) CF infants underwent the 3m ILFTs. One of the CF infants who did not have 3m test due to repeated respiratory symptoms subsequently had ILFT as well as chest CT and bronchoscopy at a year of age (**Figure 3-i**); this infant was not included in the analysis in this chapter which is limited to those infants who had paired lung function at 3m and 1yr, but was included for analysis with regards to structural changes and its relationship with lung function (**chapter 6, section 6.3)**

Seventy two infants (71% of those eligible) subsequently completed their 1yr ILFT. Of the remaining eight, three withdrew from the study after their first test and 5 were due to have their 1yr ILFT after writing up of this thesis so these infants were excluded from the analysis Hence satisfactory paired lung function data from 72 CF infants (including 7 with meconium ileus) will be presented within this chapter.

**Healthy control (HC) infants**

During the study, 560 potentially eligible healthy full-term infants were identified from the Homerton University Hospital birth lists (see **chapter 2, section 2.2.2**). Two hundred and eighty six (51% of potentially eligible) control infants were not contactable. Of those who could be contacted, 235/274 infants met the study inclusion criteria and were formally invited to participate in the study. Thirty nine of those contacted were deemed ineligible due to (a) not living within the local area (n=12), (b) language barrier which made the explanation of the study difficult (n=12) or (c) had been unwell prior to the first phone contact (n=15).

Eighty-three (35% of eligible) consented to the study but only 54 (23% of all eligible contacted controls) actually attended their 3m ILFT. Twenty nine (35%) of those who agreed to ILFT did not attend their first test, including 15 who subsequently became unwell (8 respiratory and 7 non-respiratory events), and 10 who withdrew

from the study after initially consenting; and 4 who missed the age limit for first 3m test due to repeat deferral of appointments.

Of the 54 HC infants who underwent baseline ILFT at 3m of age, eight withdrew from the study and 2 developed chronic respiratory symptoms following their first tests. The remaining 44 (81%) had successful repeat ILFT at 1yr.

## 3.3 BACKGROUND CHARACTERISTICS OF CF AND HEALTHY CONTROL INFANTS

The comparison of infant characteristics is shown in **Table 3-i**. NBS CF infants were born on average ~ a week earlier, with lower birth weights and a higher percentage born small-for-gestational age (i.e., birth weight < $10^{th}$ percentile). These differences were statistically significant but clinically trivial. A higher proportion of parents of CF infants have manual occupations, suggesting a slight bias towards recruitment of healthy infants with more favourable or stable socio-economic background, or that such families were more familiar with research therefore more keen to participate. However, other important determinants of early lung function such as ethnicity,[192] maternal smoking during pregnancy,[123] current maternal smoking and maternal history of asthma[193] were similar in both CF and healthy controls.

**Table 3-i: Comparison of background characteristics in infants with CF and healthy controls**

| | CF infants (n=72) | Control infants (n=44) | Δ (95% CI) CF– controls | p value |
|---|---|---|---|---|
| Male, n (%) | 34 (47%) | 21 (48%) | –1% (–19%; 18%) | 0.958 |
| Gestational age, weeks | 39.1 (1.4) | 40.3 (1.1) | –1.1 (–1.6; –0.6) | **<0.001** |
| Birth weight, z-score[a] | –0.64 (0.84) | 0.12 (0.81) | –0.76 (–1.07;–0.45) | **<0.001** |
| Birth weight < 10$^{th}$ percentile[a], n (%) | 13 (18%) | 2 (5%) | 14% (1%; 24%) | **0.014** |
| White mother, n (%) | 61 (85%) | 38 (86%) | -2% (–14%; 13%) | 0.806 |
| Maternal smoking during pregnancy, n (%) | 8 (11%) | 3 (7%) | 4% (–8%; 15%) | 0.419 |
| Current Maternal smoking, n (%) | 9 (13%) | 5 (11%) | 1% (–13%; 13%) | 0.854 |
| Mother in non-manual occupation | 50 (69%) | 38 (86%) | –17% (–30%; -1%) | **0.024** |
| Fathers in non-manual occupation[b] | 43 (60%) | 36 (82%) | –22% (–37%; -5%) | **0.007** |
| Maternal asthma, n (%) | 14 (19%) | 8 (18%) | 1% (–14%; 15%) | 0.865 |

***Footnote:*** *Data shown as n (%) for categorical and mean (SD) for continuous variables. [a]calculated using UK-WHO algorithms[182]; [b]n=69 CF and n=53 control infants. Abbreviations: Δ = difference between groups; CI=confidence interval of the difference between groups.*

### 3.3.1 Validation of maternal report of smoking exposure

In this study, the reported incidence of maternal smoking during pregnancy or postnatally was relatively low (7-13%; **Table 3-i**), when compared with an incidence of 21-32% from infants in London reported a decade ago by this department.[60,83] **Table 3-ii** summarises cotinine concentrations for infant urine and maternal saliva, collected from those whose mothers reported not smoking during pregnancy and postnatally (also see **section 2.2.4**). The results were well below the reported optimum cut-off values to distinguish non-smokers from smokers: i.e., 49.7ng/mL for urine and 12 ng/mL for salivary samples,[177] suggesting that parental self-reporting of non-smoking in this study was accurate, and that passive smoke exposure was likely to be minimal and therefore not likely to bias interpretation of ILFT in this study.

**Table 3-ii: Urine and salivary cotinine results to validate maternal report of 'no smoking'**

| | n | Infants with CF | n | Healthy controls |
|---|---|---|---|---|
| Infant urine cotinine (ng/ml) | 45 | 1.0 (1.0-3.0) | 27 | 1.0 (1.0-15.5) |
| Maternal saliva cotinine (ng/ml) | 11 | 0.1 (0.1-0.2) | 11 | 0.1 (0.1-0.3) |
| Missing Data | 7 | | 1 | |

***Footnote:*** *Data expressed as median (range). Due to the cost of these assessments and the unlikelihood of any false positives, sample collection and analyses were limited to mothers who reported that they did not smoke during pregnancy or postnatally.*

## 3.4 CLINICAL CHARACTERISTICS OF CF INFANTS

For NBS CF infants, the median (interquartile range (IQR)) age at diagnosis was 3.6 (3.0–4.4) weeks with 7 (10%) infants presenting with meconium ileus. Since exclusion or inclusion of these infants did not affect the results (data not shown), they were included in the analysis. Clinical characteristics of CF subjects are summarised in **Table 3-iii**.

The majority of the CF infants were in the 'severe' genotype class (Classes I-III, 82%) and were pancreatic insufficient (93%) as measured by stool elastase. By the time of the 1yr ILFT, all the CF infants had experienced respiratory symptoms, ranging from cough, runny nose, evidence of physician diagnosed wheeze (33%) or crackles (8%) on chest auscultation within the first year of life. A majority of the healthy controls also had previous experiences of cough and runny nose with 10% having had a single episode of physician-diagnosed wheeze between 3m and a year. Two healthy controls had significant lower respiratory tract infections and chronic wheeze and were excluded from testing at 1yr of age. Efforts were made to test infants at least 3 weeks after a respiratory illness, however there were times when CF infants were tested slightly earlier (a minimum of 10-14 days of being asymptomatic) as they experienced repeated episodes of exacerbations. There were no infants with evidence of wheeze, crackles or breathlessness during the 2-3 weeks preceding the 1yr ILFT. During this pre-test period, 15 (21%) of CF infants were reported to have had a cough in the absence of other symptoms.

**Table 3-iii: Clinical characteristics of CF infants (n=72)**

| | |
|---|---|
| Age at diagnosis, postnatal age in weeks | 3.9 (1.7) |
| CFTR genotype classes I-III | 59 (82%) |
| Presented with meconium ileus | 7 (10%) |
| Pancreatic sufficient | 5 (7%) |
| ***Prior to 1 year lung function assessments*** | |
| **Respiratory symptoms, ever:** | |
| Wheeze, physician diagnosed | 24 (33%) |
| Crackles, physician diagnosed | 6 (8%) |
| Cough within 3 weeks of 1 year lung function | 15 (21%) |
| **Bacterial growth on cough swab, ever[a]** | |
| *Pseudomonas aeruginosa*[194] | 25 (35%) |
| Other significant bacterial growth | 17 (24%) |
| No growth | 30 (42%) |
| **Additional treatment received[b]** | |
| rhDNase | 6 (8%) |
| Intravenous antibiotics, number of courses | 0 (0-3)[c] |
| Gastro-oesophageal reflux treatment | 38 (53%) |

***Footnote:*** *Results expressed as mean (SD) or n (%) unless otherwise stated.*
[a] *See* ***section 3.4.1****;*
[b] *in addition to the prophylactic flucloxacillin prescribed for all CF NBS infants;*
[c] *median (range).*

## 3.4.1 Microbiology results of CF infants

For infants participating in this study, a median of 9 (range: 4-17) cough swabs per child were sent for analysis during the first year of life. The results of these were obtained from the infant's CRF (**chapter 2**, **section: 2.2.5**) which were collected prospectively and sent to me every 2-3 months. Results of cough swabs sent from the lung function laboratory were also recorded on the database.

To study the effect of bacterial acquisition on lung function, infants were categorised into three groups, namely *PsA* growth ever, significant bacterial growth ever and no

growth/ non-significant bacterial growth ever. Numbers were insufficient to subdivide further for statistical analysis. The significant bacterial growth category consisted of those who isolated *SA, Haemophilus influenza (HI), Stenotrophomonas maltophilia, Acromobacter xylosidans, Methicillin Resistant Staphylococcal Aureus (MRSA)* or *Aspergillus fumigatus* with no previous *PsA* growth. No, or non-significant, bacterial growth consisted of those with isolation of coliforms and upper respiratory tract flora only.

During the first year of life, 25 (35%) of CF infants had isolated *PsA* on at least one occasion, 12 (17%) had isolated *SA*, 14 (19%) *HI*, 3 (4%) *S maltophilia*, 2 (3%) *A xylosidans*, 3 (4%) *MRSA* and 2 (3%) *A fumigatus*. Apart from one infant with chronic *PsA* and one with chronic *SA*, none had chronic bacterial growth, defined by the Leeds criteria[194] within the first year of life. 30 (42%) of CF infants never had any significant bacterial growth. Viral polymerase chain reaction and molecular microbiology studies were not undertaken in the current study. The potential impact of non-standardised microbiological analyses of cough swabs from infants in different tertiary respiratory centres and shared care clinics that could determine accuracy of bacterial isolation of these CF infants will be discussed in **chapter 7, section 7.2.2.1**.

### 3.4.2 Additional treatment received by CF infants

All CF infants were commenced on prophylactic flucloxacillin once diagnosis was confirmed (**Appendix: A7**). Antibiotics received by CF infants for respiratory symptoms (coryzal and cough) and/or positive cough swab results were recorded as additional courses; whilst those received for non-respiratory reasons were not counted as additional treatment. By 1yr of age, 19 (26%) of infants had received at least one course of intravenous antibiotics; whilst two required 3 courses. All infants had at least one additional course of oral antibiotics in the first year of life (range: 1-14) (**Table 3-iii**).

Inspection of CRFs and regular communication with consultants revealed excellent adherence to treatment protocols. 17 NBS CF infants had used an inhaled bronchodilator at some point by 1 year of age, all but one of whom commenced this

by 3 months. Of these 17 infants, only one still used it regularly by 1 year of age, with very intermittent use by the remaining 16. One infant was prescribed regular inhaled steroid by 1 year of age, while another had a single course of oral prednisolone for wheeze between 3 months and 1 year. Hypertonic saline had been used in three infants by age 1yr, one of whom started this at 3 months. Six patients received treatment with rhDNase between 3m–1yr. Within the limited power of study for such sub-group analysis, there was no significant differences for any anthropometric or lung function measurements at1yr, nor for the change in any of these measures between 3m to 1yr between those who did and did not receive rhDNase. However, there was a non–significant tendency for $FRC_{pleth}$ to be higher (mean [95% CI] difference: 0.62 [-0.35; 1.59] z-scores) and $FEV_{0.5}$ to be lower (-0.43[-1.31; 0.44] z-scores) at 1yr in the 6 infants who had been prescribed rhDNase, suggesting that this may have been prescribed for children with more severe symptoms. Fifty-three percent of the CF infants were treated medically for presumed or confirmed gastro-oesophageal reflux with proton pump inhibitors, histamine H2 antagonists or motility drugs.

After each LFT occasion, all parents received a telephone call from the research team a day later to check on the well-being of the infant and answer any further queries. The majority of CF and healthy infants did not have any adverse events apart from increased drowsiness for the first few hours after the completion of the ILFT. There were no serious adverse events.

## 3.5 LUNG FUNCTION RESULTS

### 3.5.1 Feasibility of lung function measurements at 3 months and 1year

Although 72 CF and 44 healthy controls infants completed paired measurements, not all the LFT were successful. The relative success rate in obtaining technically satisfactory measurements on each occasion is summarised in **Table 3-iv**. High success rates of ≥ 95% were seen with all three lung function tests at 1yr of age, however at 3m of age, plethysmography was less well tolerated.

**Table 3-iv: Success rates for obtaining technically satisfactory ILFT according to age in CF infants and healthy controls in whom paired assessments were undertaken**

| | Acceptable results at 3 months | | Acceptable results at 1 year | | Successful paired results: 3 months and 1 year | |
|---|---|---|---|---|---|---|
| | CF (n=72) | Controls (n=44) | CF (n=72) | Controls (n=44) | CF (n=72) | Controls (n=44) |
| LCI | 71 (99%) | 41 (93%) | 71 (99%) | 44 (100%) | 70 (97%) | 41 (93%) |
| $FRC_{pleth}$ | 57 **(79%)** | 38 **(86%)** | 70 (97%) | 42 (95%) | 55 **(76%)** | 36 **(82%)** |
| $FEV_{0.5}$ | 68 (94%) | 42 (95%) | 69 (96%) | 42 (95%) | 66 (92%) | 40 (91%) |

***Footnote:*** *Results are presented as n (%) successful measurements according to outcome. Majority of the tests were successfully performed at both test occasions for both infant groups except plethysmography which was less successful at 3 months.*

### 3.5.2 Statistical analysis of lung function results

Of the wide range of lung function tests performed on two occasions (3m and 1yr), inevitably some tests were unsuccessful due to physiological reasons (e.g., upper airway activity such as glottic closure), failure to meet quality control criteria, or measurements being omitted due to infant waking early or time constraint (hence "missing" data). In order to account for these missing values, a statistical method known as multiple imputations was used. In this procedure, all the known covariates thought to be associated with lung function at 1yr were used to help predict the value of any missing data.

The incomplete variables in this study were 3m LCI z-score, 3m $FRC_{pleth}$ z-score and 3m $FEV_{0.5}$ z-score. The observed covariates considered were sex, gestational age, birth weight z-score, maternal smoking, maternal and paternal occupations, somatic growth (between birth to 1yr and between 3m to 1yr), microbiology results (*PsA* ever, significant bacterial growth ever and no growth/ non-significant bacterial growth ever), respiratory signs (wheeze, crackles and cough) and treatment with rhDNase, intravenous antibiotics for respiratory symptoms or gastro-oesophageal reflux disease. One hundred imputations were performed using PASW Statistics v.18 (Chicago, IL, US). The results using multiple imputations were similar to those

obtained using list-wise deletion. Regression analysis was also performed using multiple imputations. Independent and paired t-tests were used to compare numerical data between CF and control infants from non-imputed data. For multiple group comparisons, Bonferroni adjustments were used for multiple t-tests.

### 3.5.3 Group analyses at 3 months and 1 year

Age at first ILFT was slightly, albeit significantly, lower in NBS CF infants [mean difference (95%CI): -1.0 (-1.79; -0.12) weeks] but not at the second test [-1.32 (-3.1; 0.5) weeks]. All lung function data were expressed as z-scores to adjust for age, sex and body size.[57-59] Results are tabulated in **Table 3-v**.

When tested at 3m, analysis of background data using independent t-test showed that CF infants had significantly lower weight, height and BMI z-scores when compared to healthy controls. They also had significantly higher z-scores for LCI and $FRC_{pleth}$, indicative of increased ventilation inhomogeneity and hyperinflation respectively. There was no evidence of gas trapping as no discrepancy was seen between FRC values measured using the MBW method and plethysmography. NBS CF infants had evidence of airway obstruction since their $FEV_{0.5}$, FVC and $FEF_{75}$ were significantly lower when compared with HC.[71]

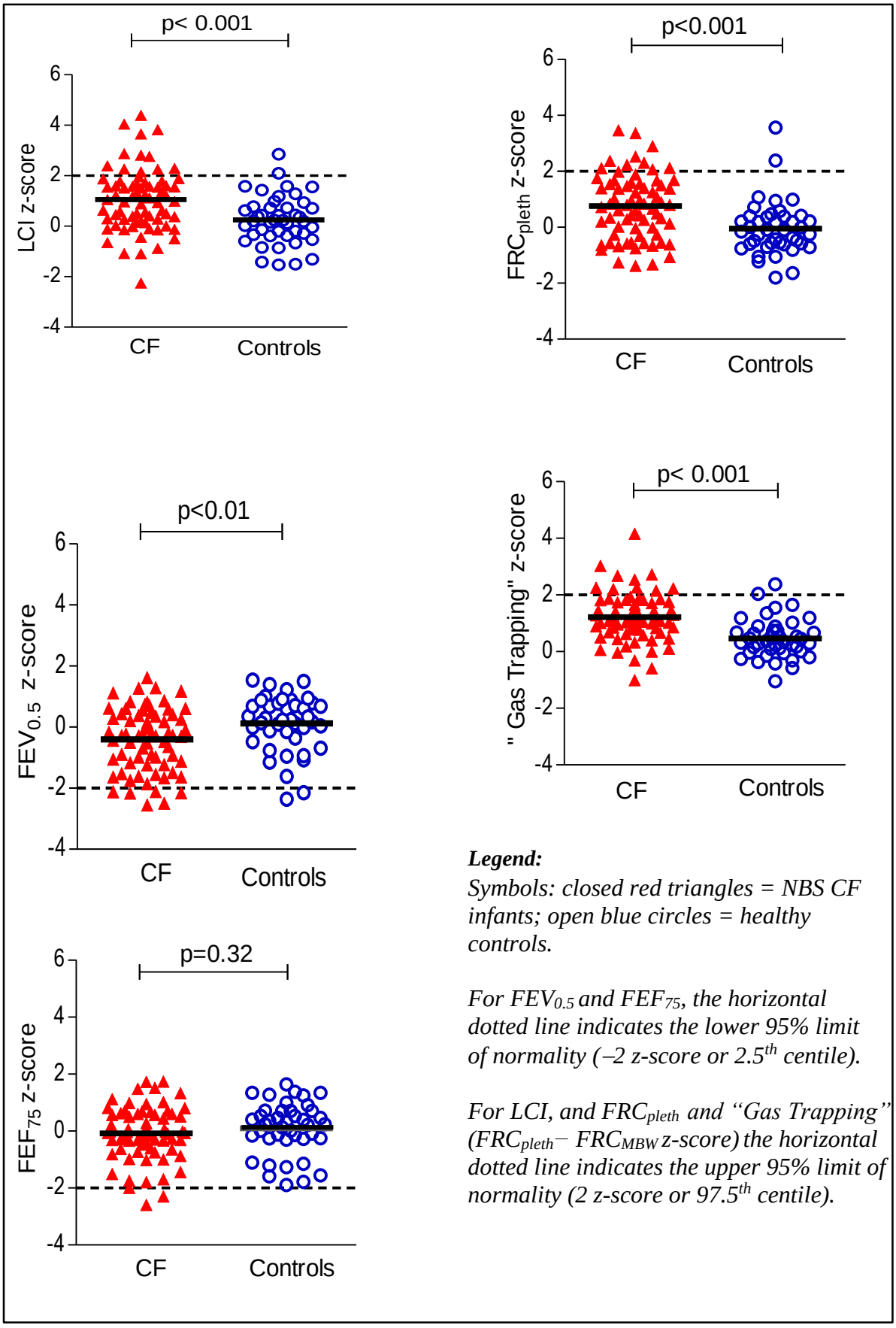
By the 1yr test, differences in height, weight and BMI between the two groups were no longer seen, but differences in lung function remained apparent on cross-sectional comparisons. As seen in **Table 3-v** and **Figure 3-ii**, NBS CF infants had significantly increased ventilation inhomogeneity (LCI: 0.8 z-score higher), hyperinflation ($FRC_{pleth}$: 0.8 z-score higher) and significant gas trapping ($\Delta FRC_{pleth-MBW}$: 0.75 z-score higher) compared with HC. NBS CF infants also had reduced airway function indicated by significantly lower forced expired volumes ($FEV_{0.5}$ 0.52 z-score and FVC 0.66 z-score lower) but there was no significant difference in $FEF_{75}$ z-score between the two groups at a year of age.

**Table 3-v: Anthropometric and lung function results at 3 months and 1 year of age**

| | ~3 months | | | ~1 year | | |
|---|---|---|---|---|---|---|
| | **CF** | **HC** | **Diff (95% CI) CF-HC** | **CF** | **HC** | **Diff (95% CI) CF-HC** |
| ***Age, weeks[a]*** | 11.2 (2.3) | 12.1 (2.1) | -1.0 (-1.8; -0.1)* | 52.4 (5.3) | 53.7 (4.4) | -1.3 (-3.1; 0.5) |
| ***Somatic growth*** | | | | | | |
| Weight, z-score[b] | -0.89 (1.03) | 0.01 (0.97) | -0.90 (-1.27; -0.52)*** | 0.32 (0.90) | 0.55 (1.21) | -0.23 (-0.64; 0.19) |
| Length, z-score | -0.21 (1.01) | 0.73 (0.0.92) | -0.94 (-1.30; -0.58)*** | 0.47 (1.01) | 0.76 (1.20) | -0.28 (-0.71; 0.15) |
| Body mass index, z-score | -1.08 (0.99) | –0.55 (0.96) | -0.53 (-0.90; -0.16)** | 0.08 (0.83) | 0.18 (1.12) | -0.10 (-0.49; 0.29) |
| ***Ventilation Inhomogeneity*** | | | | | | |
| LCI, z-score | 0.83 (1.32) | 0.36 (0.85) | 0.47 (0.06; 0.87)* | 1.05 (1.23) | 0.25 (0.95) | 0.80 (0.40; 1.21)*** |
| ***Lung Volumes*** | | | | | | |
| $FRC_{MBW}$, z-score | 0.24 (0.84) | -0.15 (0.87) | 0.39 (0.06; 0.72)* | -0.45 (0.98) | -0.52 (0.78) | 0.08 (-0.25; 0.40) |
| $FRC_{pleth}$, z-score | 0.76 (1.09) | -0.01 (1.08) | 0.77 (0.32; 1.22)** | 0.72 (1.16) | -0.05(0.96) | 0.80 (0.40; 1.20)*** |
| "Gas trapping" z-score[c] | 0.59 (0.96) | 0.22 (0.94) | 0.37 (-0.03; 0.77) | 1.21 (0.86) | 0.46 (0.69) | 0.75 (0.46; 1.05)*** |
| ***Forced Expired Volumes and Flows*** | | | | | | |
| $FEV_{0.5}$, z-score | -1.23 (1.07) | -0.16 (0.76) | -1.07 (-1.42; -0.73)*** | -0.41 (1.03) | 0.12 (0.92) | -0.52 (-0.89; -0.15)** |
| FVC, z-score | -0.50 (1.03) | 0.23 (0.67) | -0.74 (-1.06; -0.41)*** | -0.43 (1.16) | 0.23 (0.94) | -0.66 (-1.05; -0.26)** |
| $FEF_{75}$, z-score | -0.76 (1.25) | -0.07 (0.96) | -0.69 (-1.11; -0.27)** | -0.09 (0.93) | 0.09 (0.91) | -0.18 (-0.54; 0.18) |

***Footnote:*** *[a] corrected for gestational age; [b] calculated according to Cole et al[182]; [c] "Gas Trapping" represents the within- subject difference in lung volumes measured by plethysmography and MBW. CF=Cystic Fibrosis; HC=Healthy Control; CI=confidence interval of the difference; *p<0.05; **p<0.01; ***p<0.001*

**Figure 3-ii: Significantly elevated LCI, $FRC_{pleth}$ and gas trapping and reduced $FEV_{0.5}$ but not $FEF_{75}$ in NBS CF infants compared to healthy controls at 1-year of age**




***Legend:***

*Symbols: closed red triangles = NBS CF infants; open blue circles = healthy controls.*

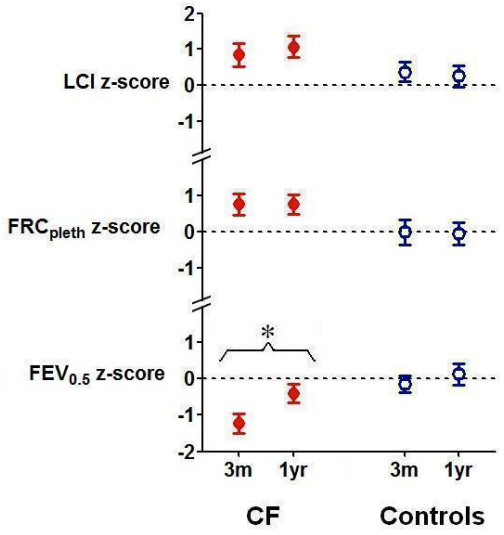
*For $FEV_{0.5}$ and $FEF_{75}$, the horizontal dotted line indicates the lower 95% limit of normality (–2 z-score or 2.5$^{th}$ centile).*

*For LCI, and $FRC_{pleth}$ and "Gas Trapping" ($FRC_{pleth}$– $FRC_{MBW}$ z-score) the horizontal dotted line indicates the upper 95% limit of normality (2 z-score or 97.5$^{th}$ centile).*

### 3.5.4 Longitudinal analysis between 3 months and 1 year

When comparing lung function between 3m and 1yr of age in NBS CF and HC infants, significant improvements were observed in $FEV_{0.5}$ and $FEF_{75}$ in CF infants, but there was no significant change in either LCI, $FRC_{pleth}$ or gas trapping (**Figure 3-iii** and **Table 3-vii).** Although LCI, $FRC_{pleth}$ and gas trapping z-scores remained significantly higher in CF than controls infants at a year, no further deterioration was observed, i.e., these lung function outcomes had remained stable since 3m of age. By contrast, airway function assessed using the RVRTC technique showed considerable improvement such that by a year, although $FEV_{0.5}$ was still significantly lower in CF compared to control infants, the mean difference between the two infants groups was much reduced, whilst $FEF_{75}$ was no longer significantly different between groups. In healthy infants, no significant changes were observed for any lung function outcome during this time period.

**Figure 3-iii: Comparison of changes in lung function z-scores between 3 months and 1 year in NBS CF infants and healthy controls**




***Legend:*** *Data are expressed as mean ± 95% confidence interval. Lung function outcomes were expressed as z- scores which adjusted for age, sex and body size as appropriate.*[57-59]

*The horizontal line represents 0 z-score which equated to 100% predicted or the 50th centile for results derived from a healthy population.*[195] *Significant improvement in $FEV_{0.5}$ observed in CF infants within the first year whilst $FRC_{pleth}$ and LCI stabilised during that time.*

** p <0.05*

Significant increases in z-scores for somatic growth were observed in both groups between 3-12 months, these changes being significantly greater in those with CF (**Table 3-vii)**. Despite lower birth weight and test weight at 3m, NBS CF infants had considerable growth catch up such that by 1yr of age, there were no significant differences in body size when compared with controls.

The percentages of CF NBS infants with abnormal lung function results at 3m and 1yr of age (i.e., beyond the 95% limits of normal, see **chapter 2, section 2.3.5.1**) are summarised in **Table 3-vi** and **Figure 3-ii**).

**Table 3-iv: Number (percentage) of CF and healthy control infants with 'abnormal' lung function at 3 months and 1 year of age.**

| CF (n=72) | $FEV_{0.5}$ < 2z | $FEF_{75}$ < 2z | LCI > 2z | $FRC_{pleth}$ >2z | "Trapped Gas" >2z |
|---|---|---|---|---|---|
| 3 months | 18/68 (**27%**) | 12/68 (**18%**) | 12/71(17%) | 9/57 (16%) | 5/56 (9%) |
| 1 year | 6/69 (**9%**) | 2/69 (**3%**) | 13/71(18%) | 11/70 (16%) | 10/69 (15%) |
| HC (n= 44) | $FEV_{0.5}$ < 2 | $FEF_{75}$ < 2 | LCI > 2 | $FRC_{pleth}$ >2 | "Trapped Gas" >2 |
| 3 months | 0 | 1/42 (2%) | 1/41 (2%) | 0 | 1/37 (3%) |
| 1 year | 2/42 (5%) | 0 | 2/44 (5%) | 2/42 (5%) | 2/42 (5%) |

***Footnote:*** *The percentage of CF infants with abnormally reduced forced expiratory volume and flow decreased by a year of age whilst the percentage with abnormally increased LCI and* $FRC_{pleth}$ *remained stable during the same time span. Although lung function at 1yr was correlated with that at 3m, those with abnormal lung function at 1yr were not necessarily abnormal at 3m. Percentage in bold represents significant difference.*

Although at a year, NBS CF infants continued to have impaired airway function compared to HC on cross-sectional analysis, the percentage with abnormally low $FEV_{0.5}$ and $FEF_{75}$ z-scores was significantly lower than at 3m. There was a 3 fold reduction in the percentage of CF infants with abnormally low forced expired volume ($FEV_{0.5}$) and a 5 fold reduction in those with abnormally low forced expired flows ($FEF_{75}$) at 1yr compared to baseline test at 3m of age. By contrast, the percentage of infants with abnormal LCI and $FRC_{pleth}$ remained stable between 3m and 1yr with no sign of deterioration. Although NBS CF infants had significant 'trapped gas' by a year of age, overall there was no significant difference over the 9-month period between the infant groups (**Table 3-vii**). Possible explanations for the

variation in lung function results using the different techniques will be discussed in **section 3.6.** No relationship was reported for change in weight z-score between 3m and 1yr and any change in lung function parameters between 3m and 1yr for the CF cohort.

**Table 3-vii: Comparison of changes in anthropometry and lung function at 3 months and 1 year in NBS CF and healthy controls infants**

| | | CF (1yr-3m) | | Healthy controls (1yr-3m) | Difference (95% CI): CF– controls | p values |
|---|---|---|---|---|---|---|
| ***Change in Somatic growth*** | n | | n | | | |
| Weight, z-score | 72 | **1.21 (0.82)** | 44 | **0.54 (0.85)** | 0.67 (0.35; 0.99)*** | <0.0005 |
| Length, z-score | 72 | **0.68 (0.71)** | 44 | 0.03 (0.73) | 0.66 (0.38; 0.93)*** | <0.0005 |
| Body mass index, z-score | 72 | **1.16 (0.90)** | 44 | **0.72 (0.98)** | 0.44 (0.07; 0.80)* | 0.019 |
| ***Change in Ventilation inhomogeneity*** | | | | | | |
| LCI, z-score | 70 | 0.24 (1.50) | 41 | -0.09 (1.18) | 0.33 (-0.18; 0.84) | 0.205 |
| ***Change in Lung Volumes*** | | | | | | |
| $FRC_{MBW}$, z-score | 70 | **-0.68 (0.90)** | 42 | **–0.37 (0.71)** | -0.31 (-0.62; -0.01)* | 0.043 |
| $FRC_{pleth}$, z-score | 55 | -0.04 (1.02) | 36 | -0.04 (1.20) | 0.001 (-0.48; 0.49) | 0.996 |
| Trapped gas, z-score[a] | 53 | **0.58 (1.14)** | 35 | 0.30 (1.12) | 0.28 (-0.21; 0.77) | 0.258 |
| ***Change in Forced Expired Volumes and Flow*** | | | | | | |
| $FEV_{0.5}$, z-score | 66 | **0.83 (1.07)** | 40 | 0.24 (0.98) | 0.59 (0.18; 0.99)** | 0.005 |
| FVC, z-score | 66 | 0.06 (0.96) | 40 | -0.02 (0.93) | 0.08 (-0.29; 0.45) | 0.688 |
| $FEF_{75}$, z-score | 66 | **0.84 (1.45)** | 40 | 0.20 (1.16) | 0.63 (0.12; 1.14)* | 0.015 |

***Footnote:*** *Data shown as mean (SD) or mean difference (95% CI) of change.* [a] *'Trapped gas' represents the within-subject difference in lung volumes measured by plethysmography and MBW.*[58,59] **p<0.05; **p<0.01; ***p<0.001 for differences in the rate of change between infants with CF and HC between 3-12 months. Significant changes over time within each group are shown in bold.*

*Significant improvements of $FEV_{0.5}$ and $FEF_{75}$ within the first year by NBS CF infants refer to a significantly greater change than that seen in healthy controls in whom lung function remained stable within the first year .The change in LCI during the first year amongst CF and healthy control infants was not significantly different.CF infants showed a much greater improvement in their anthropometry compared to controls.*

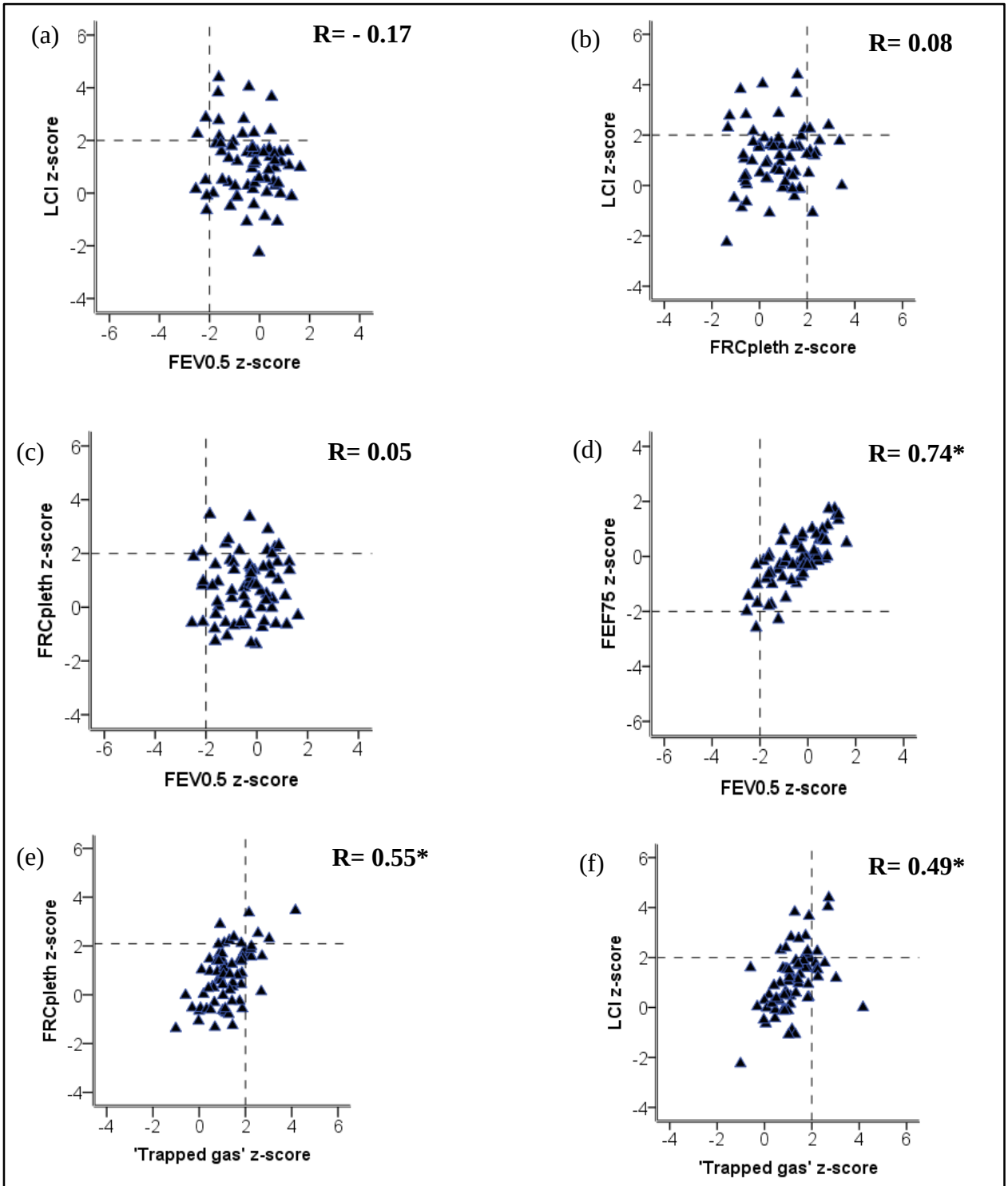
### 3.5.5 Relationship between different lung function measurements at 1 year in CF NBS infants

In this study, three methods of ILFT were performed to investigate and detect early lung disease. Contrary to previous studies involving older children that revealed greater sensitivity using LCI compared to spirometry in detecting lung disease[85,99,117], ILFT results in clinically diagnosed CF infants[83] and in this cohort of NBS CF infants at 3m of age[71] demonstrated that during infancy, both LCI and $FEV_{0.5}$ obtained using the raised volume technique were similarly sensitive in detecting lung disease although they were not necessarily detected in the same infants. The relationship between selected ILFT outcomes in 1yr old NBS CF infants in this study was explored further as shown in **Figure 3-iv.**

There was no significant relationship between the three primary outcomes: $FEV_{0.5}$ and LCI (r= –0.17, p= 0.17), $FRC_{pleth}$ and LCI (r= 0.08, p= 0.49) and $FRC_{pleth}$ and $FEV_{0.5}$ (r= 0.05, p= 0.71). As expected, $FEV_{0.5}$ and $FEF_{75}$ were significantly correlated (r=0.74, p<0.001). 'Trapped Gas' was significantly correlated with $FRC_{pleth}$ z-score (r=0.55, p<0.001) and with LCI (r = 0.49; p<0.001); indicating that gas trapping was associated with hyperinflation and increased ventilation inhomogeneity.

At one year, 17% of CF infants had an LCI >2 z-scores, 16% had $FRC_{pleth}$ >2 z-scores whereas only 9% had an $FEV_{0.5}$ <–2 z-scores (**Table 3-vi**). Only 17% (12/69) had abnormalities detected by both LCI and $FEV_{0.5}$ at a year whilst at 3m, 12% (8/69) had similar abnormalities. However, if based on abnormality in *either* test, 23% (16/69) would be identified with abnormal results in LCI or $FEV_{0.5}$ or 25% (17/69) identified with abnormal result in $FRC_{pleth}$ or $FEV_{0.5}$ at a year. At 3m, this would have risen to 35% (24/69). Thirty six percent (25/69) of CF NBS infants had at least one abnormal result if based on LCI, $FEV_{0.5}$ or $FRC_{pleth}$ at a year. Therefore, at a year of age, by increasing the number of ILFT performed, this can increase the detection rate of at least one lung function abnormality from 12% to 36%.

**Figure 3-iv: Relationship between different lung function parameters in CF infants at a year of age**

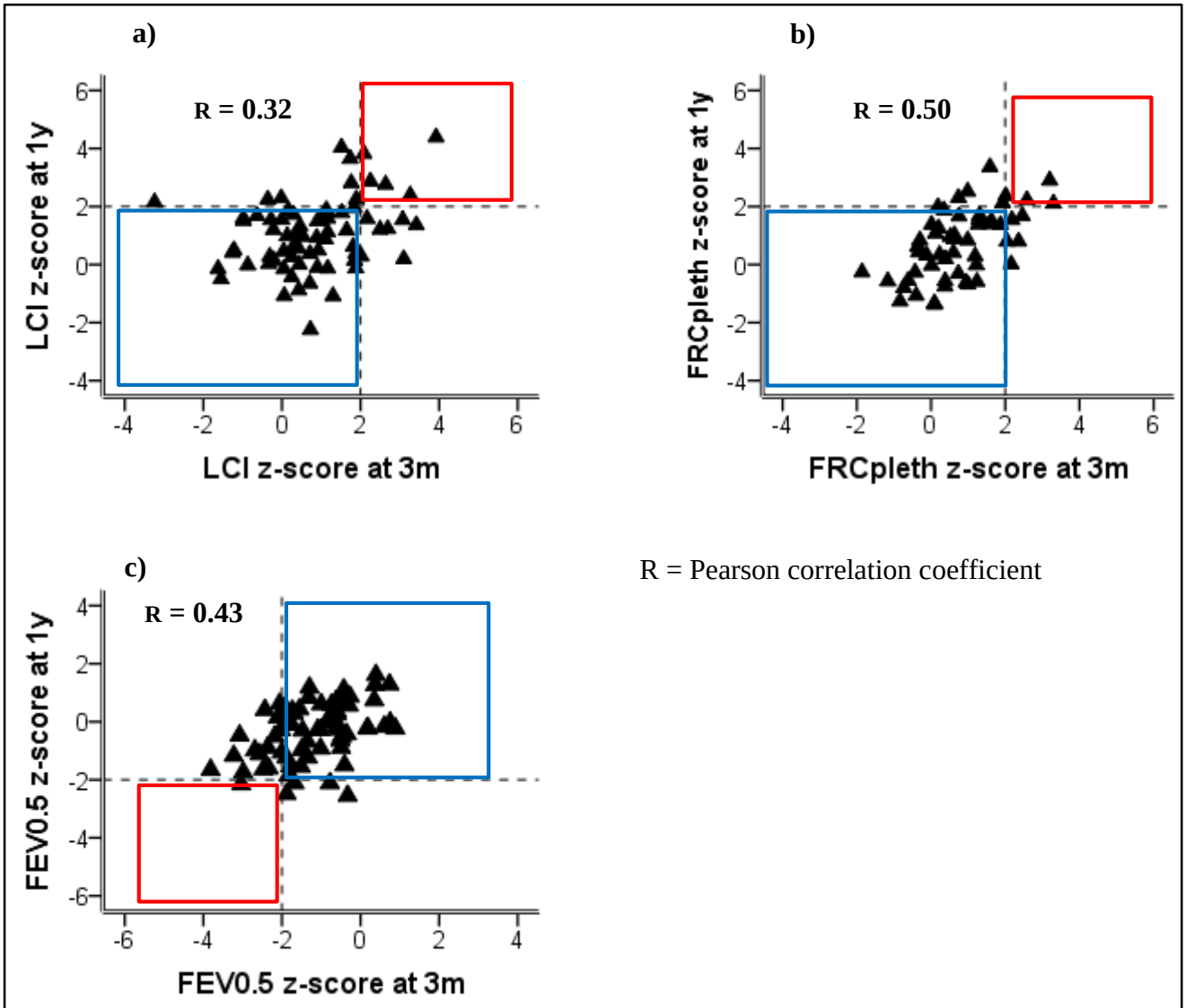



***Legend:*** *The horizontal dotted line for LCI indicates the 97.5$^{th}$ centile of normality (>2 z-score). The vertical and horizontal lines for $FRC_{pleth}$ z-score indicate the 97.5$^{th}$ centile of normality (>2 z-score). The vertical line for $FEV_{0.5}$ and the horizontal line for $FEF_{75}$ z-scores indicate the 2.5$^{th}$ centile of normality. *denotes significant Pearson correlation. Significant associations were found between $FEV_{0.5}$ and $FEF_{75}$ as well as 'trapped gas' with $FRC_{pleth}$ and LCI although the associations were moderate. The three primary outcomes ($FEV_{0.5}$, LCI and FRCpleth) were on the other hand poorly correlated with one another, suggesting they were reflecting different pathological changes.*

### 3.5.6 Relationship between 3 months and 1 year lung function results

Significant correlations were found between lung function results at 3m and 1yr of age in NBS CF infants (**Figure 3-v**). Using univariable linear regression with multiple imputations to predict 1yr lung function from the 3m data, mean (95% CI) beta coefficients were: 0.48 (0.31;0.65) for $FRC_{pleth}$, 0.42 (0.26;0.58) for $FEV_{0.5}$, and 0.32 (0.15;0.50) for LCI. These results indicate that for each unit increase in $FRC_{pleth}$, $FEV_{0.5}$ and LCI z-scores at 3m, on average $FRC_{pleth}$, $FEV_{0.5}$ and LCI would be 0.5, 0.4 and 0.3 z-scores higher, respectively, at 1 year.

**Figure 3-v: Correlations between lung function at 3 months and 1 year in NBS CF infants**




***Legend:*** *The 95% limits of 'normal range' (2 z-scores for LCI and $FRC_{pleth}$ and -2 z-scores for $FEV_{0.5}$) are represented by vertical dashed lines at 3m and horizontal lines at 1yr. Those with normal lung function on both occasions fall within the lower left quadrant for LCI and $FRC_{pleth}$, and upper right quadrant for $FEV_{0.5}$ (solid triangles lying within the blue squares); whilst those with abnormal results at 3 months and 1 year lie within the red squares. Infants with abnormal LCI and $FRC_{pleth}$ at 3 months but normal LCI and $FRC_{pleth}$ at a year respectively, lie within the lower right quadrant (panel a and b), while those with abnormal $FEV_{0.5}$ at 3 months which has normalised by 1 year are within the left upper quadrant of panel c .*

*All parameters of lung function at 3m were significantly associated with the respective parameters at 1yr.*

### 3.5.7 Clinical determinants of 1 year lung function

Previous published data from this cohort of NBS CF infants at 3m of age revealed that apart from having the status of CF, there were no significant associations between lung function and any other potential determinants such as sex, gestational age, birth weight z-score, pre- or postnatal maternal smoking and maternal asthma using multivariable analyses.[71] Among CF infants at 1yr, a significantly lower $FEV_{0.5}$ (mean regression coefficient (95%CI): –0.70 [–1.29 to –0.10] z-scores; univariable analysis) was observed in those who had received any additional antibiotics for symptoms or positive cough swab. There were no significant associations between other lung function outcomes and the infants' genotype, clinical status or treatment prior to lung function tests at ~3 months of age.[71]

**Table 3-viii** demonstrates possible determinants of 1yr lung function outcomes using **univariable regression analysis**. Besides demonstrating that 3m lung function data were predictive of lung function results at a year (**section: 3.5.6**), other significant associations were:

- **CF status**: Having CF itself was associated with impaired lung function at a year (increased LCI, $FRC_{pleth}$ and reduced $FEV_{0.5}$ z-scores).
- **1yr LCI**: This was significantly negatively associated with change in weight z-score from birth to 3m old (a greater increase in weight gain from birth led to a decrease in LCI at a year). The presence of wheeze and treatment for gastro-oesophageal reflux disease were both significantly associated with an increased LCI at 1yr.
- **1yr $FRC_{pleth}$**: A higher $FRC_{pleth}$, indicative of hyperinflation, was significantly associated with prior *PsA* infection ever, IV antibiotics use, GORD treatment and the presence of wheeze ever in the first year of life. For example, isolation of *PsA ever* in CF babies was associated with 1 z-score increase in $FRC_{pleth}$ at 1 year when compared with CF babies without *PsA.*
- **1yr $FEV_{0.5}$**: this was significantly associated with wheeze, cough within 3 weeks of 1yr LFT and treatment for GORD such that presence of any of these clinical determinants was associated with a reduction in airway function, reflected by reduced $FEV_{0.5}$ of ~0.5 z-scores.

**On multivariable linear regression**, lung function at 3 months was predictive of that at 1 yr for all lung function outcomes. Significant determinants of:

- **1yr LCI z-score** were: CF status [mean regression coefficient (95% CI): 0.48 (0.04; 0.93) z-score, p=0.032]; 3m LCI [0.24 (0.07; 0.41) z-score, p=0.005]; history of clinician diagnosed wheeze [0.59 (0.05; 1.12) z-score, p=0.031] and change in weight z-score between birth and 3m [-0.18 (-0.35; -0.01) z-score, p=0.042].
- **1yr $FRC_{pleth}$ z-score** was significantly associated with $FRC_{pleth}$ at 3m [0.43 (0.27; 0.59) z-score, p<0.0005], history of *PsA* infection [0.71 (0.24; 1.17) z-score, p=0.003] and change in weight z-score between 3m and 1yr [-0.20 (-0.41; 0.003) z-score, p=0.054]. After adjustment for these factors, other variables including CF status were no longer significantly associated with 1yr $FRC_{pleth}$.
- **1yr $FEV_{0.5}$ z-score** was only significantly associated with $FEV_{0.5}$ z-score at 3m.

**Table 3-viii: Univariable linear regression with multiple imputations: determinants of lung function at 1 year**

| | 1yr LCI z-score | 1yr $FRC_{pleth}$ z-score | 1yr $FEV_{0.5}$ z-score |
|---|---|---|---|
| CF | **0.82 (0.39; 1.24) p<0.0001** | **0.79 (0.38; 1.20) p<0.0001** | **-0.49 (-0.88; 0.10) p=0.02** |
| 3m LF | **0.32 (0.15; 0.50) p<0.0001** | **0.48 (0.31; 0.65) p<0.0001** | **0.42 (0.26; 0.58) p<0.0001** |
| Male | 0.35 (-0.09; 0.78) p=0.12 | 0.39 (-0.03; 0.81) p=0.07 | -0.01 (-0.39; 0.38) p=0.98 |
| Gestational age | -0.02 (-0.17; 0.14) p=0.83 | -0.11 (-0.26; 0.04) p=0.14 | **0.15 (0.02; 0.29) p=0.02** |
| Birth weight z-score | -0.19 (-0.43; 0.06) p=0.14 | -0.11 (-0.35; 0.13) p=0.37 | **0.27 (0.06; 0.49) p=0.01** |
| Maternal smoking during pregnancy | -0.48 (-1.22; 0.27) p=0.21 | -0.08 (-0.81; 0.65) p=0.83 | 0.52 (-0.13; 1.18) p=0.12 |
| Current maternal smoking | -0.38 (-1.04; 0.29) p=0.27 | 0.24 (-0.42; 0.89) p=0.48 | 0.47 (-0.12; 1.05) p=0.12 |
| Mother in non-manual occupation | -0.43 (-0.95; 0.09) p=0.10 | -0.30 (-0.79; 0.18) p=0.22 | 0.12 (-0.34; 0.57) p=0.62 |
| Father in non-manual occupation | -0.22 (-0.70; 0.25) p=0.35 | 0.07 (-0.38; 0.52) p=0.76 | 0.17 (-0.24; 0.58) p=0.41 |
| ΔWeight (3m-birth), z-score | **-0.19 (-0.39; -0.003) p=.04** | -0.01 (-0.20; 0.18) p=0.93 | -0.01 (-0.19; 0.16) p=0.90 |
| ΔWeight (1yr-3m), z-score | 0.20 (-0.04; 0.45) p=0.11 | -0.11 (-0.35; 0.13) p=0.36 | -0.21 (-0.42; 0.001) p=0.05 |
| *Pseudomonas aeruginosa* | 0.53 (-0.03; 1.08) p=0.06 | **0.94 (0.46; 1.42) p<0.0001** | -0.37 (-0.84; 0.11) p=0.13 |
| Significant bacterial growth | 0.35 (-0.17;0.87) p=0.18 | 0.22 (-0.26; 0.70) p=0.36 | -0.18 (-0.64; 0.28) p=0.45 |
| Wheeze, ever | **0.99 (0.48; 1.50) p<.0001** | **0.71 (0.21; 1.21) p=0.006** | **-0.52 (-0.98; -0.07) p=0.02** |
| Crackles, ever | 0.70 (-0.28; 1.68) p=0.16 | 0.85 (-0.08; 1.78) p=0.07 | -0.60 (-1.44; 0.24) p=0.16 |
| Cough, within 3 weeks of 1y lung function | 0.44 (-0.21; 1.09) p=0.19 | 0.61 (-0.01; 1.22) p=0.05 | **-0.71 (-1.25; -0.16) p=0.01** |
| rhDNase treatment, ever | 0.31 (-0.68; 1.30) p=0.54 | 0.92 (-0.01; 1.85) p=0.55 | -0.63 (-1.46; 0.21) p=0.14 |
| IV antibiotics, number of courses | 0.27 (-0.10; 0.64) p=0.16 | **0.54 (0.21; 0.87) p=0.001** | -0.28 (-0.59; 0.02) p=0.07 |
| GORD treatment, ever | **0.59 (0.13; 1.05) p=0.01** | **0.52 (0.08; 0.95) p=0.02** | **-0.44 (-0.84; -0.04) p=0.03** |

***Footnote:*** *Data are shown as mean regression coefficient (95% CI). ΔWeight: differences in weight between the 2 time periods. Significant associations are shown in bold and red. Abbreviations: IV: intravenous; rhDAase: Pulmozyme; GORD: Gastro-oesophageal reflux.*

## 3.6 SUMMARY OF LUNG FUNCTION RESULTS AND PRELIMINARY DISCUSSION

This chapter has described the results of a study undertaken to address the primary hypothesis that, despite early diagnosis and specialist, protocol-driven management, at a year of age, NBS CF infants have abnormal lung function with further deterioration since 3 months of age when compared to contemporaneous healthy controls.

Despite their lower birth-weight and test weight at 3m, there was considerable catch up growth among infants with CF such that by 1yr of age there were no significant differences in body size between the groups. These observations are in contrast to previous findings in clinically diagnosed CF infants[60] but in keeping with the growing evidence regarding the nutritional benefits of newborn screening for cystic fibrosis.[22,26]

As for the 1yr lung function results, NBS CF infants had significantly increased ventilation inhomogeneity, hyperinflation, gas trapping and central airway obstruction compared to healthy controls. Contrary to the study hypothesis, NBS CF infants did not show any deterioration in lung function but instead an improvement in forced expired volumes and flows with stability of other outcomes. The percentage of NBS CF infants with abnormal LCI and $FRC_{pleth}$ at 1yr was similar to that at 3m, and there was a significant reduction in those with abnormal $FEV_{0.5}$ during this period. Potential reasons for the discrepant results according to selected outcome will be discussed in **chapter 7, section 7.1.1**.

In considering the use of ILFT as an outcome measure, although $FRC_{pleth}$ detected abnormalities of lung function in NBS CF infants as readily as LCI or $FEV_{0.5}$, it was not as well tolerated by the younger infants. This would make $FRC_{pleth}$ less feasible as an outcome variable at 3m of age, or in longitudinal studies commencing at this age. This may be accounted by the fact that young infants arouse very readily before 3m of age and are less tolerant of brief airway occlusions than older infants. The success of obtaining plethysmographic FRC measurements improved from 79% to 97% by 1yr.

Infants were tested when asymptomatic. LFT were deferred until 3 weeks after a cold. As a result of family commitments or frequent upper respiratory tract infections, whereby only very short intervals were occurring before the infant caught another "cold", 15 CF infants were tested slightly sooner than the prescribed 3 weeks interval; although never less than 7 days after a cold.

Early lung function at 3m of age predicted lung function at 1yr, as did clinical determinants such as a history of physician diagnosed wheeze, history of *PsA* infection and suboptimal weight gain. The implications of these findings will be discussed further in (**chapter 7, section 7.4**).

The overall strengths and limitations of this study will be discussed in Chapter 7, together with the relevance of the results to both clinical practice and research, and their relationship to the published literature.

In the next chapter (Chapter 4), the development of standardised chest CT imaging for NBS CF infants in a multi-centre trial will be explored. This is followed by Chapter 5 which addresses some of the challenges faced in using chest CT as an outcome measure, including the validation of Brody-II scoring system in CF infants.

# 4 DEVELOPMENT OF STANDARDISED CHEST COMPUTED TOMOGRAPHY IN NEWBORN SCREENED CF INFANTS: METHODS

## 4.1 INTRODUCTION

Chapter 4 focuses on the use of contiguous thin section CT scanning of chest to investigate the extent of any structural changes in these NBS CF infants at 1yr of age. This chapter will address the methodology used in acquiring chest CT under GA in the current study and the challenges faced when attempting to standardise the acquisition of chest CT scans in a multicentre study to detect early lung disease. The scoring system used to quantify lung disease in NBS CF infants 1yr of age and the challenges in scoring chest CTs in the presence of mild disease will be addressed in Chapter 5. Chapter 6 will focus on exploring the relationship between structural and functional lung changes in these NBS CF infants and investigate any potential clinical determinants of pulmonary structural changes. Chapter 5 and 6 will address the secondary hypotheses of this research study i.e. that significant structural changes are present in NBS CF infants by a year of age and that these structural changes are closely related to lung function parameters.

Chest CT under GA was performed in NBS CF infants at about one year of age on a separate hospital visit shortly after their 1yr ILFT. These procedures coincided with the CF infant's first year annual review assessment which included a flexible bronchoscopy and BAL under the same GA. Healthy control infants recruited into the study did not have chest CT and flexible bronchoscopy.

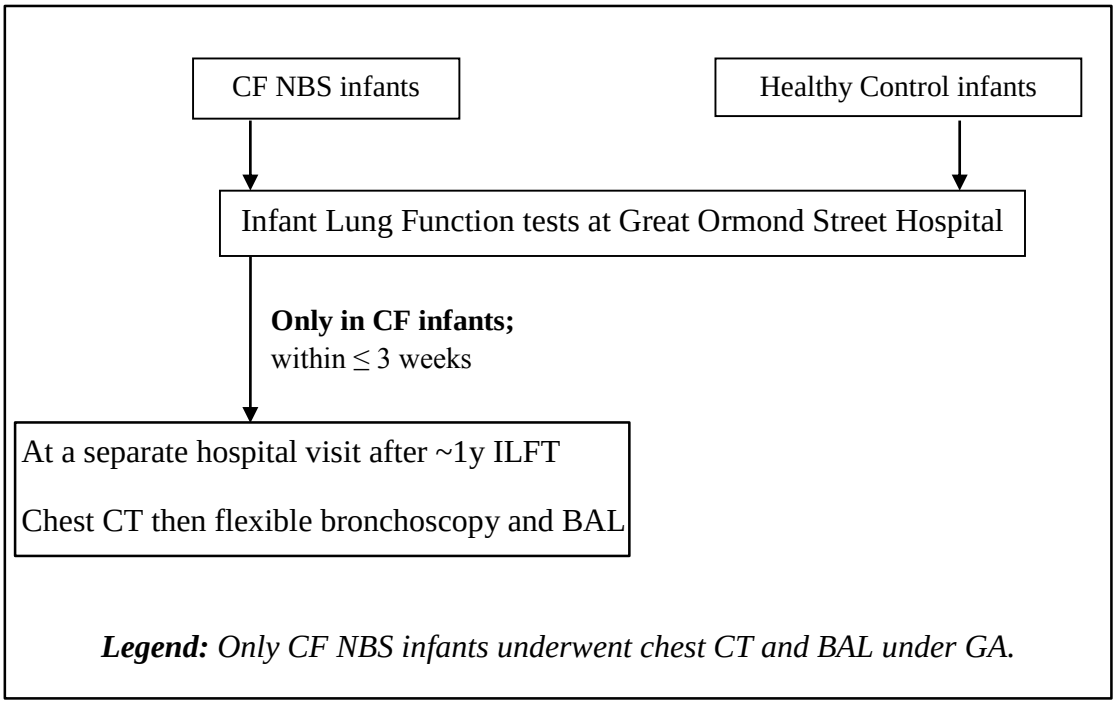
## 4.2 CHEST TOMOGRAPHY SCANNING PROTOCOL

Prior to the commencement of the combined CT scan, bronchoscopy and BAL procedure under GA, investigators from participating centres realised that specialised equipment and personnel were required to carry out these procedures. They could only be established in a standardised fashion in three of the six participating centres (Great Ormond Street Hospital, GOSH; Royal Brompton Hospital, RBH and Royal

London Hospital, RLH). Recruited infants from the remaining three centres (King's College Hospital, KCH; Lewisham University Hospital and East Surrey Teaching Hospital) underwent combined CT scan and bronchoscopy at GOSH. These procedures took place as day cases and CF infants were discharged home on the same day, after several hours of normal observation on the day-case ward of each hospital unless clinically contraindicated.

CF infants underwent chest CT, flexible bronchoscopy and BAL 2-3 weeks after their 1yr ILFT. If the interval between the two procedures was > 3 weeks or if the infant experienced any respiratory exacerbation between the ILFT and the chest CT and bronchoscopy, the ILFT was then repeated to ensure that lung functional and structural information were both obtained at the same clinical status. This occurred in five out of the 72 infants tested. **Figure 4-i** details the schedule of investigations in CF and healthy control infants at a year of age.

**Figure 4-i: Schedule of investigations at 1 year of age in CF NBS and healthy control infants**




***Legend:*** *Only CF NBS infants underwent chest CT and BAL under GA.*

### 4.2.1 Type of CT scanner and scanning parameters

Multi-detector CT scanners were used in this study across three centres. These scanners allowed thin slice volumetric inspiratory and expiratory images to be obtained with rapid scanning times (**Table 4-i**).

**Table 4-i: Details of CT scanners* used across the three centres**

| Centre | Multidetector CT scanner model |
|---|---|
| **A** | Somatom Sensation (64 slice) |
| **B** | Somatom Definition Dual- source (64 slice) |
| **C** | Somatom Definition Flash (128 slice)* |

***Footnote:*** ** Siemens Healthcare, Forchheim, Germany*

Radiographers used a pre-determined CT technique for the acquisition of CT images with controlled ventilation provided by paediatric anaesthetists. The scanning parameters were selected to produce diagnostic high quality images (images which are 'fit for purpose') with low radiation exposure.[157] The CT tube current settings used for infants in this study represented a five- fold reduction in what would normally be used in adult studies. It was possible to still obtain good image quality due to improved inherent contrast in the lung parenchyma tissue with controlled ventilation. During inspiratory scanning, the inherent contrast is most obvious so only a low tube reference current was required in the protocol. This had to be increased slightly during expiratory scans as the inherent contrast at PEEP=0 would be reduced. The gantry rotation time was reduced to half of what is normally used (1 second) in adults to proportionally reduce exposure time and hence radiation dose and its associated risks.[176]

In order to further reduce the amount of radiation exposure, a topogram (planning scan) was obtained at PIP=25 $cmH_2O$ so that, based on the topogram, scanning ranges for inspiratory and expiratory scans could be individually tailored for each infant. This allowed all essential images to be taken without exposing the infant to excessive radiation. The topogram encompassed the top of the lung apices to the costo-phrenic angles, allowing the planning for full volumetric inspiratory scanning at the same lung inflation of PIP=25 $cmH_2O$. The expiratory scanning range was

then automatically calculated as being 30mm shorter than the inspiratory range. The scanning ranges used were kept to a minimum. By adhering to this strict low dose standardised protocol for imaging across all three sites, the aim was to ensure that consistent diagnostic images were obtained, with all infants receiving similar radiation dose despite CT scanners of different models being used at different hospitals. Detailed scanning parameters used in this study can be found in the **Appendix A10**

Each CT scan was sent for clinical reporting at the child's referral hospital and anonymised for subsequent scoring at GOSH.

### 4.2.2 CT images

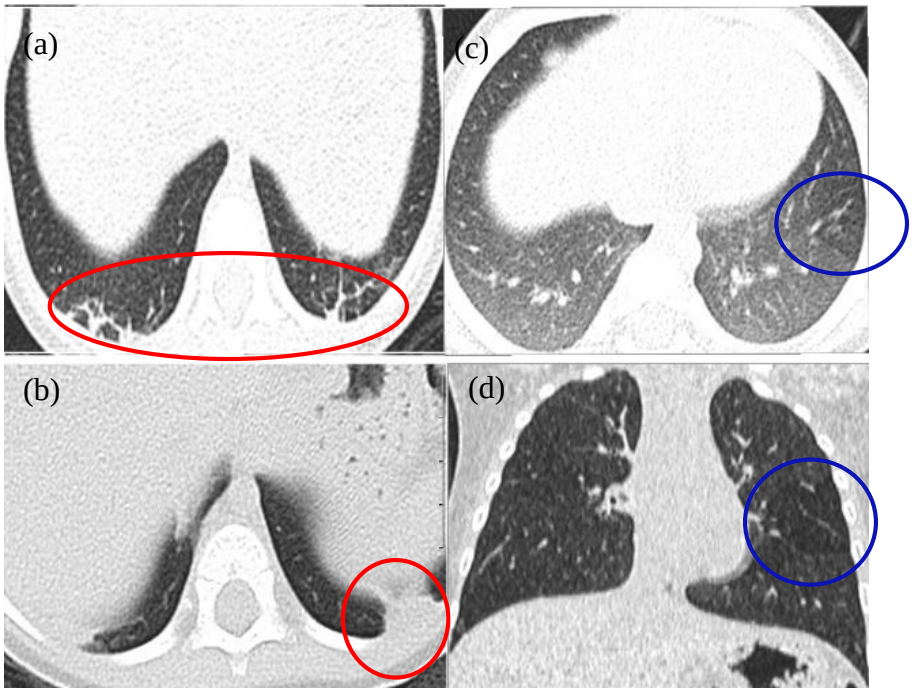
The first eight scans after the project commenced comprised a full volumetric inspiratory scan, followed by an expiratory scan which comprised only 3 sample slices of the lungs (the first slice at the carina of the trachea, the third slice at the costo-phrenic angles and finally the second slice positioned between the first and third slices, as practised in routine clinical assessments).[107,168] Initial experience from these scans showed small, subtle patchy areas of air trapping in two of the eight patients (**Figure 4-ii**).

Furthermore, evidence from emerging literature and discussions with international experts in the field of chest imaging for early lung disease in CF (Dr C Owens from GOSH, Dr H Tiddens from the Netherlands, Dr S Stick and Dr S Ranganathan from Australia, Dr A Brody from Cincinnati) suggested that subtle changes and air trapping may be missed or underestimated if only three expiratory images are performed.[169,196]

Consensus opinion was that the increased scientific information obtained from full volumetric imaging would justify the inevitable slight increase in radiation exposure. Following ethical committee approval for this amendment, the imaging protocol was therefore amended such that all subsequent scans included volumetric scanning of the entire lung in inspiration and expiration. Parents were given an amended information sheet about the slight additional radiation risk associated with the

amended CT protocol and were given the option of withdrawing from the study even if they had previously given consent. More details about parental information can be found in **section 4.2.3**.

**Figure 4-ii: Examples of CT images demonstrating anaesthetic-related basal atelectasis and small areas of focal air trapping**




***Legend:*** *These were CT images from NBS CF infants undertaken at 1yr of age in this study. Images (a) and (b) demonstrated typical linear and wedges shaped basal dependent atelectasis secondary to GA respectively (indicated by the red rings). Axial slice (c) and coronal section (d) images of expiratory scan revealed subtle areas of focal air trapping (hypodense areas indicated by blue rings).*

### 4.2.3 Radiation dose

Modern CT scanners typically display two dose indices: CT dose index ($CTDI_{vol}$, unit mGy) and dose length product (DLP, unit mGy-cm). These doses indices are based on two standard CTDI phantoms (16 or 32-cm diameter phantoms). Phantom is a standard measurement tool that all CT manufacturers have and is used in the calculation of $CTDI_{vol}$. However $CTD_{vol}$ represents the radiation produced by the CT scanner and not necessarily the radiation dose transmitted to an individual patient.[197] In paediatric patients, what the CT scanner report as the radiation dose may not represent what the patient has actually received. In order to have a better

estimate, special formula incorporating correction factors to account for paediatric imaging was devised which provided an improved estimate of the paediatric radiation dose termed as the effective dose.[197] The effective dose (E) is estimated by taking the DLP and applying a paediatric age specific conversion coefficient that is 0.026 for a child between 4 months and 1 year, and a correction factor of 2 to correct for the use of a 32 cm rather than 16 cm phantom. The formula thus used in this study was: DLP x 2 x 0.026 = estimated effective dose in milliSievert (EmSv).[198,199]

Radiation exposure in this study was minimised using automated dose modulation that performs real time assessment of body thickness and adjusts tube current to provide consistent image quality whilst keeping radiation dose to a minimum. Only one CT scan was performed in each infant during the study period and patient dose information was recorded for each examination. The above radiation formula was then applied to obtain the effective radiation dose received by each patient.

The initial estimated radiation dose of a volumetric inspiratory and limited 3-slice expiratory scan was 1.3 mSv which increased to ~1.5 mSv with the combined volumetric inspiratory and expiratory scan. The planned radiation dose range for the entire scan using the amended protocol was ≤2.0 mSv with a target of ~1.5mSv (annual background radiation exposure in the UK ~ 2.5mSv).[200-202]

***Informed consent regarding the chest CT***

Families of eligible infants were asked to provide separate written informed consent for each part of this observational study. With respect to the CT scan under GA, they were provided with written information augmented by verbal explanations about the potential risk associated with the small additional radiation exposure with having the CT scan at 1yr of age. They were advised that:

- All radiation (including the background environmental radiation to which we are all exposed) carries a small risk of damage to cells, which may lead to cancer after many years or decades.
- The natural risk of childhood cancer is 10 in 5,000. The lifetime risk of subsequent malignancy with the use of a chest CT in a young child (<2

years) will increase that risk of childhood cancer by 1 per 5000 cases or 20 per 100,000 cases.[176]

- The extra radiation from having one CT scan using the proposed protocol for this study would be equivalent to about half that which their child would receive each year from background sources.

## 4.3 GENERAL ANAESTHETIC AND IMAGING PROTOCOLS

The anaesthetic and imaging protocols used in this study were standardised in accordance with current practice[107] and international standards[140,203] for providing safe and effective GA to infants as well as producing high quality images which are 'fit for purpose'; and with low radiation exposure.[157] Specifically they were developed in collaboration with the AREST-CF team[109,111,170] in Australia through practical advice from Dr Sarath Ranganathan who has been monitoring early lung disease in NBS CF infants through annual chest CT and bronchoscopy under GA for many years.

Gaseous induction of anaesthesia using oxygen, nitrous oxide and sevoflurane was generally used unless contraindicated or at the clinical judgement of the anaesthetist. Intravenous anaesthesia with IV propofol could also be used. The infant was paralysed with atracurium and then intubated with an appropriately sized endotracheal tube to ensure minimal leak up to inflation pressures of 35 $cmH_2O$ (which were required for a few breaths prior to the scan to minimise any anaesthetic related atelectasis (see below) and sufficient calibre to ensure the passage of a 2.8mm bronchoscope. Anaesthesia was maintained for the CT scan with sevoflurane or IV propofol, oxygen and air.

During the initial mask ventilation prior to intubation, there could be a tendency for air to enter the stomach which would distort the scan image. To avoid this, a nasogastric (NG) tube or suction catheter was passed into the stomach with suction applied to the end of the NG tube or suction catheter to reduce any gastric distension prior to initial topography.

Baseline ventilatory settings prior to imaging, via the anaesthetic machine to maintain $pCO_2$ between 4.5-6kPa were:

- Pressure controlled Intermittent Positive Pressure Ventilation (IPPV)
- Respiratory rate 20 breaths per minute
- I: E ratio 1:2
- Tidal volume (VT) 8-10 ml/kg
- PEEP: 5 $cmH_2O$

A written protocol (**Appendix: A10**) was given to all anaesthetists after detailed explanations of the procedure. The importance of adhering to protocol in order to minimise anaesthesia-related atelectasis and obtain all CT images at standardised lung volumes were stressed. Whenever possible the anaesthetist and radiographer rehearsed the verbal instructions for each stage of the imaging protocol prior to commencing the procedure. A handheld manometer monitoring the ventilatory pressures delivered during the scanning protocol was attached to the handheld bagging circuit. The anaesthetist was guided by this manometer during lung inflations (**Figure 4-iii**).

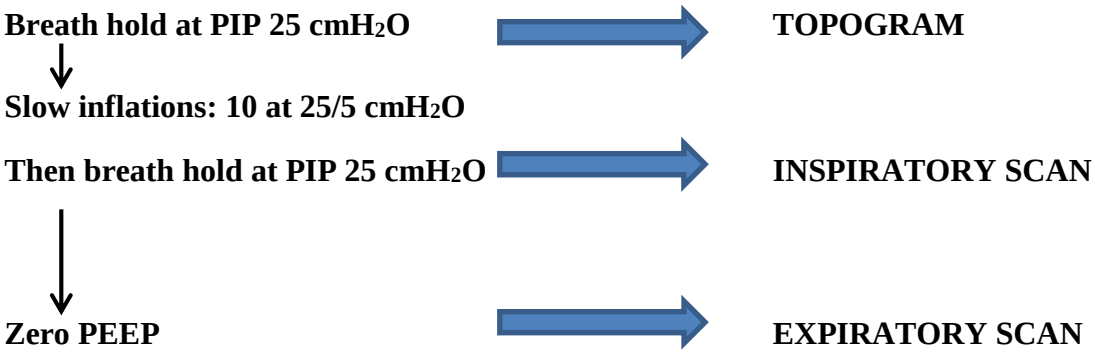
The main concern with having GA for chest CT was anaesthesia-induced atelectasis which would affect image quality and make CT scoring difficult. Densities observed in dependent regions of lungs on chest CT during anaesthesia have been previously reported to be due to atelectasis.[204,205] Atelectasis occurs with any form of anaesthesia whether inhalational or intravenous, with or without paralysing agents. Ensuring vital capacity manoeuvres (VCM) using slower and larger inflation pressures (up to a PIP 35-40 $cmH_2O$) can completely abolish atelectasis. No adverse haemodynamic or pulmonary effects have been reported when using intermittent VCM.[205,206] A high PEEP after induction and throughout GA can consistently reopen lung tissue[205] and prevent further atelectasis. Lower concentrations of oxygen during induction, maintenance of GA and/or just before extubation have also been shown to reduce the amount of atelectasis. Nonetheless inspired oxygen concentration was maintained between 30-35% to ensure normal arterial oxygen tension, even if V/Q mismatch and shunting occurred due to any atelectasis.

During the study CT-GA protocol, recruitment manoeuvres using slower and larger inflations and maintaining a PEEP of 5 $cmH_2O$ prior to scanning were applied.[206] When the radiographer was ready to perform the topogram, the anaesthetist inflated the infant's lungs to a PIP of 25 $cmH_2O$ and held them at that pressure while instructing the radiographer to perform the topogram. Upon completion of the topogram, the anaesthetist returned to normal ventilation through the handheld circuit while waiting for the radiographer to finish planning for the inspiratory and expiratory acquisition parameters. Prior to the inspiratory scan, ten slow inflations were performed at a PIP of 25 $cmH_2O$ and a PEEP of 5 $cmH_2O$. The inspiratory scan was obtained while the airway opening pressure was held steady at PIP 25 $cmH_2O$ during the last of the 10 slow inflations. Once the inspiratory scan was completed, this was immediately followed by release of the inflation bag such that there was complete deflation of the lung down to zero PEEP. There was an automatic 6-second scan delay programmed into the CT scanning protocol to ensure full lung emptying before the expiratory scan was performed. Upon completion of the CT scan, normal ventilatory support was resumed (**Figure 4-iv**). This was the ***initial*** protocol when the study first started and was used when obtaining CT under GA in the first 23 patients.

**Figure 4-iii: Chest CT scan being performed in an anaesthetised, ventilated infant**


***Legend:*** *An anaesthetist used the handheld manometer to guide delivery of the appropriate inflation pressures at different scan stages. The paralysed, intubated and ventilated infant was placed in the centre of the scanner for image acquisition. The position of the infant is important to ensure good images are acquired with minimal radiation exposure. The infant's arms were placed above the head i.e. away from the chest.*

**Figure 4-iv: Initial protocol- summary of inflation pressures during the various stages of scan acquisition**

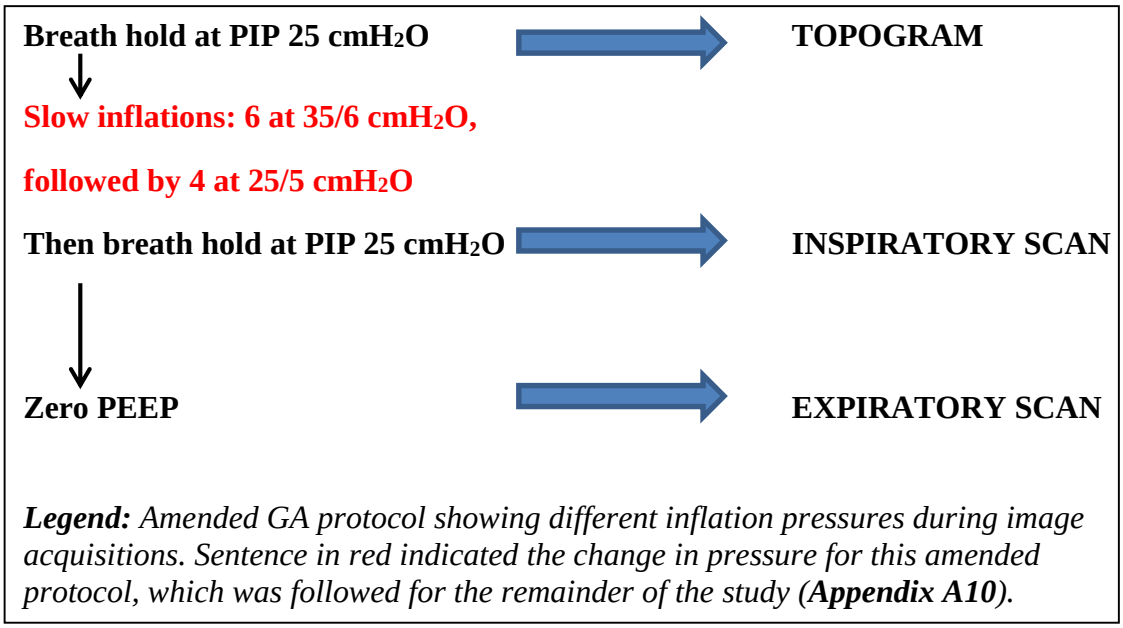



***Legend:*** *This protocol was used in the first 23 scans but the protocol was subsequently altered and replaced by the 'definitive' protocol, as described in (***Figure***).*

Despite these attempts, excessive basal atelectasis was observed in 16/23 (70%) of the initial study scans from CF infants. This raised concerns that inflations provided

during the recruitment stage or inspiratory image acquisition as stipulated in the protocol may not be sufficiently large to prevent dependent atelectasis. Following further discussions with a member of the AREST- CF team (Dr Sarath Ranganathan), the GA protocol was subsequently amended to ensure higher PIP was used to recruit lung volume.[207] The initial protocol consisted of 10 slow inflations at PIP 25 and PEEP 5 $cmH_2O$ prior to inspiratory image acquisition. This was changed to 6 slow inflations at PIP 35 and PEEP 6 $cmH_2O$ followed by 4 slow inflations at PIP 25 and PEEP 5 $cmH_2O$. See **Figure 4-v** for the amended protocol which became the final definitive protocol that was used for the rest of the study (42 scans). The aim of this amended GA protocol was to reverse any GA- related chest CT changes. With this change in GA protocol, the incidence of dependent atelectasis decreased slightly to 25/42 (59.5%) although this reduction was not statistically significant compared to that observed during the original, lower inflation pressure protocol.

**Figure 4-v: Definitive final protocol: summary of inflation pressures during the various stages of scan acquisition**




***Legend:*** *Amended GA protocol showing different inflation pressures during image acquisitions. Sentence in red indicated the change in pressure for this amended protocol, which was followed for the remainder of the study (**Appendix A10**).*

Training sessions were undertaken in the three CT assessment centres to ensure that radiographers and anaesthetists were familiar with the research protocol both before commencing the study and at regular intervals throughout the duration of the study. Every effort was made to involve a dedicated group of radiographers and anaesthetists at each site to ensure smooth execution of the standardised protocol

these CF NBS infants. Unfortunately, due to pressures from clinical NHS workload, it was not always possible to limit these procedures to a dedicated team anaesthetists or radiographers. As discussed below, it soon became apparent that when someone less familiar with the research protocol undertook the procedure, disparities were likely to occur.

## 4.4 OBJECTIVE MEASUREMENT USING $NICO_2$® RESPIRATORY MONITOR

### 4.4.1 Why was objective measurement of ventilation required?

After I was appointed and commenced work on this project, I attended all the remaining procedures (50 CT-GA) to advise and coordinate all the personnel involved in the CT and bronchoscopy under GA at the three centres. This helped to ensure smooth execution of the study protocol even if someone less familiar with the study from the three centres was involved in the procedure.

As I attended these procedures, I noticed variations in the way recruitment manoeuvres were performed and in the pressures implemented during ventilation. Uniform ventilatory pressures such as pressure during recruitment manoeuvres were not undertaken consistently, ensuring PEEP was always maintained until full expiration for expiratory film acquisition were not consistently provided during the scanning procedure by different anaesthetists in the three centres (**Figure 4-viii**). Not infrequently, when a different anaesthetist became involved in the study, there was lack of familiarity with the protocol. Consequently, I decided to objectively measure all ventilatory pressures and volumes whenever possible for the remaining procedures using the $NICO_2$® respiratory monitor (Philips Respironics, USA) [208,209] so that immediate feedback could be provided to the anaesthetists concerned.

### 4.4.2 How does $NICO_2$® measure ventilatory pattern?

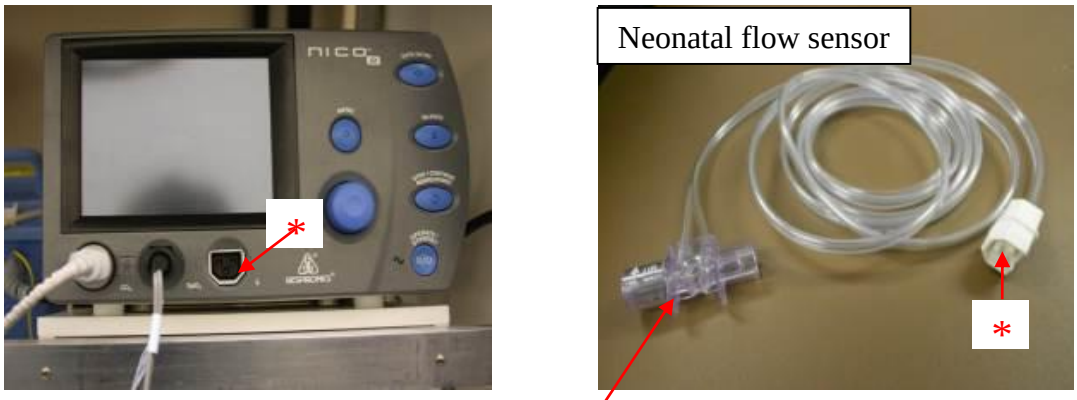
The $NICO_2$® is a respiratory monitor (**Figure 4-vi**) that measures flow, pressure and time instantaneously at the airway opening via a disposable fixed orifice differential flow-sensor and pressure transducer attached between the infant's tracheal tube and

the ventilator circuit. From the integrated flow signal, inflation volumes were calculated.

The $NICO_2$® contains the same hardware platform and algorithms as its predecessor, the $CO_2$SMO®Plus! respiratory monitor; which was thoroughly validated for use in the paediatric intensive care unit by members of the Portex respiratory unit.[208,209] The neonatal combined $CO_2$/ flow sensor (combined apparatus deadspace 0.8ml) was used for these recordings. These neonatal sensors were capable of measuring volume changes between 2-300ml within 1% accuracy, and pressure changes between 2-60 $cmH_2O$ (0.2-5.9 kilopascals) within ±2% of those measured by the electronic manometer (Digitron- pressure manometer P200UL).[208] The disposable $NICO_2$® sensors were factory calibrated and are recommended for use without further calibration. However, during previous validation studies in this department, frequent calibration checks were undertaken using a Hans Rudolf calibrated syringe and the signal was always found to be well within the narrow expected range. Hence the $NICO_2$® machine did not require calibration prior to use.

Throughout the CT scanning procedure, all the inflation breaths were recorded. Ventilatory pressures were visualised and recorded on the screen of the $NICO_2$® machine and if grossly discrepant pressures were applied at different stages of imaging, this provided immediate feedback to the anaesthetists, providing the opportunity to adhere more closely to protocol. Data were also automatically exported into an Excel file from the $NICO_2$® which allowed off-line detailed analysis at a later stage.

**Figure 4-vi: $NICO_2$® machine and neonatal flow sensor**



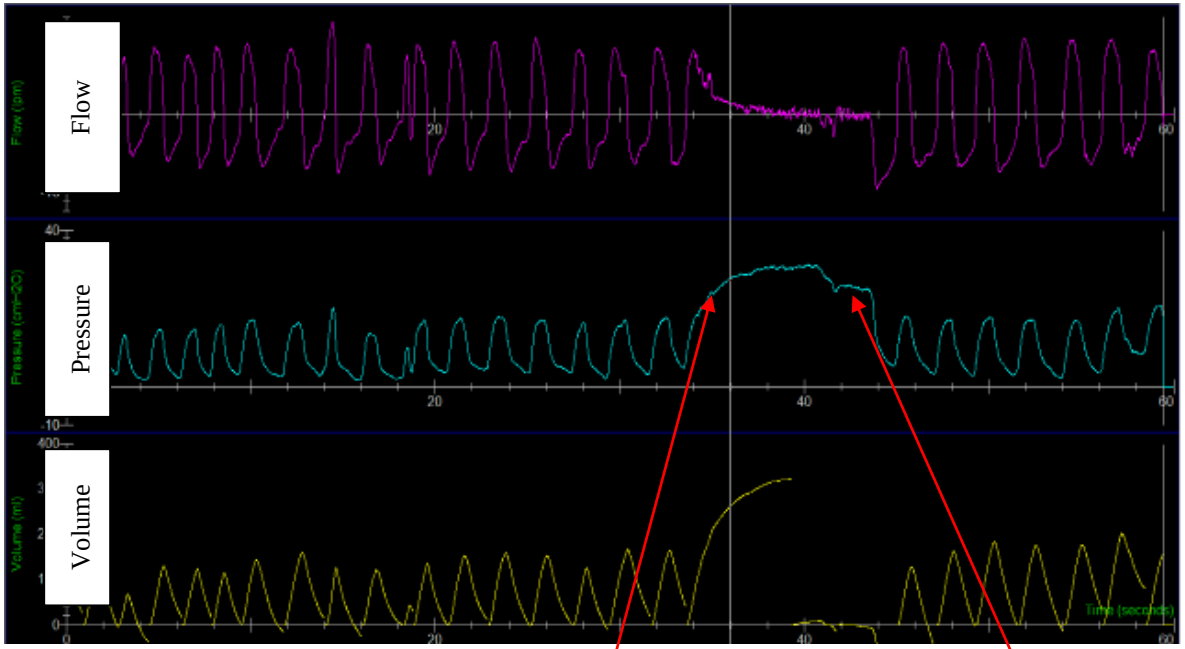

***Legend:*** *The white end of the neonatal combined $CO_2$/ flow sensor (*) is attached to the $NICO_2$® machine (*) and the other end is attached between the infant's endotracheal tube and the ventilator circuit. The $CO_2$/ flow sensor is a single use disposable respiratory sensor.*

### 4.4.3 Outcomes measured using the $NICO_2$®

Using the $NICO_2$® respiratory monitor, PIP before topogram, PIP and PEEP administered during the recruitment manoeuvres, PIP before inspiratory scan and PEEP during the expiratory scan were recorded and analysed to assess whether the scans obtained were indeed undertaken in accordance with the standardised protocols. **Figure 4-vii** are screenshots of 'ideal' measurements recorded using the respiratory monitor during different image acquisitions. Conversely, **Figure 4-viii** are examples of when the anaesthetist did not adhere closely to the protocol.

**Figure 4-vii: Examples of 'ideal' patterns of ventilatory support during various stages of the CT scan as monitored by the $NICO_2$® machine**

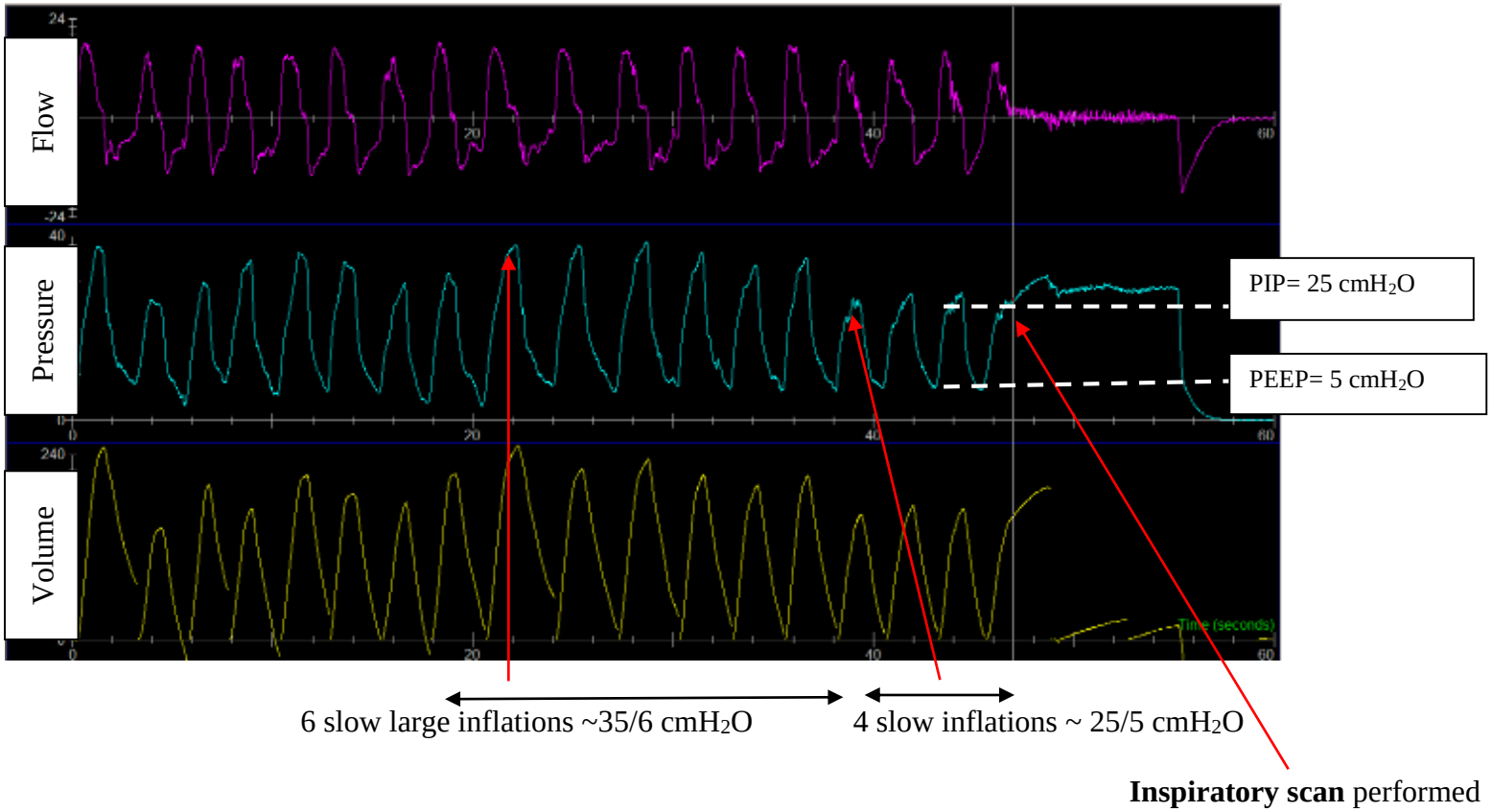
(a) During the Topogram




**Topogram** scan performed once PIP reached 25 $cmH_2O$ Throughout topography, the lung is held inflated at 25$cmH_2O$

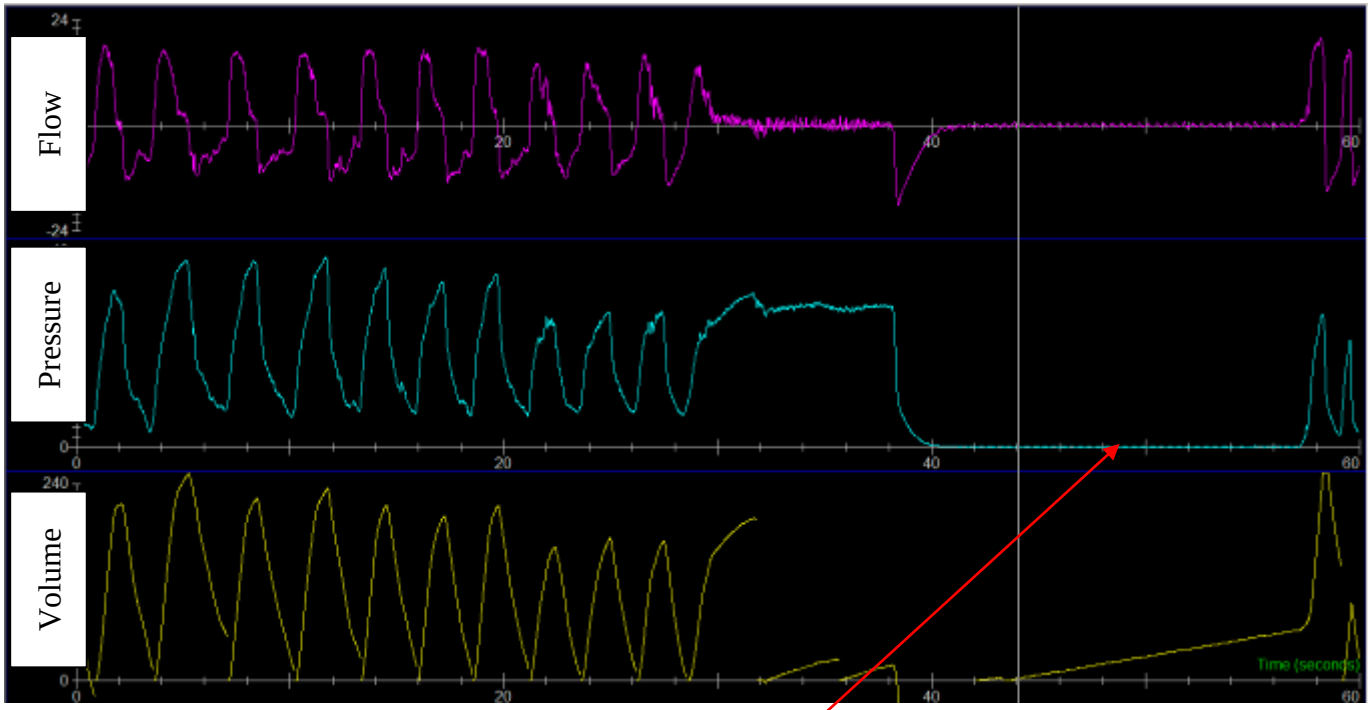
***Legend:*** *The top trace (purple) records the flow, middle trace (turquoise) records the pressure and the bottom trace (yellow) records the volume of each inflated breath during GA. Prior to performing the topogram, baseline ventilation was initially provided via the anaesthetic machine using tidal volumes of 8-10 ml/kg and PEEP 5 $cmH_2O$. Once ready for topogram, ventilation was switched to manual ventilation. During the topogram, the infant's lungs were inflated to a PIP of ~25 $cmH_2O$ and when this pressure was attained, the topogram was acquired during the breath hold at PIP 25 $cmH_2O$.*

(b) During recruitment manoeuvres and inspiratory scan




***Legend:*** *Prior to the inspiratory scan being acquired, 6 larger and slower inflations of PIP 35-40/6 cm $H_2O$ were administered to reverse any GA-related atelectasis followed by 4 smaller and slow inflations of 25/5 cm $H_2O$. During the last of the 4 smaller inflations, the inflation was held at 25 cm $H_2O$ and once attained, the inspiratory image was acquired.*

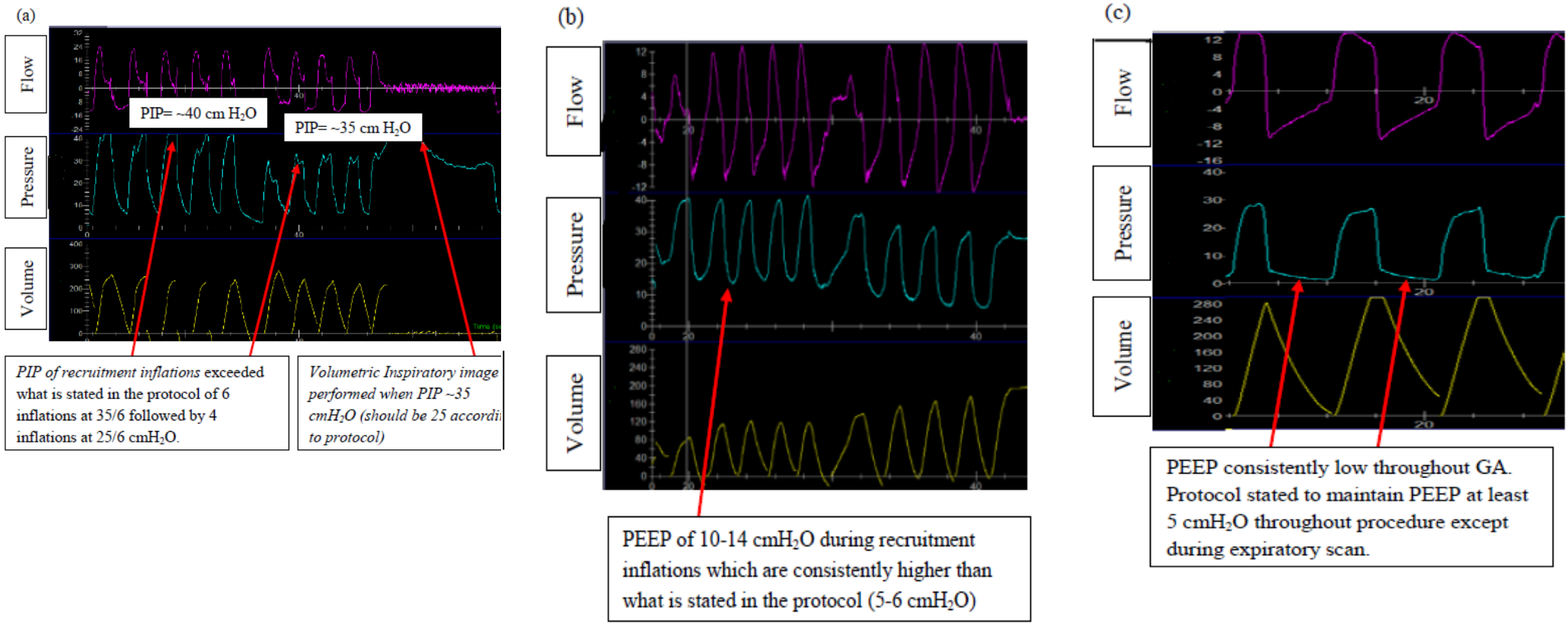
(c) During Expiratory scan




**Expiratory scan** performed once lungs were fully deflated.

***Legend:*** *Immediately following the acquisition of the inspiratory scan, the inflation was released and the infant's lungs were allowed to deflate down to their elastic equilibrium volume, FRC (zero PEEP), before the expiratory scan was performed.*

**Figure 4-viii: Screenshot of $NICO_2$® measurements showing examples of when anaesthetist did not adhere closely to protocol when manually ventilating the infant.**




***Legend:*** *Different anaesthetists from the three centres varied in the ventilation provided for the infants during the imaging protocol. Using the $NICO_2$® machine, it was possible to visualise on screen where the problems were so that changes could be implemented before the images were taken. (a)During the recruitment manoeuvre, pressures provided were higher than stipulated in the protocol. (b)PEEP was held at a higher pressure than stated in the protocol. (c)In between inflations, PEEP should be maintained at 5 but in this scenario, PEEP was zero in between inflations.*

## 4.5 RESULTS FROM $NICO_2$® MONITORING ACROSS CENTRES

65 CTs were performed at a year of age in NBS CF infants with corresponding 1yr lung function results.

I attended 50/65 (77%) of the CT procedures in all three centres; the initial 15 procedures were performed prior to my appointment to this project. I obtained objective records of manual ventilation patterns by using the $NICO_2$® respiratory monitor in 37/65 (57%) of all CT scans performed. Of the 65 scans, 15% were performed at centre A, 58% at centre B and 26% at centre C. See **Table 4-ii** for a summary of scans performed, attendance of research team and objective monitoring in each centre.

**Table 4-ii: The number (percentage) of scans performed, attendance of research team and objective monitoring in each centre**

| | n | Centre A | Centre B | Centre C |
|---|---|---|---|---|
| **No (%) scans performed/centre** | 65 | 10/65 (15%) | 38/65 (58%) | 17/65 (26%) |
| **No (%) cases attended by research team** | 50/65 (77%) | 7/10 (70%) | 28/38 (74%) | 15/17 (88%) |
| **No (%) of cases with objective monitoring** | 37/65 (58%) | 5/10 (50%) | 19/38 (50%) | 13/17 (76%) |

Due to their non-parametric distribution, ventilatory pressures used were compared using Kruskal Wallis (K-W) for significance testing of differences and post-hoc Bonferroni adjustment for multiple Mann Whitney U test to compare the median and IQR across the three centres.

Evidence from the respiratory monitor indicated that ventilatory pattern was similar across the three centres (**Table 4-iii**). A slightly higher than intended PEEP during the recruitment inflations was seen across the three centres [overall median (interquartile range) PEEP delivered being 7.2 (5.4; 8.8) $cmH_2O$], this being

significantly higher in Centre B compared to Centre C (p=0.012) (**Figure 4-ix**). No difference was observed between Centres A and B.

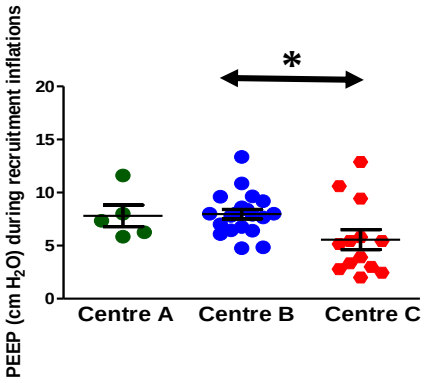
There were no significant differences between centres with respect to PIP during either recruitment inflations or the breath-hold during inspiratory image acquisition, overall median IQR across the three centres being close to that specified in the protocol [32.9(30.6; 35.1) and 26.2(24.5;27.9) $cmH_2O$ respectively].

**Table 4-iii: Ventilatory pressures during different scanning stages from the three participating centres**

| | **Procedures monitored using $NICO_2$ ®** | | | |
|---|---|---|---|---|
| | **Centre A (n=5/10)** | **Centre B (n=19/38)** | **Centre C (n=13/17)** | **Overall (n= 37/65)** |
| **PIP during recruitment** | 32.8 (30.4;34.2) | 32.6 (30.1;35.5) | 33.0 (30.7;35.5) | 32.9 (30.6;35.1) |
| **PEEP during recruitment** | 7.4 (6.1;9.8) | 8.0 (6.5;9.1)* | 5.2 (2.9;7.6)* | 7.2 (5.4;8.8) |
| **PIP during breath-hold** | 26.0 (16.3;28.8) | 27.6 (25.5;29.0) | 25.1 (23.7;26.2) | 26.2 (24.5;27.9) |

***Footnote:*** *n: number of cases monitored/number of scans performed; All results expressed as median (Inter quartile ranges) All pressures reported in $cmH_2O$. *significant difference between centres B and C: $p<0.05$, see* ***Figure 4-ix****.*

**Figure 4-ix: Diagram showing peak end-expiratory pressures during recruitment inflations across the three centres**




***Legend:*** *Median and interquartile ranges of peak end- expiratory pressure during recruitment inflations according to centre. Using Mann Whitney U test * p <0.05. This was the only ventilatory parameter that showed a significant difference between the centres.*

## 4.6 RESULTS OF RADIATION DOSES FROM CHEST CT ACROSS CENTRES

The first eight scans performed were limited to 3-slices expiratory scans so have been excluded from these calculations. With these limited expiratory scans (n=8), median (IQR) radiation dose was 1.07(0.92;1.34) mSv. Of the remaining 57 full volumetric scans, precise radiation dose for 4 of the later scans could not be calculated due to the lack of available qualified staff.

Due to their non-parametric distribution, radiation doses were also compared using Kruskal Wallis (K-W) for significance testing of differences and post-hoc Bonferroni adjustment for multiple Mann Whitney U test to compare the median and IQR across the three centres.

For the remaining 53 scans, the median (IQR) effective radiation exposure across all centres was 1.5(1.2; 1.8) mSv, with centres A and B achieving median doses close to the target dose of 1.5 mSv, exposure was significantly higher at centre C (**Table 4-v** and **Figure 4-x**). Three infants in centre C received ≥3mSv; 2 due to sub-optimal positioning. These cases were appropriately investigated and dealt with. Even when these three infants' radiation doses were not included in the analysis, median radiation dose reported in centre C (median (IQR) 2.18, (1.78 to 2.43) mSv was still

significantly higher than centres A and B. The greater variability in radiation doses observed in centre C may be due to the slightly different type of scanner (**Table 4-i**) and/or the fact that it was not possible to organise a dedicated radiographer to perform procedures within that hospital such that they were not as familiar with the tight scanning control required.

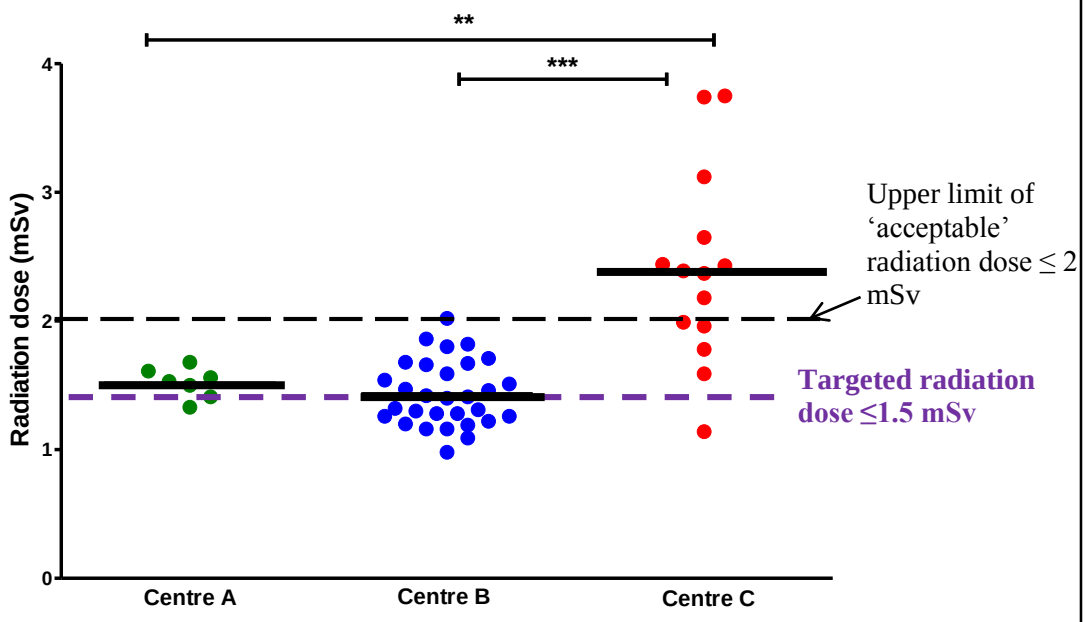
In general, exposures of ≤1.5mSv were achieved in 58% of infants; 79% received an effective dose of ≤2 mSv.

**Table 4-iv: Table of radiation doses from volumetric inspiratory and expiratory chest CT scans across three centres**

| | **Centre A (n=7)** | **Centre B (n=31)** | **Centre C (n=15)** | **Overall dose** |
|---|---|---|---|---|
| **Median (mSv)** | 1.53 | 1.31 | 2.38 | 1.50 |
| **Inter- quartile range (mSv)** | 1.37; 1.65 | 0.86; 2.02 | 1.14; 3.75 | 1.24; 1.84 |

***Footnote:*** *n= number of scans performed in each centre. mSv= milliSievert, unit of measuring ionising radiation.*

**Figure 4-x: Radiation doses from chest CT across three centres**




***Legend:*** *Solid black horizontal line demonstrates the median radiation doses from each centre. Purple dashed horizontal line denotes targeted radiation dose, which was achieved in 58% of infants (i.e those at or below the line). ~80% of infants received less than the upper limit of 'acceptable' dose (below the black* dashed line). *Radiation dose in Centre C was significantly higher than that in both centre A (**p<0.01) and B ***p<0.001 using Mann Whitney U tests.*

## 4.7 FLEXIBLE BRONCHOSCOPY, BRONCHO-ALVEOLAR LAVAGE AND POST GENERAL ANAESTHESIA PROCEDURES

Flexible bronchoscopy and BAL were performed immediately after the chest CT scan. BAL samples were obtained bilaterally; mainly 3 samples from the right middle lobe and one sample from the lingula, unless an area was identified as being the site of more severe disease either by CT scan or during bronchoscopy. This occurred in about 7/65 (~10%) of the NBS CF infants that were lavaged whereby other lobes with apparently more disease were lavaged. Lavage samples were analysed and quantified for bacterial, fungal and mycobacterial growth and virus detection through immunofluorescence (**Appendix: A11**).

Once the CT scan and BAL were completed, and the infant had roused and was clinically stable, the child was transferred back to the ward. They were observed for at least 2-4 hours to ensure that they were fully awake with stable observations before being discharged. Infants were subsequently followed up in their respective CF centres where the results from the lung function, chest CT and bronchoscopy and BAL results were conveyed to the parents of the CF infant by the consultant responsible for their care.

Detailed results of the bronchoscopy and BAL are not included in this thesis as they do not contribute towards any of the hypotheses, aims or objective in this study; being beyond the scope of the current thesis. Culture results from BAL will be reported in chapter 6, **section 6.1.1**.

## 4.8 SUMMARY

This chapter discussed the standardised protocol for obtaining chest CT scans and the associated challenges and difficulties encountered when conducting chest CT under GA in CF infants and when using chest CT as a potential outcome measure to detect early CF lung disease in multicentre studies. Despite the rigour involved in attempting to standardise procedures through written protocols, specialised training of anaesthetists and radiographers and the attendance of research personnel when available, differences occurred with respect to the pressure delivered during GA and radiation exposure. The extent to which deviations in GA and scanning protocols could affect the chest CT results will be discussed in **chapter 6, section 5.3.5**. It is vital that these challenges are anticipated and addressed when considering the use of chest CT as a potential outcome measure or clinical trial endpoint in multicentre studies, otherwise comparability of results between centres will be compromised.

In addition to the difficulties in obtaining research CT scans, there is minimal information on the best scoring system for use in CF infants diagnosed through NBS, who are likely to have much milder disease than those for whom scoring systems have been developed and validated in the past. In the next chapter (Chapter 5), the validity of using the Brody-II CT scoring system to evaluate early lung disease in

young NBS CF infants will be addressed, using inter-observer and intra-observer agreement of scores following a standardised training programme. The relationship of CT-demonstrated lung changes with physiological lung function will also be explored in Chapter 6.

# 5 CHALLENGES IN SCORING CHEST COMPUTED TOMOGRAPHY CHANGES IN NEWBORN SCREENED CF INFANTS

## 5.1 INTRODUCTION

Although there are several existing specific CT scoring systems, which identify and assess severity of various abnormalities, none has been validated for use in early infancy. In addition it is not known which scoring system is most sensitive to detect clinically relevant changes in the presence of mild lung disease. The different scoring systems that are currently available was discussed in the introductory **chapter 1, section 1.8.2.**

The Brody II (modified Brody scoring system) is one of the most widely used scores for use in subjects with CF and has been shown to provide the most comprehensive assessment of the extent and severity of lung abnormalities in CF patients. It has also been assessed in several validation studies involving older children[149,151,155,172] and the rationale for using this scoring system as compared to other scoring systems was previously discussed in **chapter 1, section 1.8.2.2 and 1.9**. Although, in keeping with all other published scoring systems [146,171,172,174,175,205], its use has yet to be established in young NBS CF infants, it has been validated in studies involving older children and adults with CF, showing low within- and between- observer variability[154,172]; and good correlation with LCI[107] and clinical outcomes such as pulmonary exacerbations[155,210]. It was therefore selected for use in this study, with the specific aim of establishing whether Brody II could detect and score changes in young CF infants with mild lung disease reliably and to measure inter and intra observer agreement using this scoring system.

This chapter consists of two sections: the first describes the CT scoring methodology while the second section reports on the scoring results of CT changes.

## 5.2 SCORING METHDOLOGY

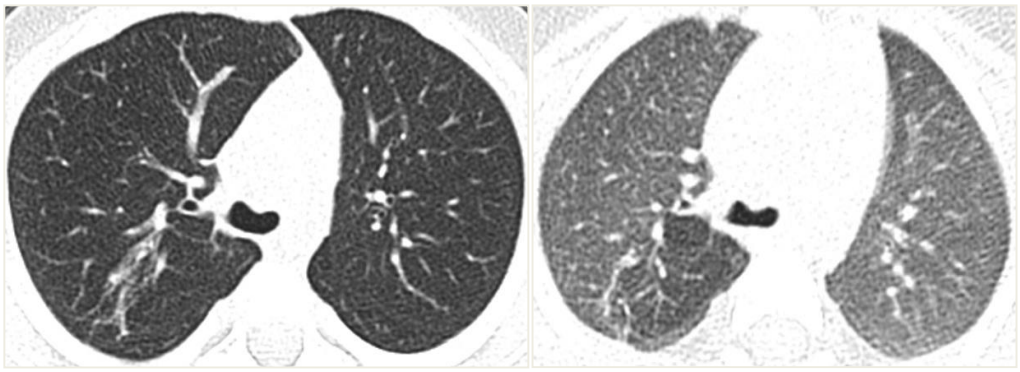
### 5.2.1 The Brody-II scoring system

Brody-II scoring is a modification of the original Brody scoring system.[211] This scoring system assesses the severity and extent of bronchial dilatation, bronchial wall thickening, parenchymal changes of consolidation and ground glass opacification, mucous plugging and air trapping (based on expiratory scans) in each lobe. Distribution of each abnormality is described according to its central or peripheral location within each lobe. Peripheral lung is defined as the portion of lung within 2 cm of the costal or diaphragmatic pleura whilst central portion accounted for the rest of the lung. Each subject's lungs are divided into 6 lobes, three on each side. A score sheet is filled out for each lobe of the lung, including the lingula as a separate lobe (**Appendix: A12**).

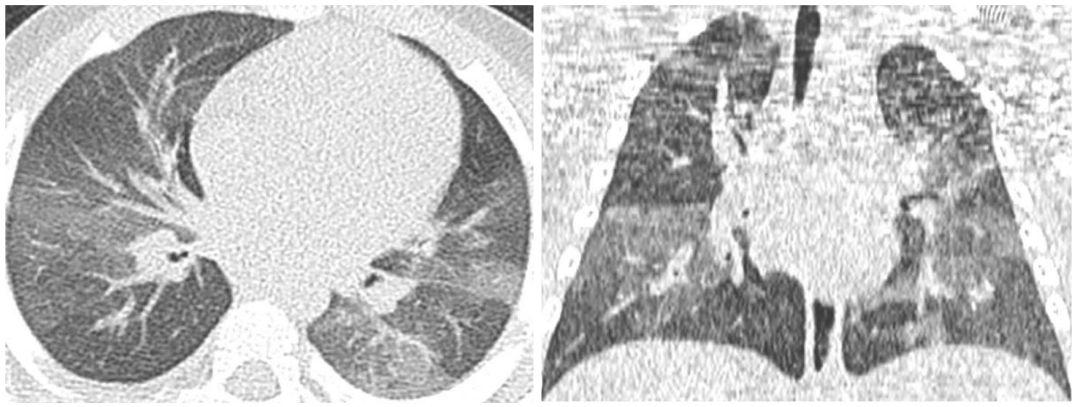
Bronchiectasis is identified by the presence of one or more of the following criteria: a broncho-arterial ratio (BAR) >1, a non-tapering bronchus, a bronchus within 1 cm of the costal pleura or abutting the mediastinal pleura.[166] A critical nuance of this is whether bronchial diameter is evaluated from outer wall to outer wall, or as luminal diameter. While rarely specified in reports, when it is, it is the luminal, rather than external diameter that should be recorded, as was used in the present study. The severity of bronchiectasis is defined as mild if the bronchus is less than twice the size of the accompanying vessel, moderate if two or three times and severe if the diameter is greater than three times the size of the vessel. Bronchial wall thickening is defined as a bronchial thickness > 2mm in the hila region, 1mm in the central portion of the lung and 0.5mm in the peripheral lung. Central mucous plugging is defined as an opacity filling a defined bronchus and peripheral plugging defined as the presence of either dilated mucus-filled bronchi or peripheral thin branching structures or centrilobular nodules in the peripheral lung. Air trapping is defined as areas of lung attenuation when compared with the appearance on the inspiratory images. Due to the decrease in lung air content during expiration, normal lung would show an increase in density on expiratory images. Air trapping is further characterised by sub-segmental or segmental distribution of the low attenuation areas.[212](**Figure 5-i**)

**Figure 5-i: Examples of Chest CT slices demonstrating air trapping**

(a) Inspiratory (b) Expiratory



(c) Coronal section (d) Sagittal section



***Legend:*** *(a) On inspiratory image, there is an area of segmental abnormality of the centrilobular bronchi in the posterior segment of the right upper lobe. No obvious reduced attenuation noted on inspiratory image, whilst in the expiratory image (b) there is a corresponding segmental area of air trapping clearly demarcated. (c) Coronal CT slice demonstrating mosaicism indicative of air trapping in another patient. (d) Sagittal CT slice demonstrating the same areas of segmental and subsegmental air trapping depicted in the coronal section.*

Assessment for severity and extent was performed by assessing the severity of the abnormality using the described criteria and estimating the volume of the lobe showing the abnormality. The lobar area was determined by estimating the area that showed the abnormality within each slice which was then combined to estimate the volume of the lobe showing the abnormality. Average bronchiectasis was defined as the degree of dilation most frequently seen. Both severity and extent contributed to the increasing score for bronchiectasis and bronchial wall thickening.

A score was calculated for each abnormality and the scores added to provide a total disease severity score for that lobe and finally scores for the six lobes were added to provide a total patient score. The weighting system used in this scoring system was based on a review of published scoring systems[171,211,213] and experience of the radiologists involved in developing the Brody-II system. The total severity score can range from 0 (normal) to 243 (severe abnormality seen in all categories affecting every lobe of the lung). Parenchymal changes (consolidation, cyst and ground glass opacity) could be found in combination in each lobe, hence these finding were grouped together as one category in this modified scoring system. The ranges for bronchiectasis and air trapping scores were 0-72 and 0-27 respectively for each scan.

A summary description of the abnormalities and the scores allocated as described in this section is in **Table 5-i**.

**Table 5-i: Brody- II scoring system**

| | | | | | | |
|---|---|---|---|---|---|---|
| Bronchiectasis score<br>(range 0 to 12) | = ( ( | Extent of bronchiectasis in central lung<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | + | Extent of bronchiectasis in peripheral lung<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | ) x ) | Average bronchiectasis size multiplier<br>0.5 = 0<br>1 = 1<br>1.5 = 1.25<br>2.0 = 1.5<br>2.5 = 1.75<br>3 = 2 |
| where | | | | | | |
| Average bronchiectasis size | = ( ( | Size of largest dilated bronchus<br>1 = <2x<br>2 = 2x-3x<br>3 = >3x | + | Average size of dilated bronchi<br>1 = <2x<br>2 = 2x-3x<br>3 = >3x | ) / ) | 2 |
| Mucous plugging score<br>(range 0 to 6) | = | Extent of mucous plugging in central lung<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | + | Extent of mucous plugging in peripheral lung<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | | |
| Peribronchial thickening score<br>(range 0 to 9) | = ( ( | Extent of peribronchial thickening in central lung<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | + | Extent of peribronchial thickening in peripheral lung<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | ) x ) | Severity of peribronchial thickening<br>1 = mild<br>1.25 = moderate<br>1.5 = severe |
| Parenchyma score<br>(range 0 to 9) | = | Extent of dense parechymal opacity<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | + | Extent of ground glass opacity<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | + | Extent of cysts or bullae<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe |
| Air Trapping score<br>(range 0 to 4.5) | | Extent of air trapping<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | X | Appearance of air trapping<br>1 = subsegmental<br>1.5 = segmental or larger | | |

***Footnote:*** *Each subscore applied to each lobe such that there are 3 lobes on the right (upper, middle and lower) and on the left (upper, lower and lingula). The maximum possible score for bronchial dilatation is 12 x 6= 72; for air trapping 4.5 x 6=27; for peribronchial thickening 9 x 6=54; for mucous plugging= 6 x 6= 36 and for parenchyma score 9 x 6= 54. The maximum possible CT total score is 243;* higher scores *indicating* more severe disease.

### 5.2.2 Training scans and scoring

All CT scans undertaken were anonymised before the commencement of scoring. Studies were scored independently without prior clinical or laboratory information by two consultant radiologists using the Brody-II scores,[151,172] Dr Alan Brody (AB: 25 years' experience of paediatric chest CT, 13 years' experience scoring CF lung disease and who had devised and validated the Brody CT scoring system.[151,154,172]) and Dr Alistair Calder (AC: 7 years' experience of paediatric chest CT, 5 years' experience scoring CF lung disease).

Prior to scoring any CTs from this study, both scorers studied a PowerPoint presentation which explained the Brody II scoring system and definitions of the different abnormalities described in the scoring system. CT images were shown in the presentation to visually illustrate these abnormalities. This training package was developed by the Eramus medical centre in Rotterdam, The Netherlands in partnership with international collaborators in an attempt to have a consensus proposal for quantifying structural abnormalities in CF. Following this, both scorers undertook an initial training period using 12 training scans provided by the AREST-CF team from children with CF aged 1- 5 years of age in whom data had been acquired using a similar protocol to the current study. Each scan comprised of a volumetric inspiratory and expiratory image. These 'training scans' were scored in two batches of 6. The two scorers independently evaluated the 1$^{st}$ training batch followed by video-conference to clarify the definitions used for bronchial dilatation and a further group of six training studies were then independently evaluated (training batch 2). Scores from these training batches were compared, results of which are reported in the second part of this chapter in **section 5.3.3**.

### 5.2.3 Process of scoring study scans

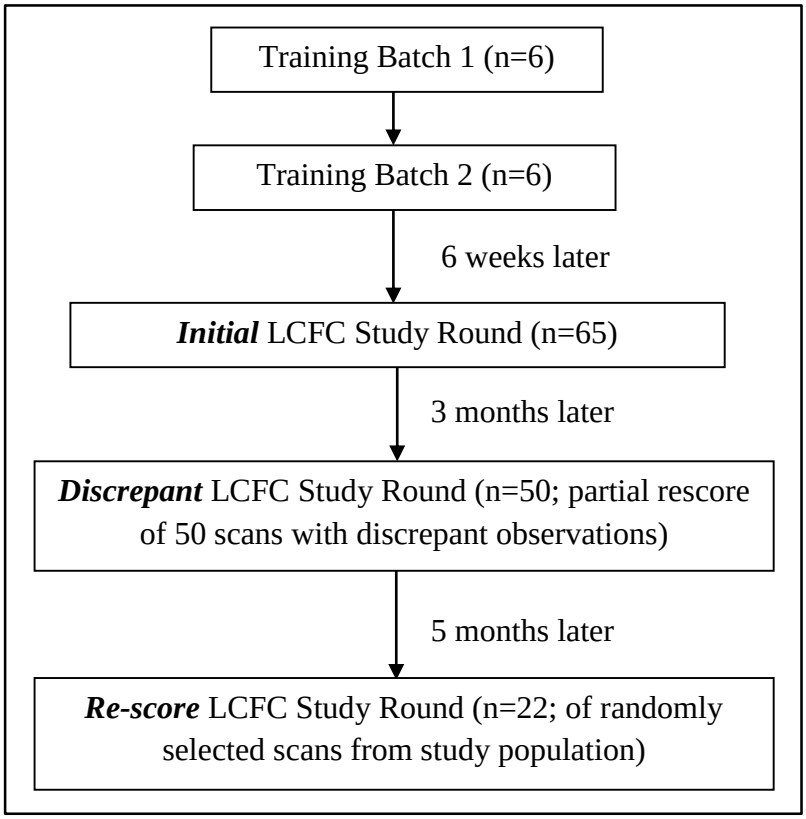
This section describes the process of the different scoring stages of the study scans performed in NBS CF infants at a year of age. Results of these scores are presented later in this chapter.

Scoring of LCFC scans took place within 6 weeks of completing training. All patient clinical information and lung function results were concealed from the scorers.

Scores from both observers were analysed and compared by me who was not involved in scoring. LCFC scans with discrepant sub-scores were returned to both scorers (without details of prior scores allocated) for subsequent re-assessment to investigate whether closer agreement could potentially be achieved. A random selection of LCFC scans was completely re-scored after ~8 months to assess inter- and intra-observer agreement over time.

After the first evaluation of study scans ***(initial LCFC study round; n=65)***, a record of all discrepant observations from this initial study round without any details of scores allocated, was sent to both observers by me for subsequent re-assessment (***discrepant LCFC study round; n=50***) to investigate whether closer agreement could potentially be achieved in future with further training and scoring only in components that were scored differently at the initial study round. Finally randomly selected studies underwent complete re-*scoring (**Rescore LCFC study round; n=22)***, 8 months after the initial LCFC study round to assess inter and intra- observer agreement of scores. Calculations and comparison of all scores in the three study rounds (scoring rounds described as stated in brackets in italics above) (**Figure 5-ii**) were undertaken by me (LT).

**Figure 5-ii: Flowchart showing the different scoring rounds, the number of scans scored and the relevant time intervals**



### 5.2.4 Outcome measures and statistical analysis of CT scores

Outcomes measures included total Brody II scores and sub-scores for bronchial dilatation, air trapping, bronchial wall thickening, mucous plugging and parenchymal opacities. The comparison of these CT abnormalities to various lung function outcomes listed in **chapter 2** (**section: 2.3.5**) and potential clinical determinants will be presented in the next chapter (chapter 6).

Data were inspected for distribution, calculation of descriptive statistics (PASW Statistics v.18, Chicago, IL, US) and summarised using n (%), mean (SD) or median (interquartile range, IQR). Each CT sub-score from each scorer was compared, for both training rounds and all three study rounds. Agreement for each sub-score was assessed using Cohen's kappa statistic with linear weighting (MedCal for Windows, statistical software version 12.3.0, Mariakerke, Belgium). Kappa coefficient with 95% confidence interval (CI) will be presented for bronchial dilatation and air trapping sub-scores and total CT scores. Results for kappa statistics are interpreted as follows:[214] 0-0.2: poor agreement; 0.21-0.4: fair agreement; 0.41-0.6: moderate agreement; 0.61-0.8: strong agreement; 0.81-1.0: excellent agreement. Kappa statistics were used to measure the level of agreement between the two scorers as a way of verifying that agreement exceeds chance level i.e. 'chance-corrected measure of agreement'.

## 5.3 SCORING RESULTS

### 5.3.1 Introduction

The previous section described the scoring process while this section will concentrate on the chest CT results and will test the secondary hypothesis which states that significant abnormal changes can be detected on chest CT even at an early age of 1yr in NBS CF infants.

Despite the availability of numerous CT scoring systems,[146,171,172,174,175,205] none have been validated for use in young CF infants. Therefore in order to validate the use of Brody II scoring system for use in CF infants, it was essential to investigate the applicability of this scoring system, with respect to its intra and inter-observer

variability in detecting and quantifying CT changes in young infants with CF when only very mild (if any) changes may be present.

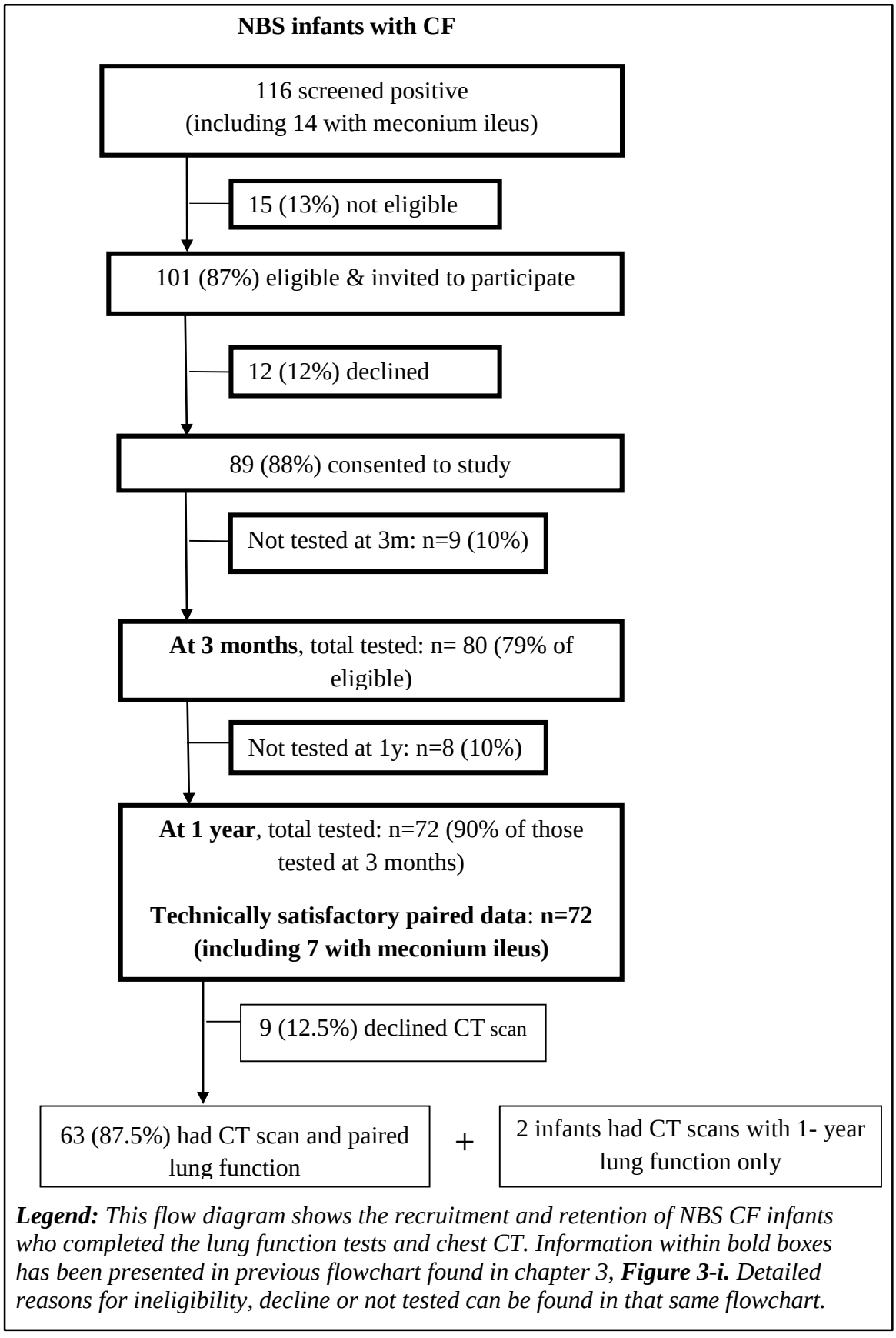
### 5.3.2 Study Population

Of the 72 NBS infants with CF who underwent paired 3 month and 1 year lung function tests, 63 (87.5%) also underwent a chest CT and flexible bronchoscopy under GA. Parents of the remaining nine (12.5%) CF infants declined these tests due to concerns about having a general anaesthetic and radiation from CT; interestingly, the concern over GA was greater than the radiation risk (**Figure 5-iii**).

In addition, there were 2 CF infants who did not have ILFT at 3m but underwent lung function and chest CT at a year of age. One of these infants did not have the three- month ILFT due to repeated respiratory illnesses within the first three months of age whilst the other had borderline screen positive CF results which were only confirmed through positive extended genotype (c.1521_1523delCTT/c.617T>G) at 3 months of age.

Hence in total, there were 65 CF infants who had 1 year lung function test and chest CT; 63 of whom also had lung function assessed at ~3m. Clinical characteristics of this subgroup of CF infants will be presented in chapter 6 (**section 6.1.1**)

**Figure 5-iii: Flow diagram showing recruitment and retention of CF only (shown previously in Figure 3-i ) who completed the 1 year study**




***Legend:*** *This flow diagram shows the recruitment and retention of NBS CF infants who completed the lung function tests and chest CT. Information within bold boxes has been presented in previous flowchart found in chapter 3,* ***Figure 3-i.*** *Detailed reasons for ineligibility, decline or not tested can be found in that same flowchart.*

### 5.3.3 Scoring results from training scans

Training scans used for practice scoring between the two scorers were provided by the AREST-CF team as described in **chapter 5, section 5.2.2.** These 12 scans were obtained from infants and young children aged 1.2 to 4.2 years (median 2.3 years), but only 5 were scanned before 1.3 years. The vast majority of the LCFC infants were studied at less than 1.2 years of age with a few as young as 0.8 years. Despite training scans being undertaken in infants of a slightly older age range, there was still an overlap age range with the current LCFC study. However, with hindsight the use of training scans in age matched infants would have been preferable.

**Table 5-ii** shows the agreement between the two scorers according to the Brody–II scoring system for the two training batches. The level of agreement for bronchial dilatation with training batch 2 improved when compared to training batch 1 and was deemed acceptable by both scorers, who then progressed to the scoring of CT scans obtained as part of the definitive LCFC study of NBS CF infants. Agreement improved during training batch 2 for the bronchial wall thickening sub-score, whereas that for mucous plugging (which initially showed strong agreement), and parenchymal change (with moderate initial agreement), showed less agreement. Agreement between scorers on the air trapping sub-score remained strong for both training batches.

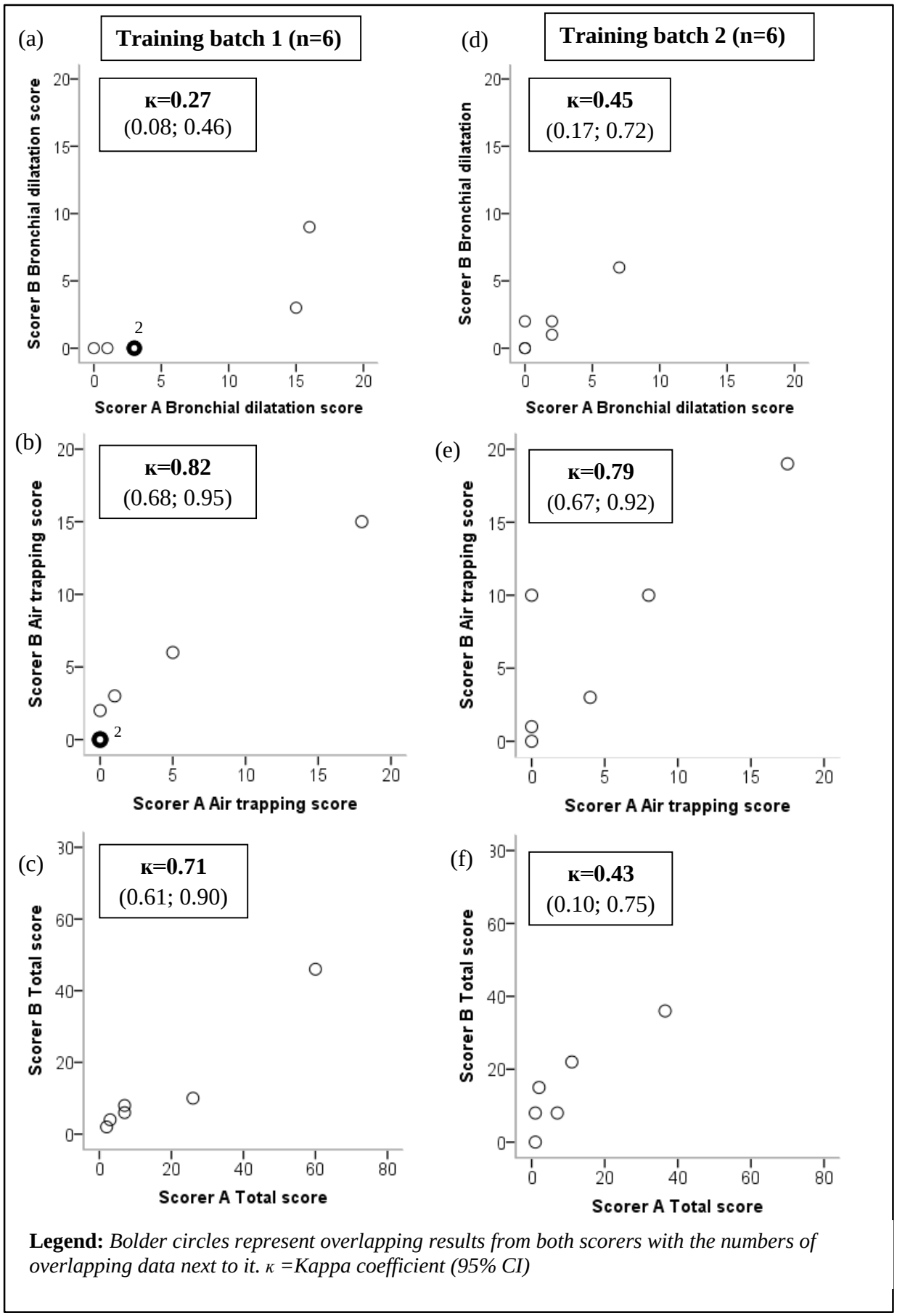
**Table 5-ii: Agreement of scores according to Brody-II scoring system during the two training batches**

| | Training batch 1 (n=6) | Training batch 2 (n=6) |
|---|---|---|
| **Age of infants (years)*** | 2.0 (1.2; 2.6) | 2.3 (1.4; 3.0) |
| **Bronchial dilatation#** | 0.27 (0.08; 0.46) | 0.45 (0.17; 0.72) |
| **Air trapping#** | 0.82 (0.68; 0.95) | 0.79 (0.67; 0.92) |
| **Bronchial wall thickening#** | 0.44 (0.19; 0.70) | 0.79 (0.67; 0.92) |
| **Mucous plugging#** | 0.62 (0.42; 0.81) | 0.00 (0.00; 0.00) |
| **Parenchymal change#** | 0.41 (0.16; 0.66) | 0.30 (0.03; 0.58) |
| **Total CT scores#** | 0.75 (0.61; 0.90) | 0.43 (0.10; 0.75) |

**Footnote:** * *Ages expressed as median (interquartile ranges) in years.*
*# Agreement expressed as mean Kappa coefficient (95% confidence interval) using linear weighted Kappa statistics. Agreement for kappa statistics are interpreted as follows:[214] 0-0.2: poor; 0.21-0.4: fair; 0.41-0.6: moderate; 0.61-0.8: strong and 0.81-1.0: excellent.*
*Agreement for bronchial dilatation improved with the second training batch although it was only moderate; whilst for air trapping, agreement remained strong for both training batches.*

**Figure 5-iv** shows graphical representation of paired scores allocated by scorer A and B for each training scan in terms of bronchial dilatation and air trapping sub-scores and total CT scores with first batch scores represented by plots a-c and second batch scores represented by plots d-f. In first training batch, scorer A gave higher bronchial dilatation score and total score compared to scorer B (**Figure 5-iv,** a & c) but subsequently allocated more similar scores during the second training batch (**Figure 5-iv,** d & f). There appeared to be higher scores during the 1st than 2nd batch for both bronchial dilatation [Batch 1: scorer A (median, range): 3(0-16) and scorer B: 0(0-9) vs. Batch 2: scorer A: 1(0-7) and scorer B: 1.5(0-6)], and for total CT scores [Batch 1: scorer A 7 (2-60) and scorer B 7 (2-46) vs. Batch 2: scorer A: 4.5 (1-37) and scorer B 11.5 (0-36)] (**Table 5-iii**). With air trapping (**Figure 5-iv,** b & e), both observers were consistent with their scores during the first and second training batches. Scans from both batches were similar in terms of severity for air trapping (**Table 5-iii**).

**Figure 5-iv: Scores allocated by scorers A and B for the two batches of training scans (n=12)**




**Legend:** *Bolder circles represent overlapping results from both scorers with the numbers of overlapping data next to it. κ =Kappa coefficient (95% CI)*

**Table 5-iii: Sub-scores and total CT scores allocated for the two training batches by both scorers A and B**

| Total scores or sub-scores | First training batch | | Second training batch | |
|---|---|---|---|---|
| | Scorer A | Scorer B | Scorer A | Scorer B |
| Bronchial dilatation (max score = 72) | 3 (0; 16) | 0 (0; 9) | 1 (0; 7) | 1.5 (0; 6) |
| Air trapping (max score = 27) | 0.5 (0; 8) | 2.5 (0; 15) | 2 (0; 18) | 6.5 (0; 19) |
| Bronchial wall thickening (max score = 54) | 0 (0; 8) | 0 (0; 7) | 0 (0; 4) | 0 (0; 9) |
| Mucous plugging (max score = 36) | 0.5 (0; 10) | 0 (0; 6) | 0 (0; 0) | 0 (0; 1) |
| Parenchymal changes (max score = 54) | 2.5 (1; 8) | 4.5 (0; 9) | 1 (1; 8) | 4.5 (0; 7) |
| Total CT scores (max score = 243) | 7 (2; 60) | 7 (2; 46) | 4.5 (1; 37) | 11.5 (0; 36) |

***Footnote:*** *CT total and sub-scores presented as median (ranges) by both scorers for each training batch.*

Although higher scores were generally allocated to older children (i.e. those ≥ 2 years old); significant bronchial dilatation and air trapping was observed in one infant who was only 1.5 years. With these training scans, there was a high prevalence of bronchial dilatation (50-67% of scans) and air trapping (50-75% of scans) reported by both scorers.

### 5.3.4 Scoring results from LCFC scans

Scoring of the 65 LCFC study scans commenced within 6 weeks of training with completion of this ***initial* LCFC** scoring round within a month. The second scoring round, which consisted of re-scoring the *discrepant* observations (***discrepant* LCFC**) that occurred in 50 scans, blinded to previous results, took place 3 months after the first *initial* LCFC study round and was completed within a month. Finally a randomly selected group of 22 scans (a third of the original cohort) was re-scored in entirety (***rescore* LCFC**) ~4 months after the discrepant LCFC study round (i.e. ~8 months after the initial LCFC round). See **Figure 5-ii** for flowchart of scoring rounds.

### 5.3.4.1 Inter-observer agreement for the initial scoring of LCFC scans

At ***initial LCFC*** scoring round consisting of 65 scans, there was fair agreement between scorers for bronchial dilatation [mean Kappa (CI) = 0.21(0.04; 0.37)] and strong agreement for air-trapping [mean Kappa (CI) = 0.66(0.49; 0.83)]. Bronchial wall thickening, parenchymal change, mucous plugging sub-scores and total scores all showed only fair agreement between both scorers at the first scoring round (**Table 5-iv**).

**Table 5-iv: Linear weighted Kappa analysis between both scorers at *initial* LCFC scoring round of entire cohort**

| | **Kappa[#] (n=65)** |
|---|---|
| **Bronchial dilatation** | 0.21 (0.05; 0.37) |
| **Air trapping** | 0.66 (0.49; 0.83) |
| **Bronchial wall thickening** | 0.27 (-0.01; 0.55) |
| **Parenchyma change** | 0.25 (0.12; 0.38) |
| **Mucous plugging** | 0.26 (-0.12; 0.63) |
| **Total score** | 0.34 (0.20; 0.49) |

***Footnote:*** *[#]Agreement expressed as mean Kappa coefficient (95% confidence interval) using linear weighted Kappa statistics. Agreement for kappa statistics are interpreted as follows:[214] 0-0.2: poor; 0.21-0.4: fair; 0.41-0.6: moderate; 0.61-0.8: strong and 0.81-1.0: excellent.*

Individual scores allocated by each scorer are represented in **Table 5-v**. Scorer A appeared to allocate lower scores compared to scorer B at the initial LCFC scoring round for bronchial dilatation and total CT scores.

**Table 5-v: Inter-observer agreement for CT scores allocated to NBS CF infants at 1 year of age during initial scoring of LCFC scans**

**a) Bronchial dilatation** (Max score 72)

| **κ= 0.21** (0.05; 0.37) | | **Scorer A** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **0** | **1** | **2** | **3** | **4** | **5** |
| **Scorer B** | **0** | 48 | - | - | - | - | |
| | **1** | 6 | - | - | - | - | - |
| | **2** | 4 | 3 | 1 | - | - | - |
| | **3** | 1 | - | - | - | - | - |
| | **4** | 1 | - | - | - | - | - |
| | **5** | - | 1 | - | - | - | - |

**b) Air trapping** (maximum possible score =27)

| **κ= 0.66** (0.49; 0.83) | | **Scorer A** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **0** | **1** | **2** | **3** | **4** | **5** | **7** | **8** | **15** | **16** |
| **Scorer B** | **0** | 37 | - | 1 | - | - | - | - | - | - | - |
| | **1** | 6 | 3 | - | - | - | - | - | - | - | - |
| | **2** | 3 | - | 1 | 1 | - | - | - | - | - | - |
| | **3** | 1 | 1 | 1 | 2 | - | - | - | - | - | - |
| | **4** | - | - | - | - | 2 | - | - | - | - | - |
| | **5** | - | - | - | - | 2 | - | - | - | - | - |
| | **7** | - | - | - | - | - | 1 | - | - | - | - |
| | **8** | 1 | - | - | 1 | - | - | - | - | - | - |
| | **15** | - | - | - | - | - | - | - | - | - | 1 |
| | **16** | - | - | - | - | - | - | - | - | - | - |

**c) Total CT score** (maximum possible score = 243)

| **κ= 0.34** (0.20; 0.49) | | **Scorer A** | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **12** | **13** | **14** | **17** | **19** | **25** | **30** |
| **Scorer B** | **0** | 7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **1** | 5 | 5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **2** | 7 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **3** | 5 | - | 2 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **4** | 3 | 1 | - | 2 | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - |
| | **5** | - | 4 | 1 | 1 | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - |
| | **6** | 1 | - | 1 | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **7** | 1 | 1 | 1 | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - |
| | **8** | 1 | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **9** | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **10** | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - |
| | **12** | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - |
| | **13** | - | - | - | - | - | 2 | - | - | - | - | - | - | - | - | - | - | - | - |
| | **14** | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **17** | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - |
| | **19** | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - |
| | **25** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **30** | - | - | - | - | - | -- | - | - | - | - | - | - | - | - | - | - | 1 | - |

***Footnote:*** *Shaded cells across the diagonals within each table represent identical results by scorers A & B. Numbers within each cell represent the number of infants with each combination of scores. For air trapping scores ≥5 and total CT scores ≥12, only*

*those for which any values were obtained are shown. Scorer A appeared to allocate lower scores compared to scorer B at the initial LCFC scoring round for bronchial dilatation and total CT scores. For total CT scores, darker shaded column and row represent total CT scores whereby it is 5% of the total maximum CT scores. For scorer A, only 2 (3%) scan whilst for scorer B 7(11%) scans had ≥ 5% of the maximum score.*

Although scorer B identified more abnormalities on scans than scorer A as indicated by values generally falling below the shaded diagonal cells [17(26%) vs 5(7%) for bronchial dilatation; 27(42%) vs 17(26%) for air trapping], the severity of changes were generally very minor, with only 7(11%) and 2(3%) of patients having a total CT score ≥12 or 5% of total possible score. It can be seen that the majority of discrepancies for bronchial dilatation occurred when changes were deemed to be very minor [1-3] by one scorer and absent [0] by the other.

#### 5.3.4.2 Re-assessment of discrepant sub-scores following initial LCFC scoring and LCFC rescores

After comparing scores between the two observers during the initial scoring of the 65 LCFC scans, a record of all discrepant observations from a total of 50 scans was prepared by me (LT). Apart from both scorers knowing that their scores differed, all discrepant observations were re-scored independently, blinded to their own and their counterpart's initial scores, following a short general discussion about the scoring system. Analysis of the discrepant cases showed that 90% of these differences were between score of 0 (normal), and 1 (minimal to mild disease) (**Table 5-v**).

Good agreement was observed for bronchial dilatation [Mean Kappa coefficient=0.62 (95% CI: 0.39; 0.86)] and excellent agreement seen for air-trapping [Mean Kappa coefficient=0.88 (95% CI: 0.81; 0.96)] when the discrepant observations were re-scored. These Kappa coefficients for agreement were higher than those obtained during initial scoring of the LCFC scans. This reassured both scorers that improved inter-observer agreement could be achieved before undertaking complete rescoring of a randomly selected subset of 22 LCFC scans 8 months after initial scoring, although as discussed below, this did not prove to be the case.

The subset of 22 scans that underwent rescoring was selected by picking out every third scan from the list of study participants by LT who was not involved in the scoring process; the process thus was not biased towards previously discrepant scans. Inter-observer agreement between *initial and re-scoring LCFC rounds* can be seen in **Table 5- vi**. When 22 randomly selected scans (re-scoring LCFC round), representing one third of the whole cohort were completely re-scored 8 months after completing the initial LCFC round, agreement between scorers was no better than on the first occasion, direct comparisons being made with respect to the same 22 scans on the two different occasions. There was fair agreement between scorers for bronchial dilatation and strong agreement for air trapping, both during initial scoring of all 65 LCFC scans, and when re-scoring the selected subset of 22 LCFC scans (**Table 5-vi** and **Figure 5-iv**).

**Table 5-vi: Kappa between both scorers at initial LCFC scoring round of whole cohort, and at initial and repeat LCFC scoring rounds of a randomly selected subset**

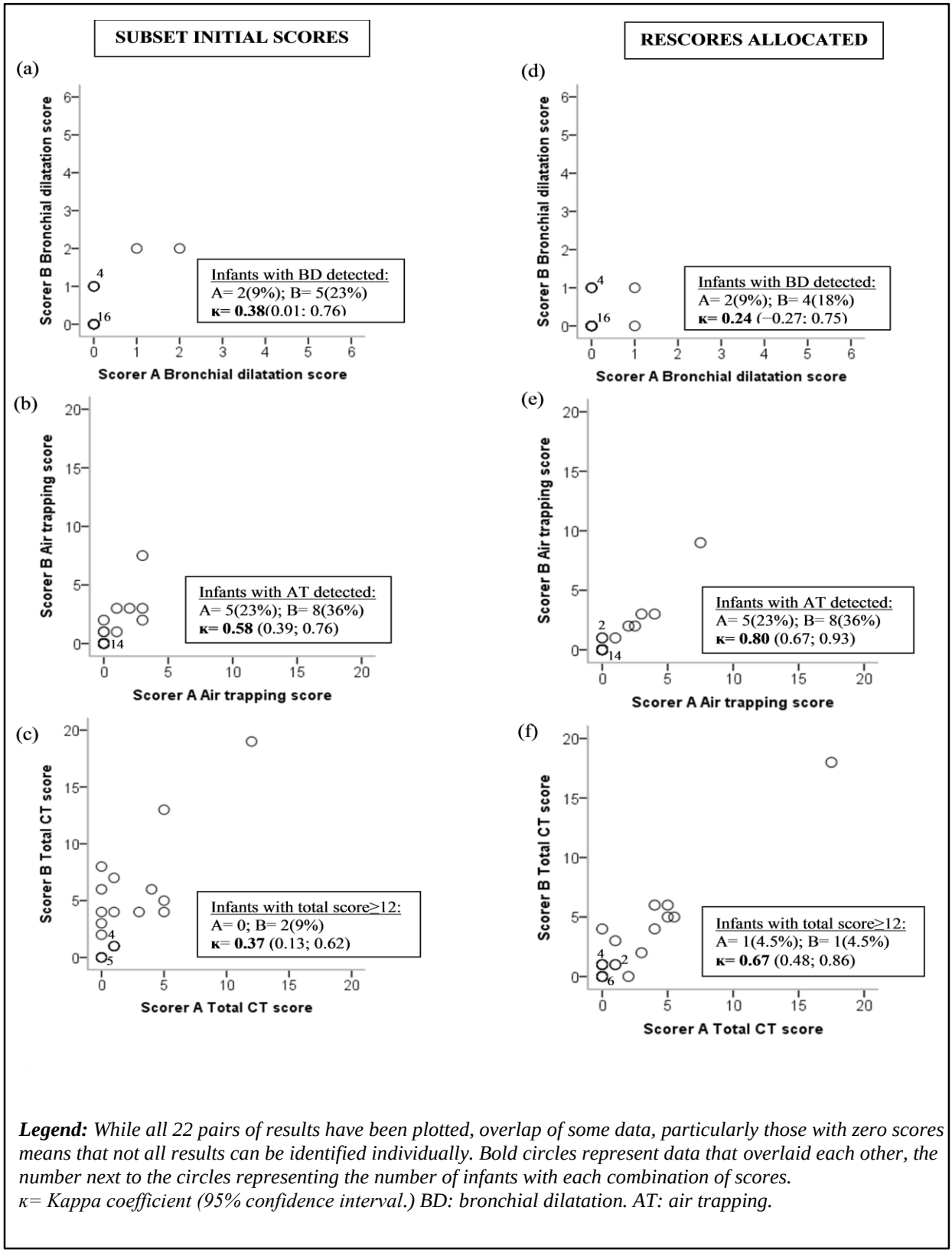
| | **Initial scoring*** **(n=65)** | | **Initial scoring†** **(Subset: n=22)** | **Rescoring†** **(n=22)** |
|---|---|---|---|---|
| **Bronchial dilatation** | 0.21 (0.05; 0.37) | | 0.38 (0.01; 0.76) | 0.24 (−0.27; 0.75) |
| **Air trapping** | 0.66 (0.49; 0.83) | | 0.58 (0.37; 0.79) | 0.80 (0.67; 0.93) |
| **Total CT scores** | 0.34 (0.20; 0.49) | | 0.38 (0.13; 0.62) | 0.67 (0.48; 0.86) |

***Footnote:*** *Results presented as mean (95% CI) linear weighted Kappa coefficient.* * *Entire study cohort of 65 scans.* † *scans from 22 infants were selected from the entire cohort (n=65 by LT), results of which are summarised for both initial and repeat scoring. Rescoring of a subset 8 months later showed no improvement in agreement for bronchial dilatation although better agreement was observed for air trapping and total CT scores.*

**Figure 5-v** illustrates CT scores allocated by scorers A and B for the subset of 22 scans during initial and rescoring rounds. During initial scoring of the subset, scores allocated by scorer B were generally higher than those by scorer A for bronchial dilatation and total scores (panels a & c). More consistent scores with good agreement were seen for

air trapping (panel b). During rescoring of this subset, scores were more similar although only fair agreement was again seen for bronchial dilatation (panel d), while good agreement was observed for air trapping and total scores (panels e & f).

**Figure 5-v: Inter-observer agreement between initial and rescoring LCFC rounds**

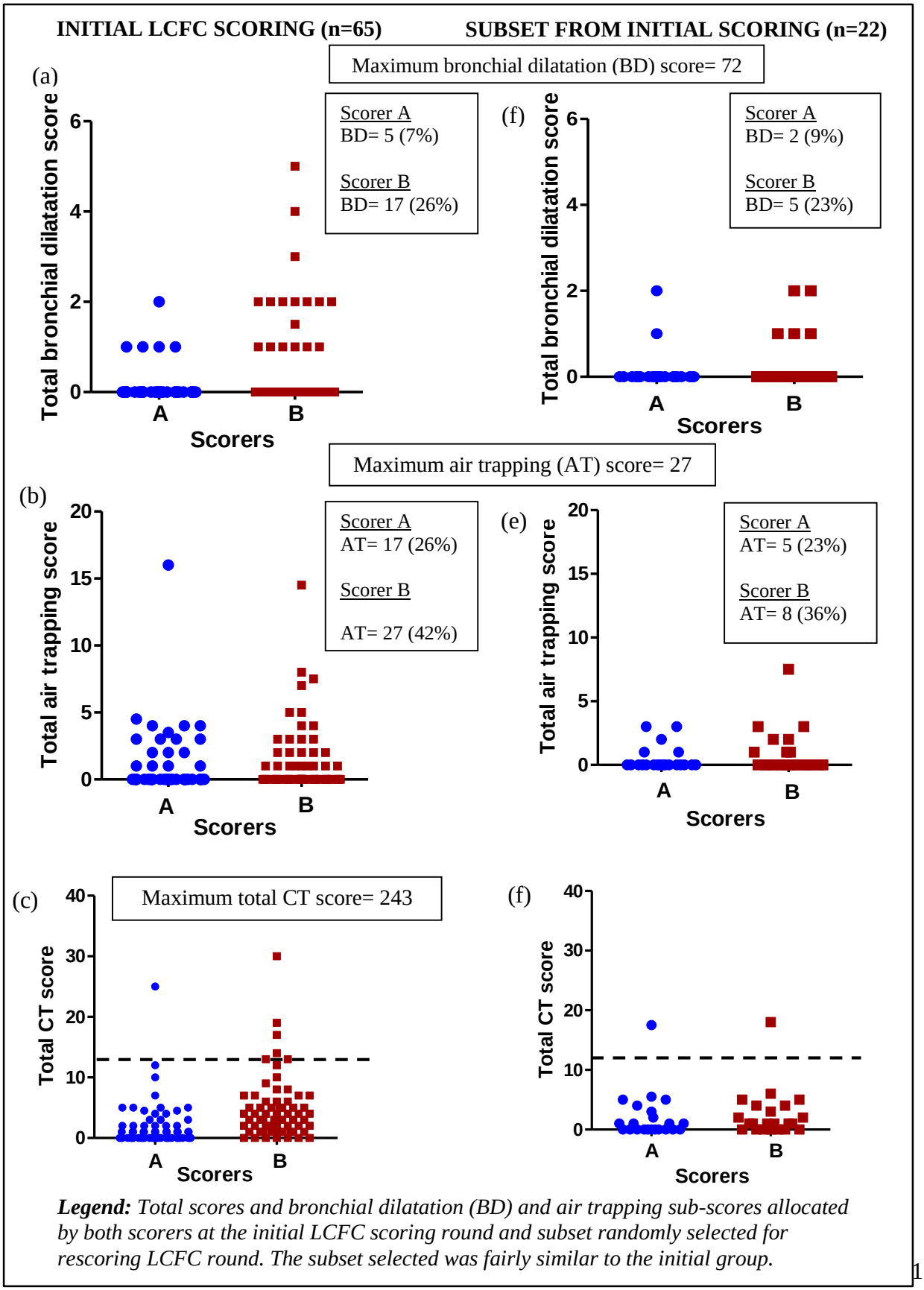



***Legend:*** *While all 22 pairs of results have been plotted, overlap of some data, particularly those with zero scores means that not all results can be identified individually. Bold circles represent data that overlaid each other, the number next to the circles representing the number of infants with each combination of scores.*
*κ= Kappa coefficient (95% confidence interval.) BD: bronchial dilatation. AT: air trapping.*

As can be seen in **Figure 5-vi**, the 22 scans selected for re-score (panels d-f) were representative of those from the entire cohort (panels a-c) in terms of changes detected on CT and extent of severity seen. Although scorer B identified a higher incidence of changes for all outcomes with respect both to the entire cohort and the selected subset, the severity of changes was generally very minor, with only 2(3%) and 7(11%) of patients having a total CT score ≥12 (≥5% of maximum score, denoted by the horizontal broken line), as can be seen by comparing the left and right-hand panels (c&f). The large number of negative (no change/zero total score) means that many data points are overlaid. At *initial* scoring, according to scorer A, no changes were detected for any Brody-II components in 31 scans (48%) whilst 7 (11%) of the scans had no changes detected by scorer B.

In summary, during the various LCFC scoring rounds, agreement between scorers in the bronchial dilatation sub-score was only fair to moderate whilst within and between occasion agreement for the air trapping sub-score was substantial. For the purpose of this thesis, since air trapping sub-score and total CT scores were more reliably scored by both scorers, these sub-scores will be utilized to investigate the relationship between structural changes or changes seen on chest CT and lung function. Despite poor agreement between scorers for bronchial dilatation, this CT sub-score will also be investigated so that comparison to previous published literature can be undertaken.

**Figure 5-vi: Comparison of results between two scorers during initial LCFC scoring of both entire cohort and the randomly selected subset**




***Legend:*** *Total scores and bronchial dilatation (BD) and air trapping sub-scores allocated by both scorers at the initial LCFC scoring round and subset randomly selected for rescoring LCFC round. The subset selected was fairly similar to the initial group.*

#### 5.3.4.3 Inter-observer agreement regarding the presence or absence of changes detected on CT

Although the Kappa score was only fair for bronchial dilatation with minimal improvement at subsequent scoring, the extent to which scorers agreed about the presence or absence of significant bronchial dilatation or air trapping was consistently achieved in >80% of the scans on initial and rescoring LCFC rounds (**Table 5-vii**).

**Table 5-vii: Inter-observer agreement with respect to presence or absence of bronchial dilatation and air trapping during initial and rescoring rounds**

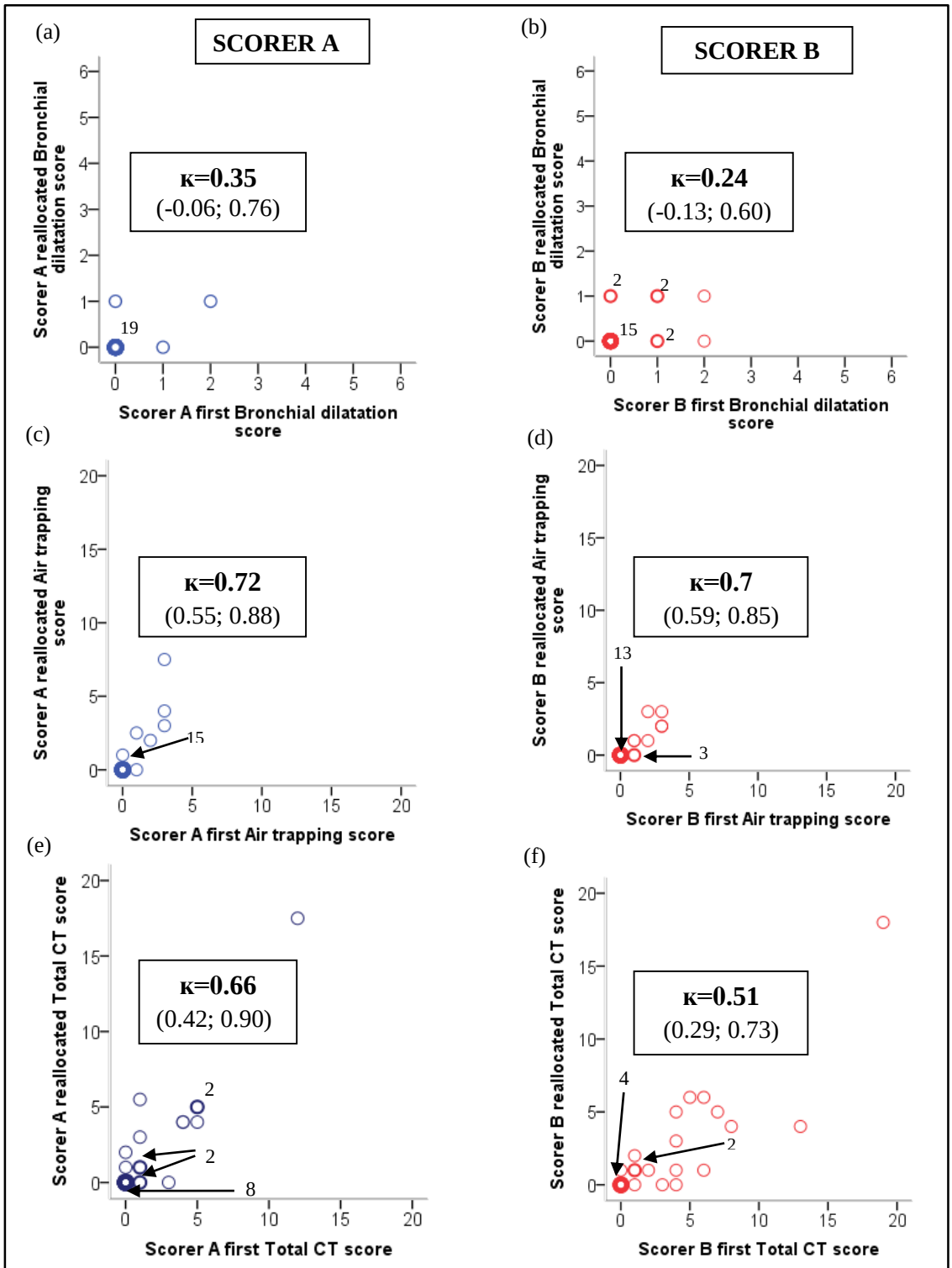
| **LCFC scans** | **Bronchial Dilatation, n (%)** | | | **Air trapping, n (%)** | | |
|---|---|---|---|---|---|---|
| | Present | Absent | **Total % agreed** | Present | Absent | **Total % agreed** |
| initial scoring of all 65 scans | 5 (8%) | 48 (74%) | **82%** | 16 (25%) | 37 (57%) | **82%** |
| initial scoring of subset (n=22) | 2 (9%) | 17 (77%) | **86%** | 5 (23%) | 14 (67%) | **90%** |
| repeat scoring of subset (n=22) | 1 (4.5%) | 17 (77%) | **81.5%** | 5 (23%) | 14 (64%) | **87%** |

***Footnote:*** *In this table, scans with bronchial dilatation or air trapping sub-scores >0 were considered as having the presence of bronchial dilatation or air trapping respectively, irrespective of the severity of the abnormality.*

#### 5.3.4.4 Intra-observer agreement of scores over time

**Figure 5-vii** presents the scores allocated by the two scorers for the subset of 22 scans that were rescored 8 months after the initial LCFC scoring round to assess intra-observer agreement. Intra-observer agreement after ~8 months was only fair for bronchial dilatation [Scorer A: Kappa=0.24 (−0.13; 0.60); B=0.35 (−0.06; 0.76)] but strong for air-trapping [A:Kappa=0.72 (0.59; 0.85); B:Kappa=0.72 (0.55; 0.88)]. For total CT score, scorer A showed strong agreement while scorer B showed moderate agreement [A:Kappa=0.66(0.42;0.90); B:Kappa=0.51(0.29;0.73)]. Both scorers detected an identical proportion of changes when re-scoring but those identified were not necessarily from the same infants.

**Figure 5-vii: Intra-observer agreement for scorers A and B when rescoring bronchial dilatation, air trapping and total score after an interval of 8 months**




***Legend:*** *Scores allocated by scorer A represented as blue circles and by scorer B represented as red circles. Bolder circles represent overlapping results with the number of overlapping data next to it. κ= Kappa coefficient (95% CI): fair intra-observer agreement for bronchial dilatation and total scores (panels a & b and e & f) and strong intra-observer agreement for air trapping (panels c & d). Although similar percentages of changes were detected on both occasions, the observers did not necessarily detect changes in the same infants during the two separate rounds.*

### 5.3.5 Relationship between ventilatory pattern during anaesthesia and CT changes

As described in **chapter 4** (**section 4.4**), the $NICO_2$® respiratory monitor was used to objectively monitor the ventilation provided during acquisition of CT scans under GA. Among the 37 (58%) infants thus assessed, ventilatory pattern was similar across the three centres, with the exception of a slightly higher PEEP in Centre B (**chapter 4, Table 4-iv**). There was no apparent relationship between pattern of ventilation, including applied PEEP, and any of the CT sub-scores or total score, irrespective of scorer. Similarly there was no significant difference in any of the CT outcomes between those with or without objective monitoring. There were also no apparent differences seen in the scoring results between scans that were performed with the original protocol of lower recruitment pressures and the amended protocol using higher pressures. Air trapping sub-scores allocated were no different between scans that had the limited 3 expiratory images and those that had volumetric expiratory images obtained.

### 5.3.6 Summary

No apparent reasons were identified for the variation in scores allocated by each scorer during the two training rounds. It did not appear to be related to the severity of changes observed as both batches showed similar total CT scores and sub-scores (**Figure 5-iv** and **Table 5-iii**) although the changes detected in the training batches were mild which may account for fair to moderate agreement only. The improved agreement with respect to bronchial dilatation during the second training batch may, however, be related to the fact that the two scorers had been through the observed discrepancies after completing training batch 1 and refined their definition of the various abnormalities.

Despite prior training and discussion on training scans with subsequent reasonable agreement, when it came to scoring the study CTs of NBS CF infants, agreement was only fair for bronchial dilatation, which did not improve when a random selection of the original study cohort was re-scored 8 months later. Although good reproducibility of air trapping sub-scores was seen between and within scorers, it is important to note that despite being present during early CF lung disease,[108,109] it's

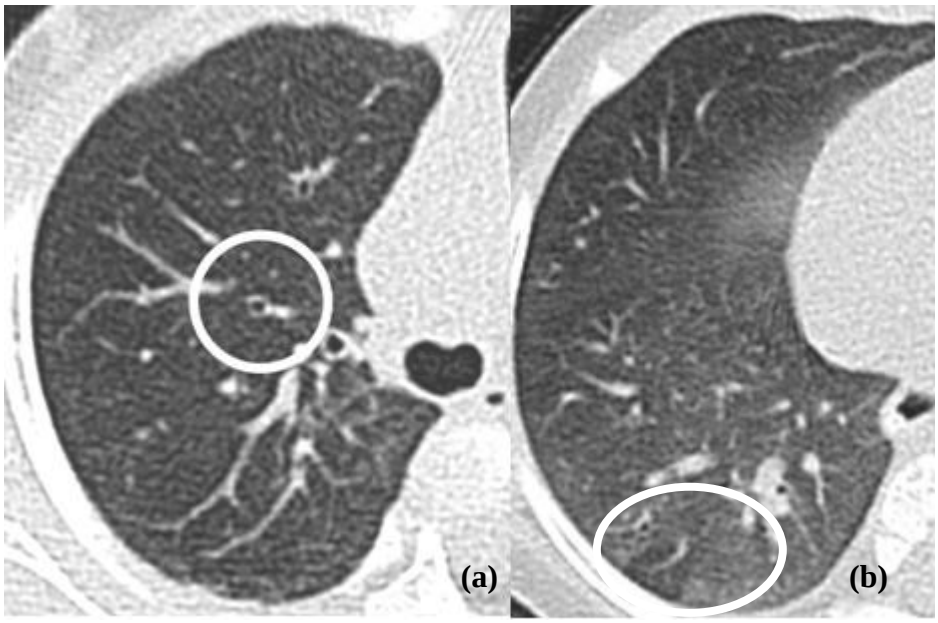
longer term clinical significance is unknown since air trapping *per se* is neither a structural abnormality nor an irreversible change seen on CT.[108] As for total CT score, this was also only fair in agreement, although this did improve slightly with rescoring. Agreement between the two scorers was better when deciding if bronchial dilatation or air trapping was present or absent as a binary outcome as opposed to the individual CT scores.

A possible explanation for the apparently better agreement seen during the scoring of training scans when compared with study scans may be related to the difference in subject's age and severity of scans between the training and study scans, this being greater in the training scans than in the study population (**Figure 5-iv** and **Table 5-iii**). Within the training scans, bronchial dilatation was detected in 50-67% of scans with higher degree of severity whilst air trapping was detected in 50-75% of scans. With the LCFC scans, very few abnormalities were detected and when they were present, these changes were so mild that they could be interpreted as either normal (thus scoring zero) or mild (gaining a score of 1) within and between observers sessions (**Figure 5-vi**). The total percentage with any 'bronchial dilatation score was only 9-26 % depending on who scored the LCFC scans. The better agreement for air trapping score during both training and LCFC study scans probably relates to the higher proportion of scans showing this abnormality in both sets of data, with air trapping being present in 26-42% of the initial LCFC scoring round. The increased agreement may also reflect the way in which air trapping is scored when using the Brody-II scoring system, whereby a score for either presence or absence of a change is allocated in a binary fashion, without the need to measure the size of change to be allocated a score. Since time constraints precluded rescoring all scans from the entire cohort, a subset of 22 LCFC scans were randomly selected. This rescoring exercise demonstrated that, as for the between-observer comparisons performed on the same occasion, inter and intra observer agreements of the CT scores over time were only fair.

Previous literature[160,215] has demonstrated the importance of performing controlled ventilation chest CT for standardisation of scans obtained and comparison of bronchial size in relation to its accompanying vessel. In this study, variations in CT scores are unlikely to have been influenced by variations in ventilatory support as the

adherence to protocolised pressures was generally achieved in the three centres, especially once procedures were monitored objectively. While findings need to be interpreted cautiously due to the limited sample size and large within-centre variability, there was no significant between-centre differences in either CT scores or pattern of support provided. The challenges faced, even by those with considerable expertise in the field, in discriminating very mild changes that could be attributed to bronchial dilation or air trapping from normal is illustrated in **Figure 5-viii**.

**Figure 5-viii: Examples of CT images from CF infants showing mild abnormalities in bronchial dilatation and air trapping leading to discrepancy in scoring**




***Legend:*** *(a) An example of thin section CT of the right lung in an infant with CF taken at 1 year of age showing discrepancies in scoring bronchial dilation (circled). This was scored as normal by scorer A, but mild by scorer B during the initial study round, whereas during the subsequent re-scoring round ~ 8 months later, scorer A scored this as mild bronchial dilatation, while scorer B scored as normal.*

*(b) Subtle tiny areas of hyperlucency in some of the scattered secondary pulmonary lobules of the lower lobes in keeping with air trapping (ringed by oval). During the initial scoring round, scorer A scored this as mild air trapping while scorer B labelled it as no air trapping. During the rescoring round, both scorers allocated mild air trapping.*

With regards to the use of CT as an outcome measure involving CF infants in a multicentre study, there are definite challenges to overcome in order to standardise the different procedures involved. Nevertheless, with close supervision and objective monitoring, adherence to protocols was good and the minor variations which did arise in the study did not appear to be of clinical significance. Although bronchial dilatation and air trapping were reported in between 9-26% and 26-42% infants respectively in this study according to either observer, even when these changes were detected, they were very mild. Hence I have disproved the secondary hypothesis by demonstrating that most NBS CF infants do not have significantly abnormal chest CT at 1 year of age.

As shown in this chapter, only air trapping sub-score and total CT scores demonstrated reasonable reproducibility between both scorers. These scores will be compared with lung function results at a year of age to establish if any relationship existed between chest CT changes and lung function. Despite poor or at best fair agreement between scorers for bronchial dilatation in this age group, comparison to lung function will also be explored as bronchial dilatation in previous studies were considered to be an established structural abnormality which might reveal better correlation with lung function compared to air trapping which strictly speaking is not a structural abnormality. The relationship between lung function and CT changes will be explored in the next chapter.

# 6 THE RELATIONSHIP BETWEEN CHEST CT CHANGES AND LUNG FUNCTION IN NBS CF INFANTS AT A YEAR OF AGE: RESULTS

## 6.1 INTRODUCTION

Clinical characteristics of the 65 CF infants (48%) boys who had chest CT (**Table 6-i**) were similar to those of the entire group of 72 CF infants who had paired lung function measurements at 3m and 1yr as described in chapter 3 (**Table 3-iii**).

Median age of diagnosis was 3.4 weeks although one infant was not diagnosed until 3m of age due to an ambiguous initial neonatal screening result, and in whom a CF genotype was only confirmed later. This infant was included in the flowchart in chapter 5 (**Figure 5-ii**) as one of the infants who only had CT and 1-year lung function.

### 6.1.1 Clinical characteristics of CF infants who had chest CT

Chest CT scans were performed at a median age of 52 (range: 43-64) weeks. There were no significant differences in anthropometry or lung function at a year of age between those who did or did not have a chest CT except for age at 1 year lung function testing whereby lung function was assessed, on average, a month earlier in those who had CT compared to those who did not have a CT (With CT vs no CT: mean difference (95% CI): −4.3 (−7.9; −0.7) weeks; p=0.02) (**Table 6-i**). This was purely due to logistical arrangements in trying to ensure that lung function was performed before bronchoscopy which took place generally at 11-12 months of age. Hence lung function appointments were prioritised for those who were having CT and bronchoscopy in precedence over those who were not having these procedures. CF infants not having CT and bronchoscopy were then scheduled for lung function slightly later. Apart from their slightly younger age, there were no significant differences in anthropometry or lung function between those who did and did not have a CT.

**Table 6-i: Anthropometry and Lung Function in ~ 1 year NBS CF infants *with or without* chest CT**

| Infants with CF | With CT (n=65) | Without CT (n=9) | Difference (95% CI): *With and without CT* |
|---|---|---|---|
| ***Age at lung function test (weeks)*** | 51.8 (4.7) | 56.1 (7.8) | -4.3 (-7.9; -0.7)* |
| ***Somatic growth*** | | | |
| Weight, z-score | 0.34 (0.90) | 0.20 (0.84) | 0.13 (-0.50; 0.76) |
| Length, z-score | 0.49 (0.97) | 0.37 (1.29) | 0.12 (-0.60; 0.83) |
| Body mass index, z-score | 0.09 (0.84) | 0.00 (0.66) | 0.09 (-0.49; 0.67) |
| ***Ventilation inhomogeneity*** | | | |
| LCI, z-score | 1.08 (1.31) [a] | 1.19 (1.48) | -0.11 (-1.05; 0.84) |
| ***Lung Volumes*** | | | |
| $FRC_{pleth}$, z-score | 0.87 (1.25) [b] | 0.51 (1.07) | 0.36 (-0.51; 1.24) |
| $FRC_{MBW}$, z-score | 0.25 (0.25) [c] | 0.17 (0.77) | 0.08 (-0.53; 0.68) |
| Trapped gas, z-score [#] | 1.23 (0.89) | 1.30 (0.86) | -0.07 (-0.70; 0.56) |
| ***Forced Expiratory Volume and Flow*** | | | |
| $FEV_{0.5}$, z-score | -0.40 (1.07)[d] | -0.36 (0.65) | -0.04 (-0.77; 0.69) |
| FVC, z-score | -0.52 (1.14) | 0.17 (1.15) | -0.69 (-1.51; 0.12) |
| $FEF_{75}$, z-score | -0.07 (1.00)[d] | -0.00 (0.44) | -0.07 (-0.75; 0.61) |

***Footnote:*** *Data shown as mean (SD).*p<0.05. [a]Successful LCI, n=64; [b]Successful $FRC_{pleth}$, n=63; [c]Successful $FRC_{MBW}$, [d]$FEV_{0.5}$, FVC and $FEF_{75}$, n=62; # Trapped gas: estimated from z-$FRC_{pleth}$ − z-$FRC_{MBW}$.*
*There was no difference in lung function or anthropometry between infants who had chest CT and those who did not. Hence the chest CT results should be representative of the whole cohort and without any significant bias related to those without chest CT.*

By the time of the 1 year assessment in CF infants, a third had physician-diagnosed wheeze, ~10% had evidence of chest crackles and all infants had experienced a cough. Only 17% had intermittent cough within 3 weeks of the 1yr ILFT whilst the remaining infants tested when well. With regards to additional treatment received within the first year, ~11% had had a trial of nebulised DNAse and 32% had had at least one course of IV antibiotics for respiratory exacerbations (**Table 6-ii**).

**Table 6-ii: Clinical characteristics of CF infants who had 1- year lung function and chest CT**

| | |
|---|---|
| Postnatal age at diagnosis: (weeks) | 3.4 (3.0;4.6)[a]. |
| CFTR genotype classes I-III | 54 (83%) |
| Presented with meconium ileus | 7 (11%) |
| Pancreatic sufficient | 4 (6%) |
| ***Prior to 1 year assessments*** | |
| **Respiratory symptoms, ever:** | |
| Wheeze, physician diagnosed | 22 (34%) |
| Crackles, physician diagnosed | 7 (11%) |
| Cough within 3 weeks of 1 year lung function | 11 (17%) |
| **Bacterial growth on cough swab ± BAL, ever[b]** | |
| *Pseudomonas aeruginosa*[c] | 21 (32%) |
| Other significant bacterial growth[d] | 18 (28%) |
| No growth[e] | 26 (40%) |
| **Additional treatment received** | |
| rhDNase | 7 (11%) |
| Intravenous antibiotics, number of courses | 0 (0; 3) [f] |
| Gastro-Oesophageal reflux treatment | 35 (54%) |

***Footnote:*** *CF infants with 1 year lung function and chest CT, n=65. Results expressed as mean (SD) or n (%) unless otherwise stated.* [a] *median (interquartile range),* [b] *based on the presence of bacteria ever isolated in the first year.* [c] *definition of colonisation according to Lee et al[194]; only 2/65 (3%) infants had any evidence of PsA on BAL or cough swab within 5 days of the CT scan.* [d] *Significant bacterial growth consisted of those who had Methicillin Sensitive or Methicillin Resistant Staphylococcus aureus (MSSA or MRSA respectively), Haemophilus influenza (HI), Stenotrophomonas maltophilia, Acromobacter xylosidans, or Aspergillus fumigatus with no previous Pseudomonas aeruginosa growth.* [e] *No bacterial growth consisted of those with isolation of coliforms and upper respiratory tract flora only.* [f] *median (range).*

*This sub group of the CF cohort who had chest CTs was similar in clinical characteristics to the whole CF cohort in this study.*

### 6.1.2 Microbiology from cough swabs and BAL

Among the NBS CF infants who had chest CT, 32% (21/65 infants) isolated *PsA* from cough swabs on at least one occasion during the first year of life. None of the infants had chronic *PsA* infection in the first year of life as defined by the Leeds criteria[194]

Significant bacterial growth on cough swabs within the first year was defined as those who had *MSSA* or *MRSA*, *HI*, *Stenotrophomonas maltophilia*, *Acromobacter xylosidans*, or *Aspergillus fumigatus* with no previous *PsA* growth. No infant had *Burkholderia cepacia*. Twenty-eight percent (18/65 infants) had at least one of these 'significant' bacterial infections which were treated with a course of antibiotics. No significant bacterial growth apart from coliforms and upper respiratory tract flora were isolated in 40% (26/65) of infants (**Table 6-ii**).

This group of NBS CF infants also underwent flexible bronchoscopy and BAL at a year of age. In their 1yr BAL samples, 6/65 (9%) isolated *SA* of which two were new cases of *SA*, not previously detected in any cough swabs within the first year. Three (3/65; 5%) isolated *HI*, whilst only 3/65 (5%) isolated *PsA* of which one was the first isolation whilst the other two had this isolated on previous cough swabs.

Among the NBS CF infants who underwent BAL, 39/65 also had cough swabs performed just prior to the BAL (**Table 6-iii**). Based on this subset and concentrating on the 3 main common CF pathogens (*PA, SA and HI*), only 3/39 (7%) of the cough swabs had positive growth whereas with BAL, 6/39 (14%) isolated positive bacterial growth. Of the three infants who had positive bacterial growth on cough swabs, there was one infant with *PsA*, one with *SA* and another with *HI*. BAL detected one with *PsA* and *SA* growth, while another infant only isolated *SA*. Four infants isolated only *HI* in BAL. All the positive BAL resulted in a new course of antibiotics being prescribed for the infants in accordance with the standardised treatment protocol (**Appendix A7**).

**Table 6-iii: Comparison of bacterial growth isolated in 1 year BAL and prior cough swabs in a cohort of 39 NBS CF infants**

| Number of infants with the bacterial growth | Cough swabs | BAL |
|---|---|---|
| ***Pseudomonas aeruginosa (PsA)*** | 1 | 1 |
| ***Staphylococcus aureus (SA)*** | 1 | 2 |
| ***Haemophilus influenza (HI)*** | 1 | 4 |

***Footnote:*** *More infants with significant bacteria were detected on lavage fluid compared to cough swab obtained just prior to BAL. Even when both cough swabs and BAL had positive bacterial growth, they did not necessarily detect the same organisms; swabs and BAL showed poor concordance.*

Even when a cough swab taken at the time of the bronchoscopy had positive bacterial growth, it did not necessarily reflect the same organism in BAL.

For ***PsA*****,** no concordance was observed (**Table 6-iii**); one infant isolated both *PsA* and *SA* on BAL but not on cough swab although this particular infant had isolated *SA* but not *PsA* previously in other surveillance cough swabs within the first year of life which was treated with oral antibiotics. The other infant isolated *PsA* on cough swab but not BAL which is somewhat surprising. In this case, the infant had isolated *PsA* previously and was already on nebulised colomycin at the time of bronchoscopy. It is possible that this infant's positive cough swab culture was reflective of oropharyngeal/ upper airway and not lower airway infection or it may be due to regional sampling during the BAL process which may have missed organisms in lobes of the lungs that were not lavaged.[216,217]

For ***SA***, concordance was 50%. The case detected by BAL but not cough swab immediately prior to the bronchoscopy had isolated *SA* on previous routine surveillance cough swab within the first year of life which was treated. Despite that, at 1yr of age, *SA* was isolated on BAL. The other child in whom *SA* was detected in both BAL and cough swab was an infant who had previously had *SA* and *PsA* on routine surveillance swabs and had already received treatment. Although *PsA* was not isolated on BAL, *SA* was still detected. It was not possible to determine if this was the same strain as previous *SA* in routine surveillance swabs as there was no further bacterial typing analysis.

For ***HI,*** concordance was 25%. There were three cases detected on BAL and not cough swabs that were completely new isolates that were not previously detected in surveillance cough swabs. One case detected on cough swab at bronchoscopy and BAL had isolated *HI* within the first year on routine surveillance swabs and was previously treated with co-amoxiclav.

Therefore, although the number of infants with positive bacterial growth in the BAL samples in this study was too small to allow any meaningful interpretation or any further statistical analysis, it echoes other studies in the literature showing poor concordance between cough swabs and BAL.[218,219] Taking into account not just cough swabs taken at the time of bronchoscopy but all swabs obtained in the first year of life from this subset of 39 infants, the result of BAL at 1 year changed the bacterial status in 4/39 (10%) infants (1 had *PsA* and never before in cough swabs whilst 3 had *HI* on BAL and never before in any swabs).

## 6.2 ASSOCIATION BETWEEN CT CHANGES AT 1 YEAR AND CLINICAL FEATURES

In the previous chapter on scoring results, inter-observer agreement was poor for many of the sub-scores, thereby shedding considerable doubt on their reliability. But for the purposes of comparisons with the literature in which comparisons have been made on basis of one observer only, as well as the fact that despite the poor agreement, one scorer's set of CT scores may demonstrate closer relationship than the other. Hence, CF score results were analysed for each scorer independently. Despite this, minimal associations with clinical features were found for both scorers, and even when these were found, the lack of intra and inter-observer agreement shed much doubt on the reliability of CT scoring in young infants.

Based on scorer A's allocated scores for CT changes, there were no significant associations between the presence of bronchial dilatation, air trapping, bronchial wall thickening or mucous plugging with any clinical features including microbiological status, presence of clinical symptoms within the first year or additional medication use ( IV antibiotics, rhDNase or gastro-oesophageal reflux treatment). Significant

associations did however exist between *PsA* infection ever during the first year of life [Logistic regression coefficient: Odds Ratio, OR= 4.64(1.51;14.26) p=0.01] and the use of IV antibiotics [OR=2.27(1.12;4.57) p=0.02] with the presence of parenchymal changes sub-score.

Similarly, when based on scorer B's allocated CT scores, there were no significant associations between the presence of bronchial dilatation, air trapping, bronchial wall thickening and parenchymal changes with any the of clinical features stated above. A history of IV antibiotics use was significantly associated with the presence of mucous plugging [OR=3.69(1.18;11.55) p=0.03]. In summary, irrespective of whose scores were used, there were minimal associations between changes observed on CT and the child's previous clinical history.

## 6.3 RELATIONSHIP BETWEEN CT CHANGES AND LUNG FUNCTION IN NBS CF INFANTS AT A YEAR OF AGE

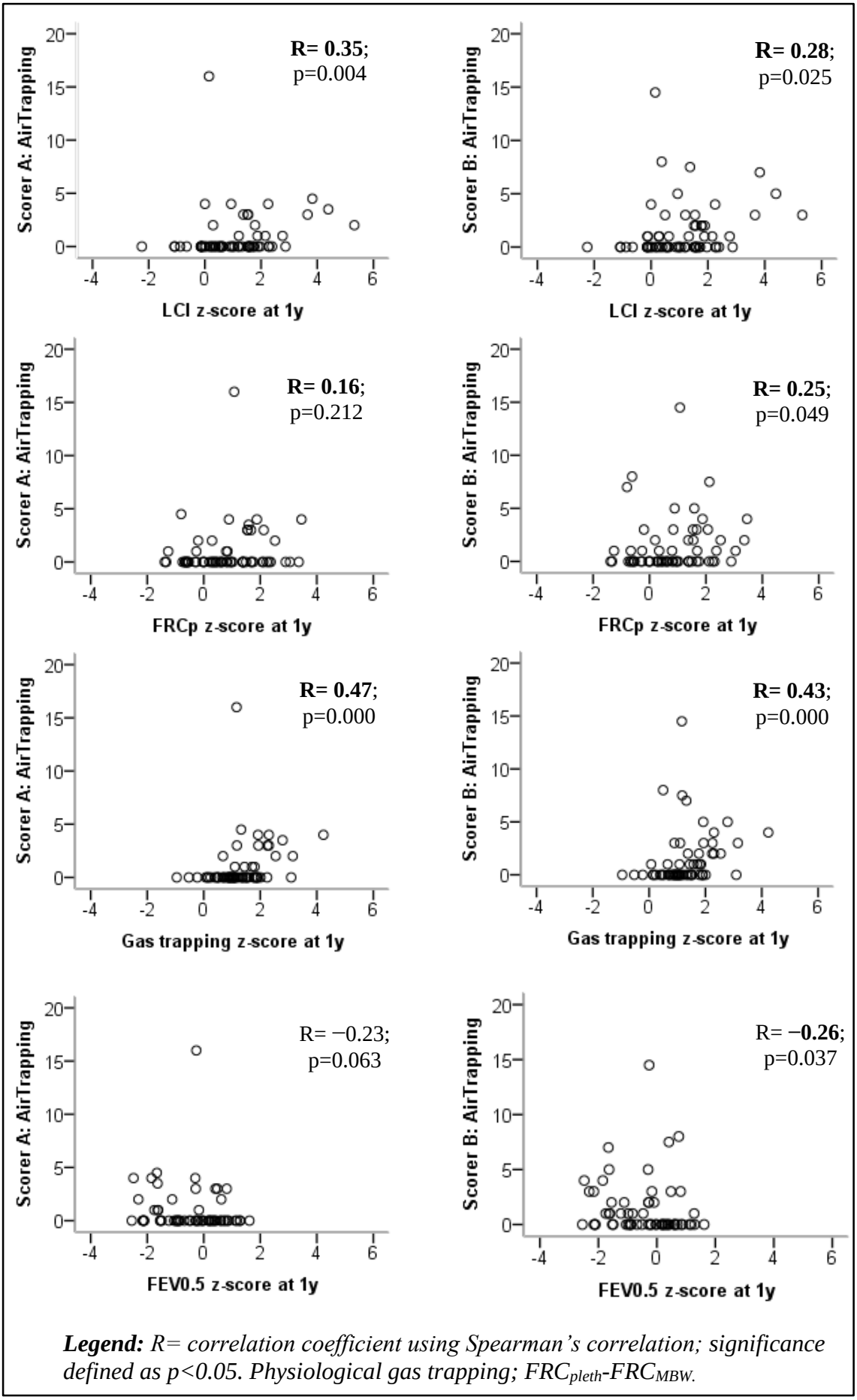
Of the 65 infants in whom CT scans were performed, lung function data at 1 year were completed for 64(98%)LCI, 63(97%)$FRC_{pleth}$ and 62(95%)$FEV_{0.5}$. The original aim of the study had been to investigate determinants of both total CT score and all the individual sub-scores as well as investigating associations between each CT outcome and selected measures of lung function. However, given the complete lack of reliability for many of these CT outcomes with poor agreement within and between observers (**section 5.3.4**), results in the following section have been limited to the relationship between the various lung function outcomes and air trapping sub-score, total CT score and a binary assessment of whether the specialist observers felt that the CT was normal or abnormal based on the presence of any bronchial dilatation, air trapping sub-score >6/27 or total CT score ≥12/243 (≥5% of the total score).[64,107] Despite its poor repeatability, the association between bronchial dilation on CT and lung function is also presented to allow comparison with previous literature in which this is a frequently reported outcome. In order to ensure the most comprehensive investigation, these analyses were undertaken for *each* scorer and also with respect to abnormalities detected by EITHER scorer.

Correlation analysis is shown in **Figure 6.i, Figure 6.ii** and **Figure 6-iii**. Lung function parameters such as LCI, $FRC_{pleth}$ and physiological gas trapping and $FEF_{75}$ may indicate more peripheral or distal airway disease and might demonstrate an association with CT air trapping sub-score. In this study, some relationship was found for CT air trapping sub-score with LCI, $FRC_{pleth}$ and physiological gas trapping but not $FEF_{75.}$ More proximal airway disease such as bronchial dilatation or bronchial wall thickening on CT may demonstrate some relationship with $FEV_{0.5}$ but in this study this was not the case.

### 6.3.1 Association between CT and lung function results

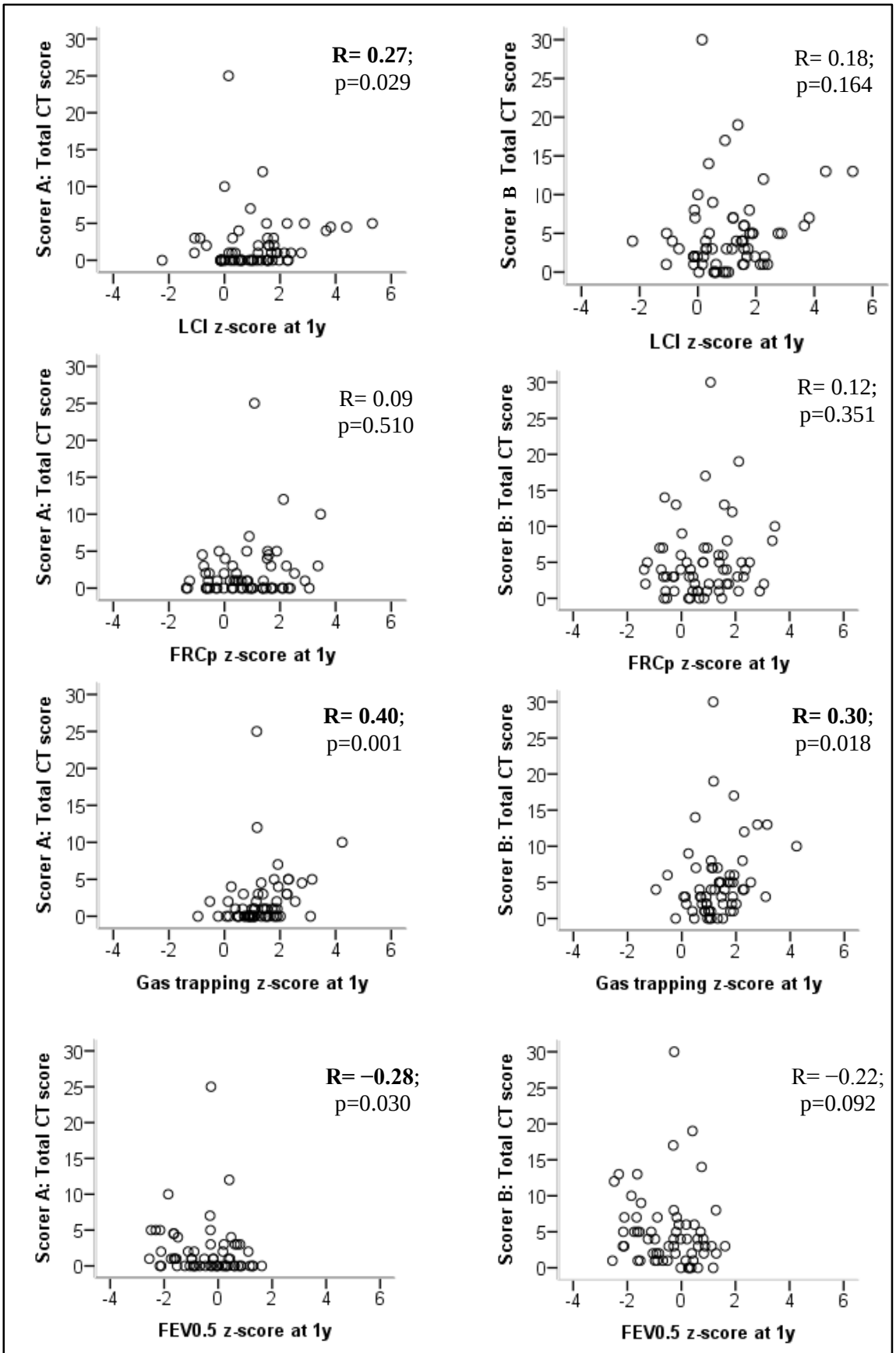
While a significant association did exist between both the CT air trapping (**Figure 6-i)** and total CT score (**Figure 6-ii**) allocated by either scorer with several of the lung function outcomes, particularly LCI and physiological 'trapped gas' ($FRC_{pleth}$-$FRC_{MBW}$), these associations were generally weak and of minimal predictive value in individual infants. The correlation coefficients were re-calculated after excluding the outlier who had an air-trapping score of ~15 and a total CT score of >25 (see **Figure 6-i** and **Figure 6-ii**) by both scorers in the presence of lung function outcomes that all fell within the normal range but this had minimal effect on results. No apparent reasons in terms of clinical characteristics were accountable for the extremely high air trapping and total CT scores in this particular infant apart from the fact that there was a longer than usual interval (4 weeks delay) between the performance of 1yr ILFT and chest CT due to hospital logistical issues. This infant was reported as being clinically well during this interval. This was the only case whereby 1yr lung function was not repeated before the chest CT when there was an interval time of more than 3 weeks. There was however no association between bronchial dilatation and any of the selected lung function outcomes (**Figure 6-iii**).

**Figure 6-i: Association between lung function and air trapping sub-score according to each scorer**



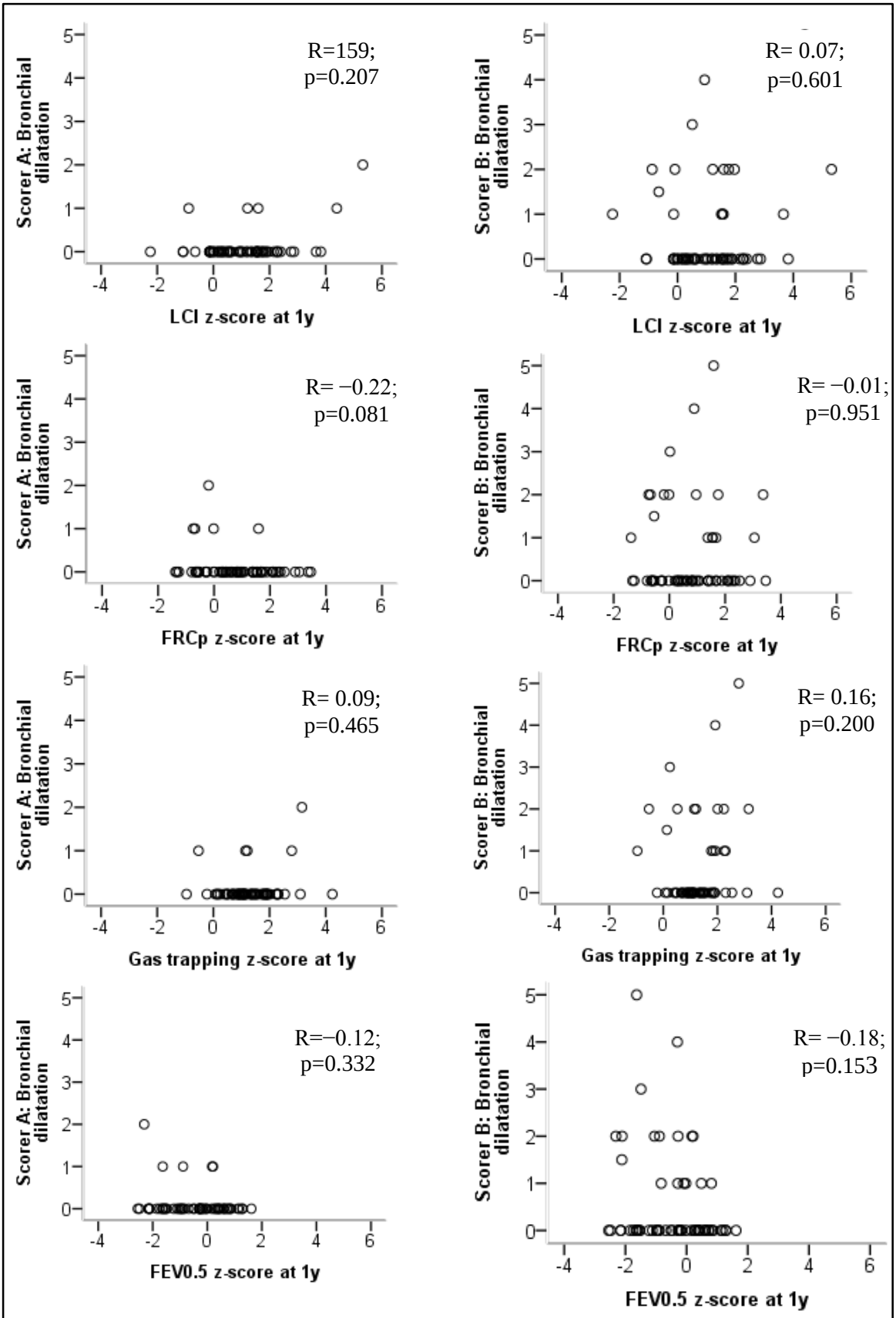

***Legend:*** *R= correlation coefficient using Spearman's correlation; significance defined as p<0.05. Physiological gas trapping; $FRC_{pleth}$-$FRC_{MBW}$.*

**Figure 6-ii: Association between lung function and total CT scores according to each scorer**




***Legend:*** *Correlation coefficient using Spearman's correlation; significance defined as p<0.05; Physiological gas trapping; $FRC_{pleth}$-$FRC_{MBW}$.*

**Figure 6-iii: Association between lung function and bronchial dilatation according to each scorer**




***Legend:*** *R= correlation coefficient using Spearman's correlation; significance defined as p<0.05; Physiological gas trapping; $FRC_{pleth}$-$FRC_{MBW}$.*

### 6.3.2 Regression analysis of CT scores and lung function

In addition to assessing the correlation between 1yr lung function and structural changes on CT (**Figure 6-i**, **Figure 6-ii** and **Figure 6-iii**), logistic regression was used to measure the size of associations between the presence or absence of bronchial dilatation or air trapping with 1yr lung function data and any potential clinical determinants. The reproducible dependent variables of interest for relating structural changes to lung function were air trapping sub-score and total CT scores at a year. These CT scores were non-parametrically distributed and were analysed as binary outcome measures (i.e. presence or absence denoted by a change seen on CT with any scan of an allocated score >0 contributing towards the category with abnormal scan).

When comparing CT and lung function results, multiple imputations was used to predict values for any missing clinical or lung function data, using all known covariates thought to be associated with 1yr CT scores. This was calculated separately for the two different scorers. The observed covariates considered were maternal and parental smoking, somatic growth between 3 months to 1 year, microbiology results (*PsA*, significant bacterial growth ever and no growth/ non-significant bacterial growth ever), respiratory signs (wheeze, crackles and cough) and history of treatment with rhDNase, anti-gastro-oesophageal reflux disease medication and IV antibiotics for respiratory symptoms; genetic mutation, presence of meconium ileus and/or pancreatic insufficiency and the CT scores allocated by each scorer. Twenty imputations were performed using IBM SPSS Statistics v.21. The results using multiple imputations were similar to those obtained using list-wise deletion.

**Table 6-iv** shows univariable binary logistic regression analysis using multiple imputations for the presence of different CT changes as allocated by either scorer in relation to the major lung function outcomes. Significant associations between lung function and CT scores allocated by either scorer were only identified between CT air trapping sub-scores and LCI and physiological 'Trapped gas' [($FRC_{pleth}$-$FRC_{MBW}$) z-score] at 1yr:

- For each unit increase in 1yr LCI z-score, the odd ratios for air trapping on CT as identified by scorers A and B were 2.5 (1.3; 4.6); p=0.004 and 1.8 (1.1; 2.9); p=0.02 respectively.
- For each unit increase in 1 yr 'Trapped gas', the odds ratios for CT air trapping were 4.6 (1.7; 12.3); p=0.002 by scorer A and 3.3(1.4; 7.8); p=0.01 by scorer B.

No associations were observed between any CT outcome and $FEV_{0.5}$ and $FEF_{75}$ z-scores at 1yr by either scorer, which is not surprising given that most children had normal $FEV_{0.5}$ and $FEF_{75}$ by that age. In addition, no relationship was observed between $FRC_{pleth}$ and the presence of any CT changes as detected by either scorer.

On multivariable analysis of scorer A's allocated scores using each of the four 1yr lung function outcomes (i.e. z-scores for $FEV_{0.5}$, LCI and $FRC_{pleth}$ and 'Trapped gas'), the only significant association was between CT air trapping sub-score and physiological 'Trapped gas' z-score, such that for any unit increase in 'Trapped gas' z-score, the odds ratio (95% CI) for air trapping was: 4.00 (1.09; 14.68); p=0.04); similar to results obtained using univariable analysis. No significant associations were observed when undertaking the same multivariable analysis using scorer B's allocated CT scores.

In **chapter 3 section 3.5.6**, significant correlations were found between all parameters of lung function at 3m and 1yr of age in NBS CF infants. In this chapter, most lung function parameters at 1yr were not significantly associated with chest CT changes at 1yr. Only LCI and 'Trapped gas' z-scores in the first year were significantly associated with CT-air trapping sub-score. Lung function at 3m did not show any significant contributions towards chest CT scores by either scorer. Hence in contrast to lung function at 1yr, 3m lung function did not predict changes seen on chest CT at a year of age.

**Table 6-iv: Univariable logistic regression with multiple imputations: Presence or absence of CT changes allocated by scorer A and B and 1- year lung function measurements**

| Per unit increase in 1-year lung function z-score | Based on scorer | Bronchial Dilatation | Air trapping | Bronchial wall thickening | Mucous Plugging | Parenchyma change | Abnormal scan[#] | Total score* |
|---|---|---|---|---|---|---|---|---|
| LCI | A | 2.0(1.0;3.8) p=0.05 | 2.5(1.3;4.6)** | 1.1(0.5;2.6) p=0.82 | 1.0(0.4;2.4) p=0.94 | 1.1(0.8;1.7) p=0.94 | 1.6(0.7;3.7) p=0.23 | −0.1(−1.0;0.7) p=0.51 |
| | B | 1.2(0.8;1.8) p=0.45 | 1.8(1.1;2.9)* | 1.2(0.8;1.8) p=0.44 | 2.3(1.0;5.0) p=0.05 | 0.9(0.6;1.4) p=0.62 | 1.2(0.7;2.0) p=0.47 | 0.2(−1.0;1.4) p=0.72 |
| $FRC_{pleth}$ | A | 0.5(0.2;1.2) p=0.12 | 1.3(0.8; 2.0) p=0.36 | 2.0(0.7;5.6) p=0.20 | 1.4(0.5;3.6) p=0.55 | 1.6(0.8;1.8) p=0.51 | 0.7(0.3;1.8) p=0.47 | 0.1(−0.9;1.1) p=0.84 |
| | B | 1.0(0.6;1.6) p=0.94 | 1.6(1.0;2.5) p=0.06 | 0.8(0.5;1.3) p=0.35 | 1.2(0.5;3.3) p=0.67 | 1.3(0.8;2.0) p=0.36 | 1.0(0.5;1.7) p=0.47 | 0.1(−1.0;1.4) p=0.88 |
| 'Trapped gas' | A | 1.4(0.5;3.7) p=0.51 | 4.6(1.7;12.3)** | 1.4(0.4;4.7) p=0.63 | 1.1(0.3;4.1) p=0.84 | 1.4(0.7;2.5) p=0.31 | 1.0(0.2;4.4) p=1.00 | 1.0(−0.5;2.6) p=0.18 |
| | B | 1.2(0.7;2.3) p=0.52 | 3.3(1.4;7.8)* | 1.1(0.6;2.0) p=0.76 | 2.3(0.7;7.5) p=0.15 | 1.0(0.5;2.0) p=1.00 | 0.9(0.4;2.3) p=0.86 | 1.0(−1.1;3.1) p=0.37 |
| $FEV_{0.5}$ | A | 0.7(0.3;1.5) p=0.34 | 0.6 (0.4;1.0) p=0.07 | 0.7(0.3;2.1) p=0.58 | 2.4(0.6;9.5) p=0.21 | 0.8(0.5;1.2) p=0.24 | 1.1(0.5;2.5) p=0.81 | −0.3(−1.2;0.6) p=0.52 |
| | B | 0.7(0.4;1.2) p=0.25 | 0.6(0.4;1.0) p=0.05 | 0.7(0.4;1.2) p=0.24 | 0.8(0.3;2.3) p=0.70 | 0.7(0.4;1.31) p=0.28 | 0.8(0.5;1.4) p=0.49 | −0.5(−1.7;0.8) p=0.47 |

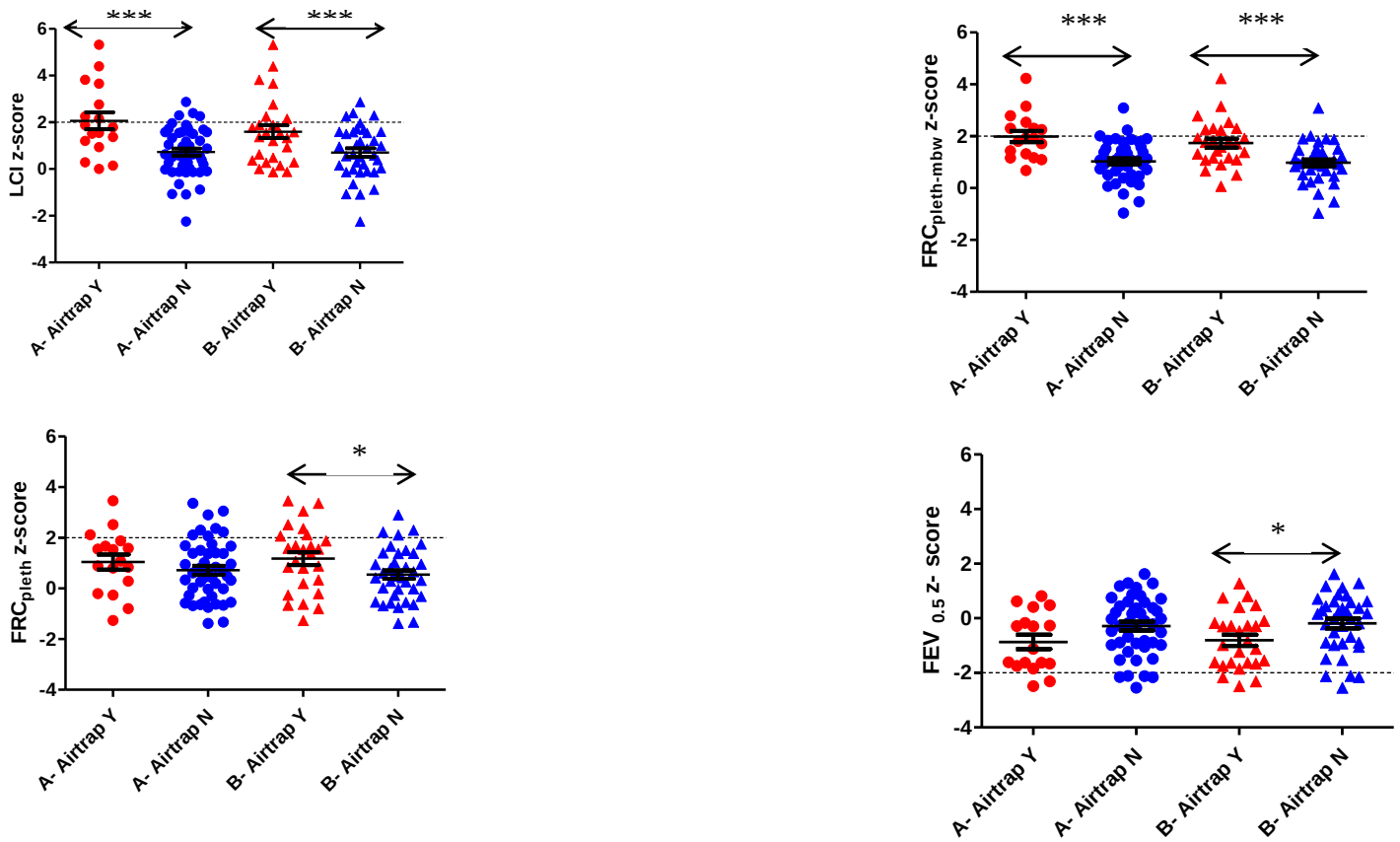
***Footnote:*** *Dependent variables are CT outcomes presented as a binary outcome ('Yes' for any score >0 and 'No' for =0) unless otherwise stated.* [#] *Abnormal scan defined as presence of any bronchial dilatation or air trapping score >6 or total score ≥12.*Total score as numerical dependent variable and linear regression used for this analysis. Independent variables are 1 yr lung function z-scores. Significant associations highlighted in red: *p<0.05; **p<0.01. Results presented as odds ratio (95% confidence interval) except for total score, where results presented as mean regression coefficient (95% confidence interval).*

### 6.3.3 Comparison of lung function between NBS CF infants with and without CT changes at a year of age

When comparing lung function at 1yr in infants with (i.e. CT-air trapping sub-score >0) and without CT evidence of air trapping, both LCI [mean difference (95% CI) = 1.33(0.66; 2.00); p<0.0001] and physiological 'Trapped gas' (ΔFRC) [0.96(0.51; 1.40); p<0.001) z-scores were significantly higher in infants with evidence of CT-air trapping when using scores allocated by scorer A. With scorer B's allocated CT-air trapping sub-score, infants with evidence of CT-air trapping also had significantly increased ventilation homogeneity [mean difference in LCI z-score = 0.89(0.26; 1.52); p=0.006) and physiological gas trapping [ΔFRC z-score mean difference=0.76(0.34; 1.18); p=0.001) but in addition, infants with CT air trapping had significant hyperinflation [$FRC_{pleth}$ z-score mean difference=0.63(0.04;1.22); p=0.04] and airway obstruction [$FEV_{0.5}$ z-score mean difference= −0.61(−1.16;−0.07); p=0.03] when compared with infants without CT- air trapping (**Figure 6-iv**).

In terms of comparing 1yr lung function with different total CT scores, infants were categorised into three groups: Group 1 (G1): zero total CT score i.e. no changes detected at all in CT, Group 2 (G2): CT score 1-10 and Group 3 (G3): CT score ≥12, i.e. >5% of the maximum Brody-II scores (**Table 6-v**). With scorer A's allocated total CT scores, significant differences were observed between infants with total scores between 1-10 (G1) and those without any changes detected (total score=0; (N)) with respect to physiological trapped gas (ΔFRC) and $FEV_{0.5}$ z-scores. There were however no significant differences in LCI and $FRC_{pleth}$ between the three subgroups. No significant differences in lung function were noted between and within the three NBS CF infant groups when using total CT scores allocated by scorer B.

**Figure 6-iv: Comparing selected 1 year lung function in infants with or without air trapping according to each scorer**




***Legend:*** *Comparing selected 1yr lung function of infants as a group; those with or without air trapping as described by either scorer. Significant increases in LCI and physiological gas trapping were observed in infants with CT air trapping compared to those without as described by scorer A and B. *** p<0.0001;* p<0.05 using unpaired t test analysis. A: Scorer A; B: Scorer B; Y: Air trapping present; N: Air trapping not present*

**Table 6-v: Comparison of lung function between infants with different total CT scores at 1 year of age by each scorer**

| | Total CT scores by scorer A | | | | | Total CT scores by scorer B | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ***>12 (G2)**** | **1-12 (G1)#** | **0 (N)†** | **p value (ANOVA)** | **Diff (95%CI): G1-N‡** | **>12 (G2)*** | **1-12 (G1)#** | **0 (N) †** | **p value (ANOVA)** | **Diff (95%CI): G2-N‡** |
| LCI, z-score‖ | *0.76 (0.87) n=2* | 1.49 (1.55) *n=31* | 0.70 (0.93) *n=31* | 0.05 | 0.79 (−0.00; 1.59) | 2.11 (2.01) *n=7* | 1.00 (1.23) *n=50* | 0.67 (0.34) *n=7* | 0.07 | 1.44 (−0.24;3.12) |
| $FRC_{pleth}$, z-score‖ | *1.60 (0.74) n=2* | 0.79 (1.23) *n=32* | 0.77 (1.17) *n=29* | 0.64 | 0.01 (−0.74; 0.76) | 0.96 (1.04) *n=7* | 0.85 (1.25) *n=49* | 0.34 (0.75) *n=7* | 0.54 | 0.62 (−0.95;2.19) |
| Trapped Gas, z-score‖ | *1.17 (0.01) n=2* | 1.58 (0.95) *n=31* | n=1.00 (0.76) *n=29* | **0.04** | 0.57 (0.03; 1.12) p=0.037 | 1.86 (0.96) *n=7* | 1.27 (0.89) *n=48* | 0.87 (0.59) *n=7* | 0.11 | 0.98 (−0.17; 2.13) |
| $FEV_{0.5}$, z-score | *0.07 (0.48) n=2* | −0.80 (1.09) *n=32* | −0.09 (1.01) *n=28* | **0.03** | −0.71(−1.37; −0.05) p=0.032 | −0.83 (1.30) *n=7* | −0.50(1.07) *n=49* | 0.44(0.41) *n=6* | 0.08 | −1.28 (−0.27; 0.17) |

***Footnote:*** *CI=confidence interval of the difference; Trapped gas is the difference in $FRC_{pleth}-FRC_{mbw,}$ a measure of physiological gas trapping. * based on those with total score ≥12 (>5% of the maximum Brody II scores) (G2); # based on those with total score 1-12 (G1); † based on those with no changes detected i.e. total score=0 (N). ‡ Based on post-hoc Bonferroni adjustment for multiple t tests between and within groups. Assessments of significant differences using ANOVA was limited to comparisons between infants with total scores between 1-12(G1) and those without any changes detected (total score=0 (N) when using scores allocated by scorer A due to the extremely low number of infants (n=2) with total score >12 . There were no significant differences in LCI and $FRC_{pleth}$ between the subgroups. For total scores allocated by scorer B, no significant difference was noted in any 1 yr lung function parameters between and within the three NBS CF infant groups.*

## 6.4 SUMMARY OF THE RELATIONSHIP BETWEEN CT CHANGES AND LUNG FUNCTION

When using scores allocated by scorer A, a unit increase in LCI z-score at a year of age increased the chance of observing bronchial dilatation by 90% (i.e. odds ratio 1.9). This relationship was not significant when using scores allocated by scorer B. On the other hand, both LCI and physiological 'gas trapping' at a year had significant associations with the presence of air trapping on CT, with a unit increase in LCI z-score increasing the odds of observing air trapping by 1.8-2.5 times according to scorer. In addition, with a unit increase in physiological 'gas trapping' z-score i.e. (ΔFRC), the odds of detecting air trapping was increased by 3.5-4.5 times. No significant associations were observed for outcomes derived from the RVRTC and the presence of CT changes by either scorer except for scorer B where a unit increase in $FEV_{0.5}$ z-score reduced the chance of seeing air trapping on CT by 40%.

The use of regression rather than simple correlation analysis allowed the strength and size of associations to be determined with confidence intervals around the mean estimate whereas with correlation analysis, only a linear relationship would have been explored with no measurement of size of impact. In addition, the data were non-parametrically distributed with small sample sizes in the modelling process. The danger of this was that even if a significant linear relationship existed with correlation analysis, this may not necessarily indicate a clinically relevant or plausible association. By using regression analysis with appropriate display of confidence intervals, it was possible to delineate and decide whether the association was potentially 'clinically significant', rather than simply being of statistical significance as the result of a small sample or biased distribution with just a few outliers that skewed the association.

In summary, some NBS CF infants had a few changes on chest CT at a year of age though the number and severity of changes seen were low. Even when such changes were observed, Brody-II scores were poorly reproducible between and within scorers. This demonstrated the limitations of the current scoring system for quantifying mild changes in young CF infants as well as the questionable utility of

chest CT to detect and quantify structural changes in young NBS CF infants. At the time of writing this thesis, no consensus has been reached with regards to the definitive CT scoring system to use in NBS CF infants. To provide further information in this field, comparison of potential structure-function relationships have been presented separately for the two scorers. As will be discussed further in **chapter 7 (section 7.4.2**), given all the challenges in acquiring standardised chest CTs under GA in infants, the lack of robust relationship between lung function and structure and the poor inter- and intra-scorer repeatability when scoring chest CT scans, these results suggests that, in their current form, chest CTs cannot be recommended for either routine clinical use or as a trial endpoint in the first year of life in NBS CF infants.

# 7 DISCUSSION

## 7.1 INTRODUCTION AND SUMMARY OF MAIN FINDINGS

Limited information about the evolution of lung function in NBS CF infants and the need to establish suitable objective outcome measures for the early detection of CF lung disease have led to the development of the hypotheses, aims and objectives of this study. If a suitable trial endpoint was available, this could potentially enable infants to be recruited into future interventional trials.

As stated in **chapter 1, section 1.10**, the primary hypothesis of this study was that NBS CF infants would demonstrate further loss in lung function from diagnosis until a year of age hence lung function at a year of age and the change in lung function within the first year were compared between NBS CF infants and contemporaneous healthy controls. Secondary hypotheses were that significant lung disease could be detected through chest CT with significant associations observed between chest CT and lung function at a year.

A summary of the main findings in addressing these hypotheses will be presented here with further discussion in the following sections (**7.1.1** and **7.1.2**).

- Diminished lung function at 3m of age in NBS CF infants improved significantly in $FEV_{0.5}$ whilst LCI and $FRC_{pleth}$ demonstrated no further deterioration within the first year of life compared to contemporaneous healthy controls who demonstrated no change in their lung function in the first year. Impaired lung function at 1yr was predicted by lung function at 3m.

- Chest CT in this study revealed mild CF changes in terms of bronchial dilatation and air trapping. No significant association existed between bronchial dilatation and any lung function parameters, whilst both CT air trapping sub-score and total CT score were weakly associated with LCI and physiological 'trapped gas' ($\Delta FRC$: $FRC_{pleth}$-$FRC_{MBW}$). Despite prior training

on the Brody-II scoring system, both scorers showed low reproducibility in the scores allocated.

### 7.1.1 Interpretation of lung function results

In this observational study investigating the evolution of lung disease in NBS CF infants, despite the presence of abnormal lung function by 3m of age, there was significant improvement in FEVF whilst maintaining stability in other lung function parameters such as LCI and $FRC_{pleth}$ by one year of age.

There was a relatively poor correlation between results from the RVRTC and other lung function outcomes (**section 3.5.5, Figure 3-iv)** reflecting the fact that different ILFT identify different types of respiratory pathophysiology. While spirometry is the most widely used outcome in older subjects with CF and is a valuable measure of airway obstruction within the conducting (proximal) airways, it is known to be less sensitive than LCI for detection of mild lung disease in preschool children with CF.[60,79,85] However, during infancy $FEV_{0.5}$ has been shown to be a sensitive outcome in clinically diagnosed CF infants. This may be due to the relatively rapid lung emptying in infancy, such that $FEV_{0.5}$ often includes expired volume down to low lung volumes, and that in the presence of highly compliant chest wall and airways, airway narrowing and flow limitation will occur more readily than in older subjects.[83] While many NBS CF infants were also found to have a diminished $FEV_{0.5}$ at 3m age[71] by 1 year far fewer NBS infants were identified by the raised volume technique than either plethysmography or LCI. This may reflect the mild nature of lung disease at 1yr in our NBS cohort when compared with those diagnosed clinically and the decreasing sensitivity of forced expiratory manoeuvres to mild lung disease as both airway and chest-wall compliance decrease with increasing maturity.[220]

By contrast LCI, as a measure of ventilation inhomogeneity, $FRC_{pleth}$, as a measure of hyperinflation, and ΔFRC ($FRC_{pleth}$- $FRC_{MBW}$), as a measure of physiological gas trapping, are more sensitive to peripheral airway obstruction and, since they are obtained during tidal breathing, are less dependent on developmental changes in the chest wall. A further reason for the relatively poor association between spirometric

outcomes and LCI is that, at least during the early stages of CF lung disease, the latter may simply reflect mucous plugging in the more peripheral airways which would not necessarily result in abnormal spirometry. In contrast to the lack of correlation between $FEV_{0.5}$ and other lung function outcomes on either test occasion, there were significant associations between LCI, $FRC_{pleth}$ and physiological gas trapping (as measured by ΔFRC), all of which are thought to be sensitive measures of peripheral airway disease throughout childhood (**Table 3-viii**).

Recent studies on newborn CF piglets have shed further light on the pathogenesis of lung disease in CF.[221,222] Newborn CF piglets have air trapping and airflow obstruction even before the onset of airway infection, inflammation or mucus accumulation compared to non CF piglets. Smaller trachea[222] and proximal airway lumen size have been found in newborn CF piglets.[221] These developmental abnormalities may be due to *CFTR* dysfunction in chondrocytes or airway smooth muscle bundles resulting in the reduction of airway size and affecting the development of airways in utero. Therefore the authors suggested that congenital airway abnormalities might in part contribute to the early airway obstruction and air trapping demonstrated in NBS CF infants.[67,71] However the improvement observed in $FEV_{0.5}$, a reflection of proximal airway function within the first year of life in NBS CF cohort in this study, may suggest that large airway cartilage abnormalities seen in CF piglets may not be entirely relevant in humans or completely accountable for the initial abnormalities and subsequent improvement in ILFT. Within the first year as one would expect continual deterioration if abnormal lung function was due to congenital abnormalities.

The likelihood of missing evidence of early lung disease in this study was reduced by applying a wide range of tests to assess different aspects of underlying pathophysiology.[71] Had only one LFT been used in this study, abnormalities would have only been detected in ~17% of infants, but this increased to 36% by using LCI, $FEV_{0.5}$ and $FRC_{pleth}$ as the three primary outcomes. Consequently, when selecting outcome measures for intervention trials in NBS CF infants,[110] reliance should not be placed solely on the raised volume technique, since measures of LCI appear essential if mild abnormalities are to be detected. While hyperinflation and gas trapping also proved to be sensitive outcomes at 1 year, routine inclusion of these outcomes

shortly after birth may be limited both by equipment costs and increased failure rate of $FRC_{pleth}$ in young infants (**Table 3-iv**). These lung function parameters were selected in view of the fact that they were sensitive markers for detecting early lung disease.

Despite the reduction in the number of infants with abnormal $FEV_{0.5}$ between 3 months to one year during this period, the percentage of NBS CF infants with abnormal LCI and $FRC_{pleth}$ at 1yr was similar to that at 3m. These were, however, not necessarily the same infants. Thus, while impaired lung function at 1yr was predicted by lung function at 3m such that assessment of lung function at 3m could allow groups of infants most at risk of impaired lung function at a later stage to be identified, such tests would be poorly predictive for individual infants .

Clinical determinants such as a history of clinician-diagnosed wheeze (LCI), poor weight gain (LCI and $FRC_{pleth}$) and prior $FRC_{pleth}$ were also useful indicators for detecting groups of CF infants most at risk of early lung disease (**Table 3-viii)**. Such infants may be potential candidates for early interventional trials as they represent those in whom any effects of interventions are most likely to be demonstrated. Furthermore, since they represent the sub-set of NBS CF infants most likely to benefit from more intensive therapy, parental consent to and compliance with such a trial are likely to be enhanced.

Discussion on the clinical implications of these lung function results in relation to selecting outcome measures for clinical trials and the calculation of number required for trials will be discussed in detail in **section 7.4**.

### 7.1.2 Interpretation of Chest CT results

There is no consensus on which CT scoring system should be used to quantify the severity of changes, particularly in the presence of mild CF lung disease.[175] The most widely used scoring system is the modified version of the Brody score (Brody-II CT score).[172] This has been widely validated and used in school-aged children with moderate to severe CF and shown to objectively quantify CF lung disease in such

children.[107,203] However, its use in infants with very mild disease, such as may occur following diagnosis by NBS, has yet to be established.

This is the first study specifically to assess the reproducibility, and hence validity, of CT evaluation of lung disease in CF infants. Despite scoring being undertaken by experienced observers with prior training, with the exception of air trapping, the Brody-II score was not reproducible in this age range. The most obvious interpretation of these findings is that the mild nature of any CT changes at 1yr of age precluded reproducible evaluation of most parameters.

Very few abnormalities were found in this group of CF infants and when present were mild in severity. Seventy five percent (50/65) of the scans showed inconsistent agreement of the CT scores between both scorers in terms of the abnormalities found. Although bronchial dilatation is the best validated and most reproducible score in children in other studies,[172] this was not demonstrated in this age group of NBS CF infants. The only reproducible score between scorers on any one occasion or within scorers across time was that relating to CT air trapping which in studies involving older NBS CF infants and young children was present relatively frequently.[108,109,111,223] In the AREST-CF study, although a significant proportion (~88%) of infants demonstrated air trapping on chest CT, these changes were not consistently present in the first few years of life.[108] Therefore, air trapping as a CT feature of early lung disease may not necessarily represent or predict irreversible structural lung changes. During infancy, it also remains unclear how these changes are related to functional abnormalities.

Compared to studies in older subjects[64,107,117,154] which have reported good correlations between CT changes and lung function results, especially LCI, this study on 1yr old NBS CF infants did not show a close relationship between lung function and structure. This may be due to the mild structural disease seen in these asymptomatic CF infants who were diagnosed early and rapidly commenced on CF therapy. Such mild changes did not necessarily result in functional decline, and may in fact be reversible. In addition, infants in this age group were tested with sensitive methods of lung function that could differentiate and detect subtle changes in lung

disease which may not be revealed on CT scans, resulting in the discordant, or lack of, relationship between lung function and structure observed in this study.

As for clinical determinants of lung structure, there were no indicators to predict the presence of bronchial dilatation or air trapping. This is in contrast to other studies which have reported increased incidence of bronchial dilatation with *PsA* infection and wheeze with presence of air trapping on CT scan.[109,111] The only associations found were between a) use of IV antibiotics and either parenchymal changes detected by scorer A or mucous plugging by scorer B; and b) a history of *PsA* infection within the first year with the parenchymal changes detected by scorer A . However parenchymal change and mucous plugging were not reproducible CT sub-scores as shown in Table **5-iv.** Thus irrespective of whose scores were used, there were minimal associations between changes observed on CT and the child's previous clinical history. This lack of association likely reflects the very mild nature of CT changes observed in these NBS CF infants and the fact that CT represents a single snap-shot of the child's disease when clinically stable. In general, this cohort of NBS CF infants were clinically well with significant catch-up growth profile.

In contrast to 1-year lung function outcomes whereby significant associations were noted with clinical determinants such as *PsA* infection, weight gain between 3 months and birth, presence of wheeze, cough or gastro-oesophageal reflex disease ever and the use of IV antibiotics, such clinical determinants were not associated with any reproducible CT scores. Hence neither lung function at 1 year of age nor clinical determinants were predictive of any CT outcomes.

An informal survey conducted to establish whether CT findings resulted in any change in management among the clinicians responsible for the care of these NBS CF infants confirmed that very few management plans were instituted based purely on the results of the scans. Amongst scans with the most changes seen, only 1 infant had a change in treatment while 2 had additional investigations looking for gastro-oesophageal reflux. Of the 65 infants in whom scans were conducted for this study, only 3 (4.6%) infants had a change in management plans following reporting of CT results.

A label of bronchial dilatation in the presence of very mild lung disease should therefore be applied cautiously, at least using current methods and definitions. In addition to all the challenges in performing GA and the lack of relationship observed between lung function and structure, the poor inter and intra-subject variability when scoring chest CT scans suggests that CT in its current form is not ready for either widespread clinical use or as a trial endpoint in the first year of life in NBS CF infants.

## 7.2 STRENGTHS AND LIMITATIONS OF THE STUDY

### 7.2.1 Strengths

The major strength of this study was that a large cohort of NBS CF infants and local healthy controls were recruited and measured within a 2.5y period by a highly experienced team within a single location. With exception of socio-economic circumstances which were slightly less favourable among the CF infants than in the controls (see below), the two groups were well matched. Maternal report of smoking history was validated using cotinine analysis.

All lung function measurements were performed at defined time points using ATS/ERS international standards to minimise methodological or analytical bias.[57-59] Furthermore all results, including the LCI, which has now been shown to be dependent on body size during early life,[58] were interpreted using appropriate reference equations. These were derived from a large number of healthy subjects studied with identical equipment and protocols,[57-59] over the past decade which facilitated accurate interpretation of results. The confidence with which changes over time due to CF lung disease could be detected after adjusting for growth was greatly increased by serial measures in contemporaneous healthy infants over an identical time period and by the extremely high retention rate both for infants with CF and controls.[224] This increases the power of the study and once results expressed and compared as z-scores, it simplifies the statistical analysis between the two groups. As mentioned above, the likelihood of missing evidence of early lung disease was decreased by applying a wide range of tests to assess different aspects of underlying pathophysiology.[71] The tidal and RVRTC techniques were always performed at the

end of the lung function protocol, to ensure that the subsequent forced expirations and/or lung inflations did not bias results from MBW and plethysmography by altering ventilation distribution and/or the extent of gas trapping.

In terms of investigating structural changes in NBS CF infants, in addition to being the first study to examine the within- and between-observer reproducibility of CT scorers, this is the first study to investigate NBS CF infants using both volumetric inspiratory and expiratory images which enabled detailed imaging to be performed. This method of performing CT is particularly useful for longitudinal assessment in research studies as it allows images and airways to be matched for direct comparison.[168,169,196] Use of both inspiratory and expiratory volumetric scans reduced the risk of missing subtle abnormalities, thereby increasing the likely accuracy of the reported changes.

Radiation dose was kept to a minimum through expertise from the radiology department by altering scanning parameters and individualising scanning ranges of infants in order to obtain 'fit for purpose' images.[157]

This study took place at a defined time point of age (1yr) with all CF infants diagnosed through NBS. Functional assessment was timed to be close to the performance of chest CT when the child was as well as possible, which allowed the relationship between structure and function to be investigated under clinically stable conditions. This is in contrast to the AREST-CF study whereby 35% of infants studied were symptomatic at the test of testing.[67]

Several challenges in performing thoracic CT in this age group were experienced in this study. Despite clear protocols and briefing the anaesthetic and radiology teams across all centres, there was variability in the image acquisition parameters in terms of airway pressures and radiation doses delivered. The greater variability in radiation doses in centre C may be due to the slightly different type of scanner (**Table 4-i**) and/or the fact that it was not possible to organise a dedicated radiographer to perform procedures within that hospital, the latter being a problem likely to be encountered in clinical practice as well as multi-centre trials. Presence of an investigator to monitor all procedures did improve compliance, but is unlikely to be

feasible in clinical practice or most clinical trials. This is the only study to ascertain pressures delivered objectively through the use of the $NICO_2$® during controlled volume ventilation to obtain volumetric CT images. Therefore, as an essential part of designing a multicentre study, considerable effort was invested to ensure that all procedures were standardised according to international guidelines. Quality of data collection was further enhanced by establishment of a dedicated research team, objective measurement of adherence to protocols and my attendance at the majority of CTs to ensure the smooth running of these tests. Prior to this study the challenges encountered when using CT as a multicentre outcome measure had never been reported, let alone any information about adherence to standardised protocols, issues that were addressed in this study.

Finally, in this study the use of Brody-II scoring system in infants was evaluated by measuring inter- and intra-agreement of scores by two highly experienced scorers. These two scorers underwent training using scans from young CF children immediately before scoring the LCFC scans so as to ensure consistent interpretation. Previous studies involving infants have only reported results from non-validated scoring system involving only one scorer.[108,148]

## 7.2.2 Limitations

### 7.2.2.1 Recruitment of subjects and study design

There are inevitably some limitations to this study; the observation period is currently short and further follow-up will be essential to establish the clinical implications of any abnormalities detected through lung function tests and chest CT at 1 year of age. With an observational study, we can only demonstrate association not causation of potential determinants of 1 year lung function. Despite the high retention of CF and healthy control infants, there were several who were lost to follow up for different reasons. Although the CF and control groups were equally matched with respect to their background characteristics, there was a significant difference with regard to their socioeconomic status. More CF infants were from lower socioeconomic groups compared to healthy controls. Some studies have suggested that lower socioeconomic group may be associated with a more chaotic life style, increased exposure to tobacco smoking and sub-optimal adherence to

treatment for the CF families[225-227] that could bias the results observed. Since this study was not designed to measure adherence we were unable to comment on how this would vary with different socioeconomic groups. However, the percentage of infants exposed to antenatal, maternal and postnatal household smoking which is strongly associated with reduced lung function[228] was relatively low and similar between the CF infants and healthy controls.

Another potential weakness of the study was that healthy controls were only eligible for recruitment if they had no prior history of respiratory symptoms or illness before the first baseline study at 3 months. These criteria could potentially mean that the cohort of NBS CF infants were compared to a 'super-healthy' group of controls which is not representative of the general population. This in turn could amplify the observed difference in lung function at 3m of age between CF infants compared to controls. The decision to limit recruitment of healthy infants to those without prior illness was pragmatic and arose from the need to study all infants within the first few months of life. In contrast to NBS CF infants virtually all of whom were diagnosed within the first month of life, it took longer to identify and screen controls to ascertain suitability for recruitment (including waiting for GP approval). Appointments then had to be organised rapidly, such that there was usually insufficient time to wait for at least 3-weeks before re-arranging the lung function tests in an otherwise healthy child who developed a respiratory infection, if the ILFT were to be performed within the first 4 months of life. Healthy control infants with a history of neonatal lung disease, or coexistent heart, lung, neuromuscular or renal disease that could impact on the respiratory system were ineligible for the study. However once healthy controls infants were recruited and had had their first lung function performed at 3m of age, they were not excluded from further follow up during the first year of life if they subsequently developed a lower respiratory tract infection or wheezing illness, unless this necessitated hospital admission or symptoms were chronic. Only 2 healthy control infants were withdrawn from the study at a year of age due to chronic respiratory symptoms and none of the healthy control cohort had failure to thrive.

At time of study, very few CT scans from 1yr old NBS CF infants were available, on which to train. The lack of normal chest CTs measured under the same conditions

from healthy age-matched infants or appropriate controls at a similarly young age for comparison with NBS CF infants is an obvious limitation, but one that would have been almost impossible to overcome due to ethical issues regarding radiation exposure in healthy individuals for a research study. Since clinical CT scans in children with normal lungs undertaken for other clinical indications (for example, screening for metastases) would not have included volume controlled inspiratory or expiratory images under GA unless specifically requested for research purposes, even this group would not provide adequate controls.

#### 7.2.2.2 Clinical details of CF infants

Prospective clinical information with regards to clinical status, antibiotic usage and bacterial infections over the course of the first year were collected at regular time intervals through CRF sent to me by the respective tertiary centres. In common with all multicentre studies such as the current one which recruited CF infants who were managed in different tertiary respiratory centres, it was difficult to be absolutely certain that there was complete adherence to the standardised treatment protocol. Consequently, it is possible that some variation in treatment across different centres might have influenced the evolution of lung disease. There was variation in the number of CRFs submitted for each CF infant from the different centres. In order not to miss any vital information on clinical status, bacterial infection and treatment the CF infants would have received, I gathered additional clinical information from clinic letters, annual review letters, parental study questionnaires obtained during lung function testing and where possible, respective hospital microbiology laboratory results.

In the CRF, as parental identification of wheezing was more difficult, no quantification of this symptom was asked except for whether the infant wheezed or not. The fact that identification of wheeze was based on parental identification without any formal training or use of video sleep questionnaires rather than being physician-diagnosed; is a potential weakness of this study. Cough swabs were taken from CF infants, who were seen regularly once every 2 months in respiratory clinics. Additional cough swabs were undertaken whenever respiratory symptoms were reported. Since cough swabs were analysed in the different microbiology laboratories within the tertiary respiratory centres and shared care clinics, the

accuracy of bacterial isolation could have potentially been influenced by the use of non-standardised microbiological analyses of cough swabs. Nevertheless even though there was a wide range of cough swabs obtained, at least four were collected per child in the course of the year which was compliant with local hospital guidelines on obtaining surveillance cough swabs in CF children.

When 1yr lung function results were compared among the 4 CF centres who recruited most of the CF infants (RLH, GOSH, RBH and KCH), there were no significant differences between the groups in terms of 1yr LCI, $FRC_{pleth}$, $FEV_{0.5}$ and $FEF_{75}$ z-scores. This suggests that even if there were slight variations from the standardised treatment protocol that were not apparent from the CRFs, this did not result in any major differences in primary outcome measures at a year (**Table 7-i**). However it is important to be aware that numbers in each group were small and the study was not powered to detect anything other than large differences between the different centres.

**Table 7-i: Table showing 1 year lung function results among the 4 main CF centres**

| **1 year ILFT (z-scores)** | **Centre 1** | **Centre 2** | **Centre 3** | **Centre 4** | **Overall** |
|---|---|---|---|---|---|
| **LCI** | 1.3 (1.1) | 1.0 (1.1) | 1.0 (1.2) | 0.9 (1.7) | 1.0 (1.2) |
| *n* | *11* | *16* | *31* | *11* | *69* |
| **$FRC_{pleth}$** | 0.8 (1.0) | 0.6 (1.1) | 0.6 (1.2) | 1.0 (1.1) | 0.7 (1.1) |
| *n* | *11* | *17* | *30* | *10* | 68 |
| **$FEV_{0.5}$** | -0.6 (0.9) | -0.4 (1.2) | -0.5 (1.0) | -0.1 (0.9) | -0.4 (1.0) |
| *n* | *11* | *17* | *29* | *10* | *67* |
| **$FEF_{75}$** | -0.1 (0.6) | -0.1 (1.0) | -0.0 (0.9) | -0.2 (1.3) | -0.1 (0.9) |
| *n* | *11* | *17* | *29* | *10* | *67* |

***Footnote:*** *Lung function results presented as mean (SD). Using ANOVA for comparison between the different centres, no significant differences were detected for any of the 1yr outcomes. n= number of successful tests.*

#### 7.2.2.3 Technical challenges in acquiring standardised CTs

Despite clear protocols and briefing the anaesthetic and radiology teams across all centres, there was variability in the image acquisition parameters in terms of airway pressures and radiation doses delivered. The greater variability in radiation doses in centre C may be due to the slightly different type of scanner (**Table 4-i**) and/or the fact that it was not possible to organise a dedicated radiographer to perform procedures within that hospital, the latter being a problem likely to be encountered in clinical practice as well as multi-centre trials. Presence of an investigator and/ or dedicated radiographer and anaesthetist whenever possible to monitor all procedures improved compliance, but is unlikely to be feasible in clinical practice or most clinical trials. It would also be unrealistic to completely standardise all CT scanners used in the different centres. When these potential limitations were recognised in the design of the study and during the first few months of data collection, every effort was made to address these issues through rigorous objective monitoring.

#### 7.2.2.4 CT scoring system

Another limitation of this study was the time and expertise involved in the use of the Brody-II scoring system. A CT scan scored through Brody-II is time consuming, especially in scans with significant and numerous CT changes where it can take up to 30mins to score each scan. Ideally all the scans in this study would have been re-scored after 8 months, but this was precluded by time constraints – this exercise being limited instead to the subgroup of 22 LCFC scans. Fortunately, the random selection of scans for re-score several months later proved to be representative of the whole cohort. Due to the time-consuming nature of the reproducibility studies, no other CT scoring system was used, but given that most CT scoring system use components which overlap with Brody-II, it is unlikely that results would have been very different.

The Brody –II scoring system in its present format has limitations in scoring minor CT changes such that with study scans, changes in bronchial size were so mild and subtle that both scorers changed their minds with regard to the score allocated during different scoring rounds. Furthermore, measuring changes in small bronchial luminal size in young infants to define bronchial dilatation may be beyond current CT spatial resolving ability, resulting in the whole scoring process becoming a real challenge.

## 7.3 COMPARISON WITH THE LITERATURE

### 7.3.1 Clinical status

The percentage of NBS CF infants in this study with at least one infection of *PsA* within the first year of life was 35%. The median age of first infection with *PsA* was 27.7 weeks (range: 6.1-52.4 weeks.). By the first year ILFT, only 1 infant had chronic *PsA* infection, defined by the Leeds criteria.[194] The incidence of *PsA* found in this study is comparable to that reported previously, which ranges between 9 to 42% in the first 2 years of life,[229] depending on the clinical status at time of sampling[54,230,231] and type of microbiological samples (cough swab, sputum, BAL).[219,232]

Anthropometric measurements in NBS CF infants were significantly lower compared to contemporaneous healthy controls despite early detection through NBS. This LCFC NBS cohort demonstrated improved somatic growth compared to the previous LCFC clinically diagnosed cohort.[233]Comparable to previous studies on the nutritional benefits of NBS,[29,39,234] significant catch up growth took place such that by a year, there were no differences observed between LCFC NBS CF infants and healthy controls.

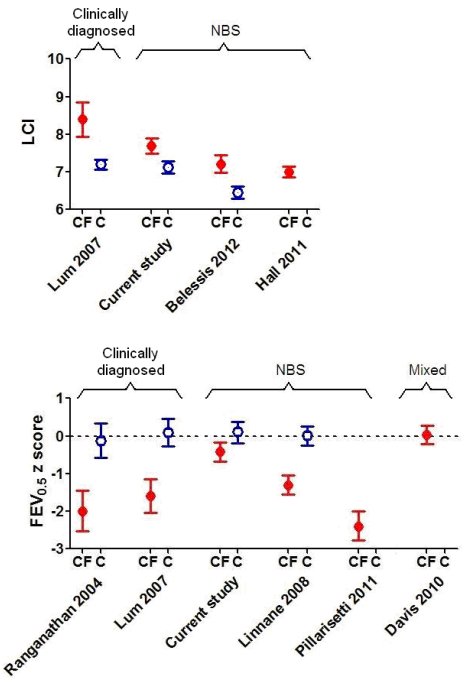
### 7.3.2 Infant lung function studies

Results regarding evolution of early lung disease in CF infants diagnosed by NBS have been conflicting (**Figure 7-i**). The AREST-CF study has reported both normal[82] and reduced[67] ILFT in such infants within the first 6 months of life, with further rapid deterioration over the first year of life (mean $FEV_{0.5}$ being -2.4 z-scores by ~1yr of age).

In the current study, lung function was abnormal by 3m,[71] but stabilised or improved thereafter. As can be seen from **Figure 7.i**, 1yr lung function in the LCFC NBS cohort was significantly better than that in previous clinically-diagnosed LCFC cohorts[60,83] or in the AREST-CF NBS cohort at similar age.[67,82] Reasons for the discrepancies between our results and those for AREST-CF are unclear. While the standardised protocol adhered to by the LCFC differs in some respects from that used by most centres in the USA, Australia and Europe (e.g., use of flucloxacillin

prophylaxis), the results should be a benchmark for other centres, and could serve as the basis for quality improvement.[235] Median age at first test in this study is younger than that in AREST-CF, which may reflect earlier diagnosis and implementation of treatment within the narrow geographical area of S.E England from which infants were recruited for this study, thereby halting progression of any early lung disease. It is possible that infants recruited to AREST-CF were sicker, or deteriorated faster due to differences in modifier genes, environment or adherence to treatment, when compared with those in London. Most importantly, in contrast to the current study, AREST-CF data were not compared with contemporaneous controls, historical controls being used initially,[82] with subsequent results (obtained using higher inflation pressures[67]) being interpreted using reference data based on different equipment, which can bias interpretation.[55,57]

**Figure 7-i: Comparison of current lung function results in infants with cystic fibrosis and healthy controls**




***Legend:*** *Comparison of current lung function results in infants with CF and healthy controls (C) at ~1yr of age, with previously published results. NBS: newborn screening. Data expressed as mean (95% CI). To allow direct comparison with previously published studies, Lung Clearance Index is presented in absolute units, whereas $FEV_{0.5}$ is expressed as z-scores, based on different reference equations according to each author. The dashed horizontal line at 0 z-scores equates to 100% predicted based on a healthy population. Control data were not available in all studies.*

In keeping with the study by Lum *et al* in clinically diagnosed CF infants which found elevated levels of LCI in the first year of life[83], Belessis *et al* demonstrated in a prospective cross-sectional study that it has the ability to detect pre-symptomatic disease in NBS CF infants and very young children (≤ 3 years of age). Fifty five CF (mean age: 1.96 years) and 36 healthy control (mean age: 1.26 years) infants and young children showed good repeatability and reproducibility of LCI in both groups and LCI was elevated in CF compared to healthy control infants. This study also demonstrated greater airway inflammation and elevated LCI in those with *PsA* infections.[86] Another study published by the AREST-CF team using the same commercially available, ultrasonic device, found similar LCI readings as the Belessis *et al* study even though LCI was not significantly associated with CT air trapping or bronchiectasis in NBS CF infants in contrast to what have been published in older children and adults. [121] When interpreting published data using commercial devices, discrepancies can occur between studies due to the fact that only prototype versions of the commercial device were available at the time of testing, with frequent amendments of software and algorithms[236] that would require substantial re-analysis of results. This can lead to discrepant results between different devices, despite being produced by the same company.[237]

In the US multicentre evaluation of infant lung function, compared to historical healthy controls, CF infants showed significantly diminished flow [$FEF_{75}$ z-score; mean (95% CI): -0.52 (-0.78; -0.25)] and elevated lung volumes [$FRC_{pleth}$ z-score; mean (95% CI): 1.92 (1.39; 2.45)]; with no reductions seen in forced expiratory volumes. Although some hyperinflation is expected, it is somewhat surprising to see the extent of elevated $FRC_{pleth}$ without corresponding reduced airway function. This might be related to the quality of the test centres in performing these tests. The low success rate for RVRTC measures due to great variability in skill-mix and experience of the laboratories and hence measurement acceptability rates in lung function results could account for the lack of abnormal results seen in forced expiratory volumes. Furthermore lung function results were only compared to historical controls in this study. Hence the feasibility of performing different ILFT is important to take into account when considering it as a multicentre clinical trial endpoint.

While improvements in lung function following treatment with IV antibiotics for acute exacerbations in 11 infants with CF have been demonstrated in a retrospective study;[118] the current LCFC study of NBS infants is the first to document improvements in $FEV_{0.5}$ in infants treated with standard therapy, studied during periods of clinical stability. The prospective Infant Study of Inhaled Saline (ISIS) in Cystic Fibrosis trial reported greater increases in $FEV_{0.5}$ over a 48-week period in 22 infants and young children treated with hypertonic saline compared with 23 randomised to isotonic saline (mean (95% CI) difference:38(1-76) mL).[229,238] However, from the data presented, it was impossible to ascertain whether this reflected stability, improvement or simply less deterioration over time with active treatment, once effects of lung and somatic growth had been accounted for. The authors did not discuss the reason why results were not expressed as z-scores which would have accounted for growth.

A small group of infants and preschool children in the ISIS trial also underwent LCI measurements using mass spectrometry and $SF_6$ in one centre. The authors concluded that all infants in this pilot study had normal LCI. All except one infant on hypertonic saline showed stabilisation of LCI whilst those treated with isotonic saline showed worsening of LCI during the 48 weeks trial. As for pre-schoolers, those on hypertonic saline showed an improvement in LCI compared to those on isotonic saline; however there is a great variation in the LCI results within each individual. Findings suggested a beneficial treatment effect but this is a small study comprising of only 25 patients, 12 on hypertonic saline and 13 on isotonic saline.[238] Despite the apparent improvement in infant lung function in this subgroup of CF infants, the use of inhaled hypertonic saline compared to isotonic saline did not reduce the rate of pulmonary exacerbations over the treatment period.

### 7.3.3 Comparison of CT findings

Use of different scoring systems makes direct comparisons difficult, particularly when attempting to quantify severity of changes. While changes could be identified on at least one Brody-II sub-score in 34/65 (52 %) of the LCFC infants, the magnitude of these changes was often trivial. Important changes (defined either by

visual inspection and/or a total CT score ≥5% maximum possible) were only detected in 2% of infants by scorer A and 11% by scorer B (**Table 5-v**).

Although AREST-CF detected chest CT changes in 81% of NBS CF infants at a median age of 3.6 months, bronchial dilation was only found in 11/57(19%) at this age,[109] and remained low through the first two years of life (~8% at both 1 and 2yrs of age) before increasing markedly to ~36% by 4yr.[111] In the most recent publication from this group, prevalence of bronchial dilatation in CF children during the first 4yrs of life was ~60%,[19,239] ~80% of whom had evidence of bronchial dilatation at some time during the first 3 years. Bronchiectasis as classically defined refers to irreversible dilatation due to damaged bronchi. The "apparent improvement" in bronchiectasis reported in some of the AREST-CF children could be associated with mild and borderline normal bronchi (see below). The AREST-CF studies also report more air-trapping (67% at ~4m,[109] 62% at ~1y[111] and 69% at ~3y[19]) than in the current study. These discrepancies may be partially explained by the fact that in contrast to the AREST-CF study, LCFC children were only studied when asymptomatic. Bronchial dilatation was significantly more likely (60.0% vs 10.2% in asymptomatic) and more severe in AREST-CF infants with respiratory symptoms at the time of CT.[109] In this LCFC study, all the infants were tested close to their first birthday whereas in the AREST-CF study most infants were studied at a slightly older age. While not emphasised in the various reports from the AREST-CF study, only minimal changes CT were detected at around 1 year of age, the increase in frequency and severity commencing beyond 2-3 years of age.

#### 7.3.3.1 Comparison of inter and intra-observer agreement for CT scores with previous studies

The poor inter-observer agreement when using Brody-II in NBS CF infants contrasts markedly with previous studies in older subjects (including those in which scorers A and B participated, **Table 7-ii**). Previous studies have found bronchial dilatation to be the most reliably reproducible element when evaluating CF lung disease.[154,172,240] The poor agreement in the current study likely reflects the subtlety of changes observed. A single scorer scored all the AREST-CF scans with good intra-observer agreement after a 6-12 month interval.[111] (**Table 7-ii**) Separate assessments for younger children in whom bronchial dilatation was infrequent and milder were not

however reported. While use of a single dedicated observer to score all scans [109,111,241] could provide more consistent outcomes, such an approach is impractical in clinical practice and unlikely to be either generalisable or feasible in large multi-centre trials. In the absence of measures of repeatability, the extent to which inter- and intra-observer variation contributes to reported CT findings in other studies cannot be established.

**Table 7-ii: Comparison of measures of within- and between-observer variability used in the current and selected previous studies**

| Study | Current study | | Brody[*172] | | Owens[†107] | Brody*[151] | De Jong [154] | Stick[109] |
|---|---|---|---|---|---|---|---|---|
| Population studied | NBS CF | | NBS and clinically diagnosed CF | | Clinically diagnosed CF | Clinically diagnosed CF | Clinically diagnosed CF | NBS CF |
| Age: years [‡] | 1.0 (0.1) | | 10.5 (0.7) | | 7.8 (1.3) | 6-10 [§] | 5-52 [§] | 1.1(0.3-3.3)[‖] |
| Scoring system | Brody-II | | Brody-II | | Brody-II | Brody-II | Brody-II | Specific[**] |
| Measure of variability | Between Obs kappa | Within Obs kappa | Between Obs variability | Within Obs variability | Between Obs Kendall's tau | Within Obs kappa | Between Obs ICC | Within Obs kappa |
| Bronchial dilatation | 0.21 | 0.24/0.35 | 0.04 | 0.06 | 0.77 | 0.64 | 0.88 | 0.64 |
| Air trapping | 0.66 | 0.72/0.72 | 0.07 | 0.04 | 0.59 | 0.55 | 0.27 | 0.55 |

***Footnote:*** *[*]Studies included scorer A as an observer. [†]Studies including scorer B as an observer. Obs = Observer; ICC = Intraclass correlation [‡]Age at time of CT scan, expressed as mean (SD) unless otherwise stated. [§] Age expressed as range. [‖]Age as median (inter-quartile range) [**] AREST-CF CT scoring system*

Additional problems in interpreting CT scans relate to lack of international consensus on how to define bronchial dilation, especially in infants. A broncho-arterial ratio (BAR) >1 as specified in Brody II was used both in the current study and AREST-CF. This speeds up evaluation as judging whether the bronchus is bigger than the adjoining vessel can be assessed subjectively more easily than calculating a ratio. It has been suggested that a threshold of 0.76, rather than 1, should be applied in children[137,167] but given the poor inter- and intra-observer agreement even when using BAR≥1 in infants with mild CF lung disease, it is unlikely that this would be effective without the use of calliper measurements which would be time and labour intensive. This would limit its use as an outcome measure in multicentre studies. Furthermore measuring changes in small bronchial luminal size to define bronchial dilatation may be beyond current CT spatial resolving ability. The accuracy of assessing BARs, especially in health, is also critically dependent on reliably achieving full lung inflations. Therefore it is vitally crucial for any multi-centre study to obtain standardised CT for accurate and consistent interpretation.

#### 7.3.3.2 Methodological differences in acquiring chest CT in infants and young children

To date there is no consensus on the optimal method of acquiring CT scans in young children to ensure maximum information with minimal radiation exposure. Following discussions with the AREST-CF team, the approach of obtaining end-inspiratory scans at 25 $cmH_2O$ PIP and end-expiratory scans at 0 $cmH_2O$, together with recruitment manoeuvres to minimise procedure-related atelectasis were undertaken. However, in this study a volumetric technique that images the entire lung volume was used in contrast to the initial studies by AREST-CF where only 3 thin-slice scans were obtained during inspiration and expiration.[109,111,241] Volumetric imaging should enable fewer changes to be missed.[169,196] Limiting the dataset to 3 images, compared to ≥20 for the volumetric technique, severely limits the number of airways that can be evaluated and matched for comparison in longitudinal studies, such that if bronchi were sampled and imaged at the point of bifurcation, this would over-estimate the size of the bronchial lumen, potentially leading to over-detection of bronchial dilatation. This may have contributed to the differences in reported CT changes between this study and those published by AREST-CF. [109,111,241] In addition

concerns about radiation exposure warrant further studies to elucidate the minimum number of CT slices required for optimum evaluation of early CF lung disease at the lowest possible radiation burden.

Volumetric CT scanning in infants and young children requires sedation or GA if lung volumes are to be standardised within- or between centres during image acquisition. Images obtained at varying lung volumes can greatly affect the reporting of changes detected on CT thus creating inaccuracies both within individuals studied longitudinally and between subjects studied within and between centres. If inspiratory images were obtained not at TLC, this may underestimate the bronchial dilatation sub-score while air trapping sub-score may be underestimated if expiratory images were obtained not at FRC. Without standardisation, any changes reported may not be a true reflection of the extent of disease in patients but rather a result of the technical variation during imaging. However, the optimum inflation pressure at which to acquire standardised lung volumes has yet to be ascertained.

#### 7.3.3.3 Association between lung function and CT changes

Compared to studies in older subjects[64,107,117,154] which have reported good correlations between lung function outcomes, especially LCI, and CT changes, Hall *et al* showed that among 49 NBS CF infants studied at one of the CF centres in the AREST-CF study, air trapping on chest CT was only weakly associated with moment ratios as a measure of ventilation inhomogeneity, and not with LCI.[121] In this LCFC study on 1 year old NBS CF infants, there was lack of close relationship between lung function and structure. This may be due to the mild structural disease seen in these asymptomatic CF infants who were diagnosed early and rapidly commenced on CF therapy. Such mild CT changes did not necessarily result in functional decline, and may in fact be reversible.[108] In addition, infants in this age group were tested with sensitive methods of lung function that could differentiate and detect subtle changes in lung disease which may not yet be detected through CT changes. Hence a discordant or lack of relationship was observed between lung function and CT changes in this study.

In the study by Belessis *et al*, no chest CT was performed. Structural changes were described using the CF-specific CXR scoring system. Only mild structural lung

disease was identified on CXR which could not be distinguished between CF children with infection and those without. There was no correlation found between LCI and CXR structural changes as very few changes were detected. In the multicentre study by Davis *et al*, there was no chest CT performed for comparison.

## 7.4 CLINICAL IMPLICATIONS

The need for sensitive measures to be developed for the detection of early CF lung disease is crucial. CF lung disease starts early in life[139] and often progresses even in the absence of clinical symptoms. With the emergent therapeutic options and increasing drug development,[5,7] it would be important for infants and young children to be involved in clinical trials involving these therapeutic options to halt disease progression. Other than the ISIS trial,[229] there is no single randomised controlled trial in infants or pre-school children, so all recommendations are based on the weakest level of evidence, namely consensus documents. To choose appropriate outcome measures in infants and young children for interventional trials is extremely challenging as not only are these measurements complex and time consuming to undertake, but pulmonary changes may be so mild that any tests performed may be unable to differentiate between those due to disease and those associated with ongoing lung growth and development in this age group, unless very large numbers of subjects are studied.

Clinical endpoints such as respiratory exacerbations were not explored in this thesis. Defining these clinical endpoints is both challenging and complex within the CF adult population let alone among infants and young children when often many of these criteria do not apply. Therefore for the purpose of this thesis, only infant lung function and chest CT changes were explored as objective outcome measures.

### 7.4.1 Infant lung function tests as an outcome measure

Results in this study have implications for both clinical practice and research. This study indicates that despite early diagnosis and rapid implementation of therapy, including prophylactic antibiotics, a substantial number of NBS infants with CF have abnormalities of lung function within the first 3m of life. The apparent wellness of

the cohort should not lead to complacency, and prompt and aggressive treatment of any abnormal symptoms or signs is vital.

Although ILFT represent only one of the potential outcomes that can be used during early life,[110] with additional information gleaned from inflammatory markers and CT,[242] they represent the mainstay of clinical management and a major outcome in randomised controlled trials in both older children and adults. Since lung function tracks from late infancy into later life, in both clinically diagnosed CF[60,102] and non-CF cohort studies[130,132] accurate identification of early abnormalities is imperative. Furthermore, given the increasing number of centres undertaking 'clinical' ILFT,[243] the current study may facilitate more meaningful interpretation of results by providing vital evidence regarding the natural changes that can occur over time in both healthy infants and those with lung disease, in the absence of any specific interventions. How well ILFT tracks from early infancy especially in those diagnosed through NBS is still unclear.

Even when both physiological gas trapping and CT air trapping are present at a year, they are likely to represent dynamic changes dependent on current or recent clinical status which may be reversible with standard treatment during infancy. It is possible that abnormalities in lung function and CT changes in young infants are more readily reversible than when observed in older subjects. Based on this observational study, 24 CF infants had abnormal $FEV_{0.5}$ or LCI identified at 3m but by a year of age, 14/24 (58%) of these infants normalised both $FEV_{0.5}$ and LCI. This would have meant that more than half of the infants would have been unnecessarily treated if they were commenced on specific treatment in an interventional study based on abnormal lung function at 3m. This throws doubt on the use of lung function testing as outcome measures during infancy and raises the question as to whether objective lung function monitoring should wait until the preschool years.

We have shown that both lung function and somatic growth during the first year of life are significantly better in infants diagnosed by NBS in the UK than in their counterparts who were clinically diagnosed a decade earlier[60,83] (**Figure 7-i**). It is, however, of concern that LCI remains abnormal at 1yr (**Figure 7-i)**, albeit to a mild degree.[83] Further follow-up is required to establish the extent to which these changes

predict later outcome beyond infancy. Nevertheless, in this study, normal lung function was sustained in at least 50% NBS CF infants to 1yr of age. The significant improvement in $FEV_{0.5}$ and stability of distal airway function during early life when on 'standard therapy', and the relatively small deficits in lung function in NBS CF infants at 1yr have important implications for design of future randomised intervention trials in this age group. Despite considerable within-subject variability, the main predictor of lung function at 1yr was that at 3m, allowing us to identify a 'high-risk' group who could potentially be targeted for future intervention trials.

Using data from this study, results from ~ 85 infants per arm would be required to detect relatively small differences in lung function (i.e. equivalent to 0.5 z-scores which equates to differences of ~ 4-7% depending on outcome) that might occur in response to an intervention if unselected NBS CF were recruited to such a trial. By contrast, were recruitment to such a randomised controlled trial limited to a 'high risk group' (i.e. abnormal ILFT by 3m, see **Table 7-iii** and **Table 7-iv**), a larger treatment effect would be expected, with only 22 infants per arm being required to detect a difference of 1 z-score (equivalent to ~ 9% for LCI), with 90% power. Such an approach could optimise recruitment since not only would parents of infants with early lung function abnormalities be more likely to consent, but this approach would minimise exposure of children to unnecessary side effects with potentially little to gain from therapy.

**Table 7-iii: Summary of anthropometry and pulmonary function at ~3 months and 1 year in CF NBS infants with normal and abnormal lung function on the 3-months test occasion versus healthy controls**

| | At 3 months | | | | At 1 year | | | |
|---|---|---|---|---|---|---|---|---|
| | **Abnormal[*] (A)** | **Normal[†] (N)** | **Controls (C)** | **p value (ANOVA)[‡]** | **Abnormal* (A)** | **Normal[†] (N)** | **Controls (C)** | **p value (ANOVA)[‡]** |
| ***n*** | 19 | 45 | 37 | | 19 | 45 | 37 | |
| ***Age, weeks***[§] | 11.1 (2.3) | 11.1 (2.2) | 11.9 (2.0) | 0.22 | 55.0 (5.1) | 51.7 (5.3) | 53.5 (4.5) | 0.05 |
| Weight, z-score[‖] | −0.67 (0.89) | −1.00 (1.07) | −0.03 (0.97) | **<0.001** | 0.36 (0.80) | 0.25 (0.95) | 0.51 (1.29) | 0.56 |
| Length, z-score[‖] | 0.12 (0.92) | −0.40 (0.96) | 0.67 (0.93) | **<0.001** | 0.76 (0.98) | 0.28 (0.96) | 0.73 (1.25) | 0.11 |
| LCI, z-score | 1.22 (1.85) | 0.51 (0.91) | 0.37 (0.89) | **0.03** | 1.64 (0.98) | 0.78 (1.23) | 0.31 (0.97) | **<0.001** |
| $FEV_{0.5}$, z-score | −2.29 (0.79) | −0.71 (0.80) | −0.13 (0.77) | **<0.001** | −0.67 (0.95) | −0.24 (1.04) | 0.13 (0.94) | **0.02** |

***Footnote:*** *Comparisons between groups were undertaken using ANOVA. Dataset used for this analysis were limited to those infants with technically successful LCI and $FEV_{0.5}$ results on both test occasions. [*] (A): based on those with abnormal LCI and/or $FEV_{0.5}$ at 3m (i.e. outside the 95% limits of normality found in healthy infants); [†] (N): based on those with normal LCI and $FEV_{0.5}$ at 3m. [§]corrected for gestational age; [‖]calculated according to Cole et al*[182]

[‡]*Based on post-hoc Bonferroni adjustment for multiple t tests between and within groups. Significant difference in anthropometry at 3m identified by ANOVA was seen between healthy controls and CF infants with abnormal or normal ILFT at 3m (**Table 7-iv**). There were no significant anthropometric differences between the two subgroups of CF infants, nor between those with abnormal or normal lung function and controls at 1 year. Significantly reduced lung function was found between those with abnormal lung function and healthy controls at 3 months which persist to 1 year of age.*

**Table 7-iv: Comparison of anthropometry and pulmonary function at ~3 months and 1 year between CF NBS infants with normal and abnormal lung function at 3 months and healthy controls.**

| | At 3 months | | | At 1 year | | |
|---|---|---|---|---|---|---|
| | **Diff (95%CI):A-N* p value** | **Diff (95%CI):N-C* p value** | **Diff (95%CI):A-C* p value** | **Diff (95%CI):A-N* p value** | **Diff (95%CI):N-C* p value** | **Diff (95%CI):A-C* p value** |
| *Age, weeks*[†] | -0.02 (-1.30; 1.25) 0.97 | -0.77 (-1.70; 0.15) 0.10 | -0.80 (-2.07; 0.46) 0.20 | 3.3 (0.38; 6.16) 0.028 | -1.79 (-3.94; 0.37) 0.10 | 1.48 (-1.35; 4.31) 0.29 |
| Weight, z-score[‡] | 0.34 (-0.19; 0.86) 0.620 | **-0.97 (-1.42; -0.52) <0.001** | **-0.64 (-1.16; -0.11) 0.018** | 0.10 (-0.37; 0.57) 0.66 | -0.25 (-0.76; 0.25) 0.32 | -0.15 (-0.71; 0.41) 0.59 |
| Length, z-score[‡] | 0.52 (0.004; 1.04) 0.048 | **-1.07 (-1.49; -0.65) <0.001** | **-0.55 (-1.08; -0.02) 0.041** | 0.48 (-0.06; 1.02) 0.082 | -0.45 (-0.94; 0.04) 0.076 | 0.03 (-0.59; 0.64) 0.93 |
| LCI, z-score | 0.71 (-0.21; 1.63) 0.12 | 0.14 (-0.26; 0.54) 0.49 | **0.85 (0.12; 1.58) 0.023** | **0.87 (0.28; 1.45) 0.005** | 0.46 (-0.02; 0.95) 0.059 | **1.33 (0.787; 1.89) <0.001** |
| $FEV_{0.5}$, z-score | **-1.57 (-2.01; -1.13) <0.001** | **-0.59 (-0.93; -0.24) 0.001** | **-2.16 (-2.61; -1.71) <0.001** | -0.44 (-0.98; 0.11) 0.11 | -0.36 (-0.80; 0.07) 0.10 | **-0.80 (-1.34; -0.26) 0.005** |

***Footnote:*** *Dataset used for this analysis were limited to those infants with technically successful LCI and $FEV_{0.5}$ results on both test occasions. CI=confidence interval of the difference; A: CF infants with abnormal LCI and/or $FEV_{0.5}$ at 3m**;** N: CF infants with normal LCI and $FEV_{0.5}$ at 3m; C: controls *Based on student's t-test; [†]corrected for gestational age; [‡]calculated according to Cole et al [182]; significant differences are shown in bold.*

*When the CF cohort was subdivided into those with normal or abnormal lung function at 3m, there was a greater significant difference detected between those with abnormal lung function results compared to healthy controls which may be important in designing interventional trials (in grey shaded boxes).*

Thus a trial designed to detect a 1 z-score improvement in lung function in response to an intervention would only require 22 infants in each arm for 90% power at the 5% significance level. Nevertheless, since abnormalities at 3m were only observed in 30% of our infants when based on the 2 most feasible PFTs (LCI and $FEV_{0.5}$), after allowing for attrition and exclusions it would still be necessary to access a population of (22 x 2) x (100/62) x (100/30) i.e. ~237 NBS CF infants to obtain 90% power in a RCT. This is more than double the number identified in the South-East of England over a 2.5 year period during the present study and would hence inevitably require a multi-centre study if to be completed in a timely manner. This adds to the body of evidence that if ILFT is used as an outcome measure, such a study would require multicentre collaboration for adequate number of patients to be recruited.

### 7.4.2 Chest CT as outcome measure

In terms of the evaluation of chest CT changes seen in early CF lung disease, results from this study suggest that both the acquisition and interpretation of CT scans need further evaluation before being applicable either as a clinical tool or research outcome measure in NBS CF infants at least at a year of age. This study highlighted the complexities involved in obtaining standardised best quality images that are fit for accurate interpretation. It also revealed that only half of NBS CF infants had any changes seen on chest CT, and of these the majority of changes were very mild. There is currently no knowledge about the long-term clinical significance of such mild changes in young infants with CF, nor any data to suggest that mild changes lead to alterations in clinical management or long term clinical outcomes. It is questionable whether the risks of exposing young infants to additional ionising radiation currently outweigh the benefits.

An informal survey conducted to establish whether CT findings resulted in any change in management among the clinicians responsible for the care of these NBS CF infants within the LCFC collaboration confirmed that very few management plans were instituted based purely on the results of the scans. Amongst scans with the most changes seen, only 1 infant had a change in treatment, while 2 had additional investigations looking for gastro-oesophageal reflux. Hence out of the 65 scans conducted in this study, only 3 (4.6%) infants had a change in management

plans. As a result of the findings in this study, none of the respiratory paediatricians who participated in the LCFC study of NBS CF infants are performing chest CT unless clinically indicated.

With increased longevity of CF patients, repeated use of chest CT to monitor progression has raised concerns especially in growing children.[176,244] Consequently, there have been several studies conducted to establish the relationship between LCI and structural CT changes, as both are considered sensitive. Older CF children diagnosed clinically with significant changes seen on CT have demonstrated a correspondingly high LCI.[64,107] The prospect of LCI being a potential surrogate marker for changes observed on CT is exciting as MBW can potentially be performed across all ages. By using LCI as a screening or monitoring tool of CF lung disease, the number of CT scans performed could be reduced hence minimising the risks associated with repeated radiation exposure. However, whether this approach could be extended to infancy would depend on how well LCI tracks between infancy and later childhood and the availability of robust commercially available equipment for assessing LCI during the first few years of life. Preliminary analysis of this NBS CF cohort suggests that LCI tracks poorly from early infancy to 2 years of age.[245] These issues will be discussed in detail in the following **sections 7.5.1** and **7.5.2**.

In contrast to other studies which have reported increased incidence of bronchial dilatation with *PsA* infection and wheeze with presence of air trapping on CT scan,[109,111] in this study there were no indicators to predict the presence of bronchial dilatation or air trapping. Although the use of IV antibiotics was associated with parenchymal changes in scorer A and mucous plugging in scorer B and a history of *PsA* infection within the first year significantly associated with parenchymal changes by scorer A only; both parenchymal change and mucous plugging were not reproducible CT sub-scores as shown in **Table 5-iv.** In contrast to 1yr lung function outcomes whereby significant associations were noted with clinical associations such as *PsA* infection, weight gain between 3 months and birth, presence of wheeze, cough or gastro-oesophageal reflex disease ever and the use of IV antibiotics, such clinical determinants did not appear to be associated with any reproducible CT

scores. Hence neither lung function at 1yr of age nor any clinical determinants were associated with CT outcomes.

As a research endpoint, there is an international momentum advocating the use of chest CT as a sensitive objective measure of early CF lung disease.[110] Until refinement of CT scoring has been established and validated for mild disease, caution should be exercised when reporting bronchial dilatation in NBS CF infants, the incidence of which appears low in the first year of life. If CT changes are used as outcome measures in their current form, based on the incidence of bronchial dilatation detected by both scorers in this study, between 190-850 infants per group would be required in order to detect a reduction in bronchial dilatation by 50% in the interventional group with 90% power at 5% significance level at 1yr of age, this number rising further after accounting for those ineligible for such a trial or whose parents decline.[191] It should be noted that recent suggestions that such a study would be feasible with only 100/group were based on incidence of bronchiectasis at four, not one year of age.[111] Ensuring standardisation in the performance of chest CT and obtaining images in multicentre studies for scoring must be considered carefully. In this study, formal monitoring during the GA process took place during CT imaging which was useful to show the variation that could potentially occur across different centres. If imaging was to be used as outcome measure in a multicentre study, it would be impractical and labour intensive to have formal monitoring of the GA process across different centres. Therefore the utility of these tests during infancy requires rigorous investigation if they are to be employed in interventional studies involving infants.

Determining useful outcomes as clinical trial endpoints whether ILFT or CT have been much more difficult than we had previously envisaged. Using lung function or chest CT as outcome measures will require a large population to adequately power the study in view of the mild abnormalities detected in this NBS CF cohort. Besides there is uncertainty about the long term tracking of these mild changes detected during infancy. It may be safer to defer novel CF therapies with potential toxicity to the growing lung beyond the first year of life.

## 7.5 UNANSWERED QUESTIONS AND FUTURE RESEARCH

### 7.5.1 The evolution of lung function beyond 1 year of age

In this LCFC NBS CF study, lung function was already impaired compared to HC infants by 3m of age despite early diagnosis through NBS and early intervention to optimise respiratory and nutritional support. One year after the positive diagnosis, significant improvement was observed in some measures of airway function. It is, however, of concern that LCI remains abnormal at 1-year (**Figure 7-i**), albeit to a mild degree. The need for long term follow-up to evaluate the significance and consistency of outcomes during infancy requires a major ongoing effort. Further funding has already been obtained from the CF Trust and Action Medical Research to follow-up this cohort of NBS CF infants and HC to 2yrs of age using ILFT under sedation, and from 3 years upwards in awake children using specially adapted preschool tests to assess LCI, plethysmographic specific airways resistance and spirometry.

Preliminary analysis of data from 55 CF NBS and 28 healthy control infants who have completed 3 tests occasions to date ( 3m, 1yr and 2yrs) (**Table 7-v**) has been completed recently.[245,246] Mildly elevated LCI and $FRC_{pleth}$ at 3m remained stable from 3m to 1yr with no further deterioration by 2yrs even though LCI and $FRC_{pleth}$ were on average higher in CF than in HC across all three tests occasions. In contrast, a significant reduction in $FEV_{0.5}$ was only evident at 3m of age in CF NBS infants with significant improvement observed from 3m to 1yr such that by 1 and 2 yrs, $FEV_{0.5}$ was comparable between CF NBS and HC infants. Sustained improvement in $FEV_{0.5}$ was evident to 2yrs of age in CF NBS infants. Thus far, this is also the first study to demonstrate stable lung function to 2 years in NBS CF infants managed on standard CF therapy.[245,246]

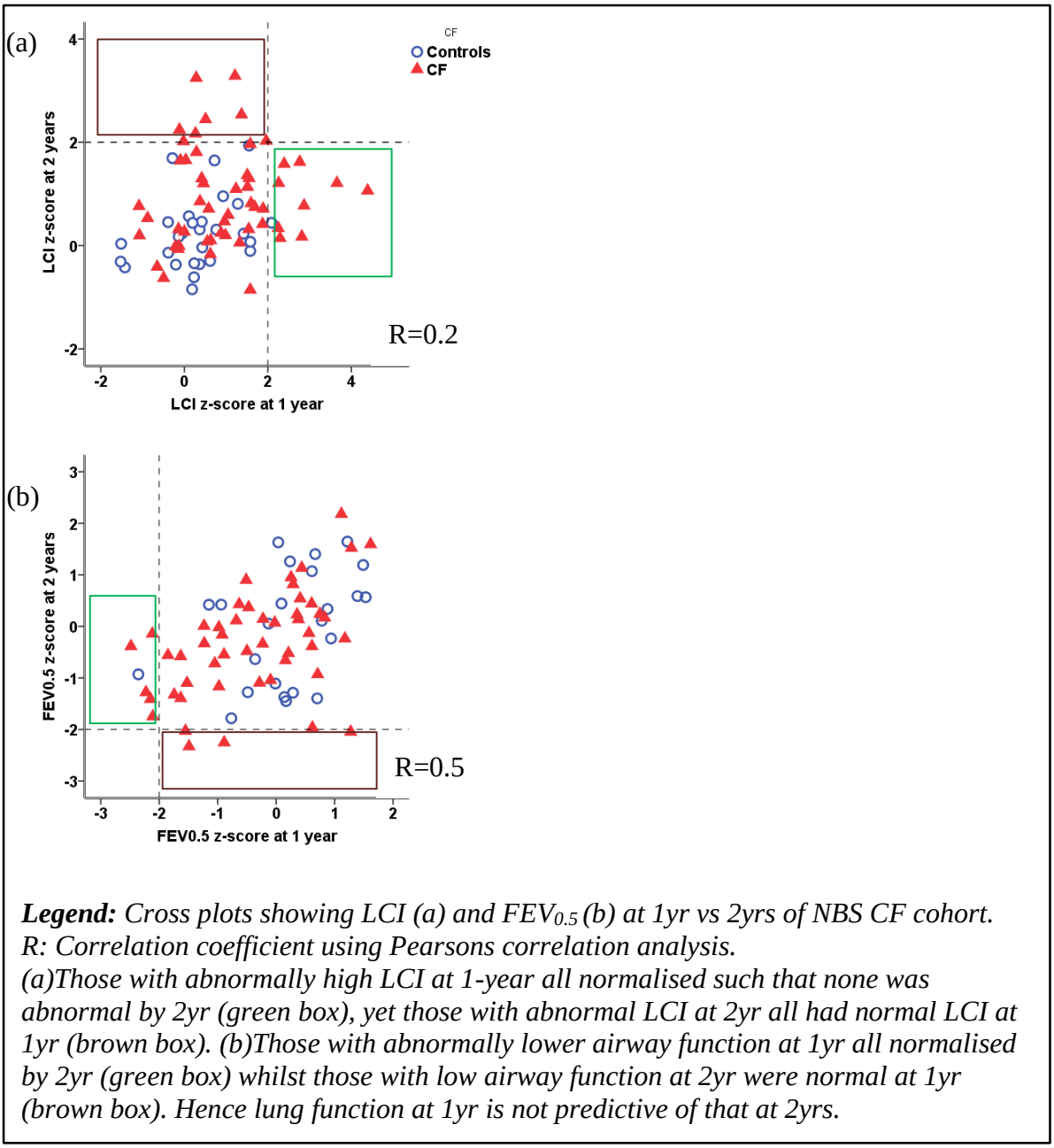
**Table 7-v: Lung function results (in z-scores) at 3 months, 1 year and 2 year in CF NBS infants**

| | Mean (SD) | | | Difference mean (95% CI) | |
|---|---|---|---|---|---|
| | 3m | 1y | 2y | 1y-3m | 2y-1y |
| Z-$FEV_{0.5}$ | **−1.4 (1.1)** | −0.4 (1.1) | −0.4 (1.0) | **1.0 (0.7;1.3)*** | 0.0 (−0.3;0.3) |
| Z-LCI | 0.7 (1.3) | **1.0 (1.2)** | **0.9 (1.0)** | 0.3 (−0.1;0.7) | −0.0 (−0.4;0.4) |
| Z-$FRC_{pleth}$ | **1.0 (1.0)** | **0.9 (1.0)** | **1.0 (1.5)** | −0.2 (−0.5;0.2) | 0.2 (−0.2;0.5) |

***Footnote:*** *Results from healthy controls are not shown in the table. Results highlighted in bold denote significant difference ($p<0.05$) in lung function between CF and healthy controls at the three test occasions and the interval change between the test occasions.* *Represent significant difference observed in $FEV_{0.5}$ in CF infants at 1 yr to 3m ($p<0.001$).*

Although tracking of lung function may be present in clinically diagnosed CF infants[60] and children[102] this does not appear to be so clearly demonstrated in this NBS CF cohort. As shown in **Figure 7-ii**, as a group analysis, there was weak correlation in LCI from 1yr to 2yrs with better correlation observed in $FEV_{0.5}$. **Figure 7-iii** demonstrates individual lung function measurements of CF infants with abnormal lung function detected at 3m of age and the evolution of lung function within the first 2 years. For individual patients, variation in lung function was evident in the first two years of life. Lung function at 1yr was not predictive of that at 2yrs of age. These inconsistencies suggest that perhaps lung function should wait until preschool years for objective outcomes. These results are currently being prepared for publication.

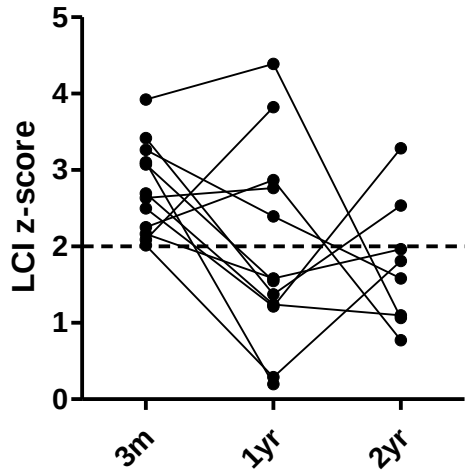
**Figure 7-ii: Association between 1-year and 2-year lung function in NBS CF infants.**



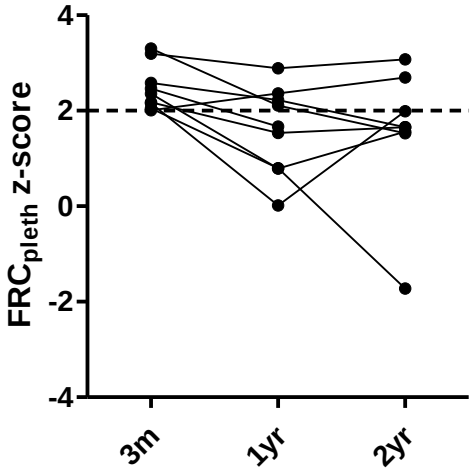

***Legend:*** *Cross plots showing LCI (a) and $FEV_{0.5}$ (b) at 1yr vs 2yrs of NBS CF cohort. R: Correlation coefficient using Pearsons correlation analysis.*
*(a)Those with abnormally high LCI at 1-year all normalised such that none was abnormal by 2yr (green box), yet those with abnormal LCI at 2yr all had normal LCI at 1yr (brown box). (b)Those with abnormally lower airway function at 1yr all normalised by 2yr (green box) whilst those with low airway function at 2yr were normal at 1yr (brown box). Hence lung function at 1yr is not predictive of that at 2yrs.*

**Figure 7-iii: Longitudinal lung function results of CF infants with abnormal lung function at 3months**

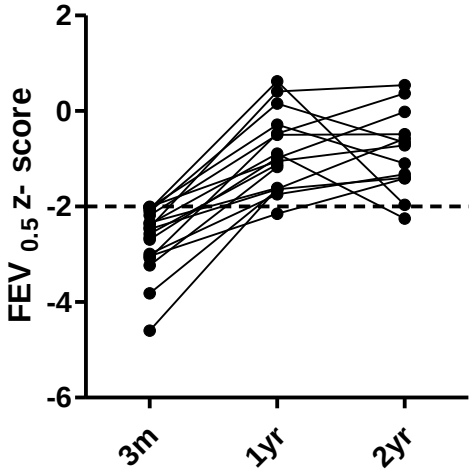
(a)




(b)




(c)




***Legend:*** *(a &b)Majority of CF infants who had abnormally high LCI and $FRC_{pleth}$ z-score at 3m of age improved by 1yr with sustained improvement seen at 2y. (c) All with abnormally low $FEV_{0.5}$ z-score improved significantly at 1yr with normal airway function by 1yr which was sustained through to 2yr.*

Although spirometric measurements have long been recognised as a valid surrogate measure for lung disease in the older CF population and have been shown to be related to structural lung damage and clinical parameters such as pulmonary exacerbations,[145,155] quality of life[247] and survival,[248] it is well recognised that they are relatively insensitive to early CF lung disease.[77,85,102] While LCI has been shown to be more sensitive in detecting early lung disease than spirometry[64,83,90,107] with more centres advocating its use for clinical purposes and as a clinical trial endpoint, the long term clinical implication of an abnormal LCI has yet to be established. Among clinically diagnosed CF children, preschool LCI predicted the LCI at school-age.[102] It will be important to continue to monitor LCI in these NBS CF infants beyond the first year of age to ascertain whether the same evolution can be observed in the NBS cohort. LCI and physiological gas trapping (ΔFRC) may represent dynamic changes dependent on current or recent clinical status which are reversible with appropriate treatment. This would make LCI and ΔFRC potentially sensitive short term measures for monitoring response to an intervention but would make them less suitable for predicting long term prognosis.

Despite our attempts only to study children when well, the effects of any exacerbations may last several weeks and it is possible that mild abnormalities in lung function in infants and young children primarily reflect the current clinical status with respect to airway inflammation and/ or mild obstruction and ventilation inhomogeneities due to mucus and secretions, and as such are far more reversible than when such changes are observed in later childhood. Further research, including follow up through the preschool and school-age years is essential to address these uncertainties.

### 7.5.2 Future validation studies of commercial lung function equipment

The ability to undertake longitudinal studies during infancy is currently limited by the time consuming nature of the tests and the need for sedation. Equipment required for infant lung function testing has cost implications. In the past, most MBW studies were performed using mass spectrometry and $SF_6$ as a tracer gas, both of which are available only in specialist centres. This has impeded the universal use of MBW

technique to measure ventilation inhomogeneity. Reference data for LCI are limited when using commercial equipment in infants and young children and are currently only available from assessments over a limited age range using $SF_6$ in infants.[105]

There are three commercially available recording systems (ultrasonic mainstream Exhalyzer D Ecomedics,[86] ultrasonic sidestream EasyOne Pro Lab NDD,[249] and infrared Innocor Innovision[91]) that are potentially available for use in children. In view of the different deadspace or response time, measurements are currently possible for children from at least 5 years of age for the two ultrasonic systems and from 10 years of age with the infrared system. Modifications to these commercially available systems are required for their use in younger children and infants. Rigorous quality control is essential for consistent reporting of lung function results if these systems are to be utilised for clinical or research purposes.[237] Studies involving older children and adults are available using nitrogen washout with the ultrasonic systems[72,249] whilst the infra- red system is based on the use of $SF_6$ as inert gas washout.[91] Only the Exhalyzer D Ecomedics system has been modified for use in infants based on $SF_6$[86,121] but recent adaptations have been designed to allow this system to be applied to infants using nitrogen washout.[250]

As mentioned in chapter 1 (**section 1.4.2.3**), the influence of hyperoxia during nitrogen washout on the respiratory control of infants is unclear. In a recent study by Singer *et al*,[250] CF and HC infants (n=31; age range 3-13 weeks were studied during natural sleep whilst older infants (n=8) age range of 13-14 months were sedated with chloral hydrate) were prospectively allocated to protocols comprising of classical 1-step nitrogen washout protocol with a switch from ambient to 100% oxygen or to a new 2-step protocol, introducing infants first to 40% oxygen for 30 breaths before the introduction of 100% oxygen. LCI was measured using a mainstream ultrasonic flow meter (Exhalyzer D, Ecomedics). This is the first published study to report the feasibility of using this approach in healthy and CF infants even though it does influence the ventilatory control of infants. However if infants were first introduced to 40% oxygen prior to the nitrogen washout with 100% oxygen, this appeared to induce tolerance to hyperoxia with less impact on the quality of tidal breathing. The preliminary safety result looked promising and will warrant further work to investigate the use of nitrogen washout commercial systems in infants.

A recent study has compared $N_2$-LCI using the Exhalyzer D and $SF_6$-LCI using Amis 2000 mass spectrometer in 62 HC and 61 CF children from 3-18 years old.[251] In CF children, $N_2$-LCI was higher than $SF_6$-LCI and the difference in values found between the two systems was double that seen in HC which in contrast demonstrated good agreement between the two systems. There was a clear bias towards disproportionately higher LCI obtained through $N_2$ washout than $SF_6$; the higher the mean values were for LCI, the higher the discrepancy. The authors concluded that although both systems have similar discriminative power and repeatability, values obtained by both systems were not inter-changeable. Any future studies should include independent normative values. It would simply be inappropriate and scientifically unfounded to switch from $SF_6$ to $N_2$ LCI without further validation studies. Important issues such as problems associated with the indirect measurement of nitrogen, the physiological effects of pure oxygen in young children and infants and the back diffusion of nitrogen from blood and tissue within the time frame of a normal washout test must be addressed.

In addition to utilising commercial systems for MBW tests, there are research studies in progress to determine the possibility of reducing testing time for MBW which will be particularly useful when measuring infants and pre-schoolers. Adult MBW guidelines recommend three technically acceptable tests with FRC values within 10% whilst preschool guidelines recommend two such tests for the calculation of LCI. No recommendations were made for infants although for the purpose of this study and other validation studies, three technically acceptable tests were used. Robinson *et al*[252] in a retrospective study demonstrated that if adult MBW guidelines were used in paediatric subjects, LCI would not be routinely applicable across the paediatric age range and would lengthen the test session. Using two technically acceptable tests irrespective of FRC repeatability did not significantly affect the mean LCI or compromise the sensitivity of the test to detect abnormal peripheral airway function in CF subjects. The findings from this cohort will need further replication and corroboration in other cohorts using cross sectional and interventional study designs and in other disease groups. The ability to shorten test duration without compromising quality would be beneficial for incorporating the assessment of MBW for routine clinical testing protocol.

### 7.5.3 The evolution of mild chest CT changes and its clinical impact

Bronchiectasis and trapped air are important components of end stage CF lung disease.[253] Total CT scores and other sub-scores (except bronchiectasis sub-score) have been shown to improve with treatment in previous studies.[144,147] Although strictly speaking bronchiectasis is irreversible structural change, the AREST-CF longitudinal study has shown improvement in the bronchiectasis sub-score.[108] This 'apparent improvement' in bronchiectasis suggests that when changes are very mild, there may still be reversible. Therefore if changes observed were so mild in NBS CF infants that they normalised within a year, the clinical impact associated with these CT changes may not be significant enough to justify exposing all CF infants to a routine clinical chest CT at such a young age.

Before chest CT can be advocated for widespread usage, especially in infants and very young children, standardised CT scanning protocols, which can be readily applied across multiple centres are also essential. Given the radiation burden and the costly expense of even limited, low dose annual CT scans, it is essential to ensure that information obtained is useful. Indeed there is a strong case for a randomised controlled study of whether CT actually improves outcome, analogous to the recent Australasian bronchoscopy study.[254] It will be important to study the effects of antibiotics, mucolytics or disease modifiers in the development of bronchiectasis to further validate the response to treatment. It would also be necessary to demonstrate the effect of an intervention on the CT score and to predict the effect on true clinical endpoints such as respiratory tract infections and quality of life which have been seen in a few studies involving older children and adults.[145,148,149,155,169,248]

Furthermore, the clinical significance of 'mild' changes detected on chest CT is not known and would require repeat follow up scans to monitor the evolution of these CT changes, which in itself has ethical implications with respect to subjecting CF infants to potentially unnecessary ionising radiation without current evidence of any real benefit. This highlights the urgent need for a randomised study in assessing the usefulness of routine clinical CT scans in infants and young children. There are currently several international consensus groups who are trying to tackle the problems associated with standardising the acquisition of images, establishing

scoring systems for mild disease for clinical monitoring[255] or investigating the feasibility of CT as an outcome measure in trials.[52,110,136,256]

### 7.5.4 Validated CT scoring system as trial endpoint in infants

Although emerging technology may allow high quality volumetric inspiratory and expiratory chest CT images to be obtained at low radiation doses, image analysis remains problematic. This study has shown that due to the mild subtle changes observed, scores from the commonly used Brody- II CF CT scoring system, were not reproducible in NBS CF infants at a year of age and hence not suitable as a research trial endpoint in such infants. A more robust approach to CT scoring in CF infants may be required. Analysis strategies that can identify early disease more sensitively than dichotomous outcomes for a given abnormality may reflect disease heterogeneity better than a crude discrete score with low resolution. International collaborations are investigating improved scoring systems for documenting mild disease. Current relatively subjective methods by expert scorers could be augmented by publishing visual standards for comparison or by the more widespread use of formal airway measurements and quantitative assessment of air-trapping through semi or fully automated scoring software.[147,257] While expert visual scoring like the Brody scoring system may include more features of CF lung disease than can be evaluated by automated software, it is time consuming, labour intensive and limited by the number of expert scorers. In contrast, automated computer analysis does not require specially trained scorers which may avoid inter-observer bias and variability, offer better standardisation and may be more sensitive in detecting subtle changes.[257] Automated systems are able to report on the number of visible airways seen especially in the periphery which may be a surrogate of bronchiectasis on CT scans, whilst air trapping due to small airway obstruction may be indicated by abnormally low attenuation on expiratory CT images which have been quantified using a variety of lung density approaches.[147,167,257,258] Such systems may improve the accuracy of chest CT as a surrogate endpoint. However, currently available automated quantitative scoring systems are still in their infancy and require extensive validation in CF patients of all ages, during both cross sectional and longitudinal studies. Furthermore, whether CT changes are scored by expert observers or through automated CT software, it is extremely important to establish what CT changes are

clinically relevant, which changes are responsive to the different interventions and the time taken for these changes to occur; hence necessitating the need for future comparative and longitudinal studies to establish the trends of different CT features over time. A greater understanding of these CT changes may allow the development of suitable inclusion and exclusion criteria during the recruitment process as well as the study design for interventional trials involving different therapeutic options[259] using CT as trial endpoints.

### 7.5.5 Magnetic resonance imaging of chest as a possible imaging modality

Magnetic resonance imaging (MRI) of the chest is gaining popularity as a radiation free imaging modality and has been shown to be comparable to chest CT in detecting morphological changes in children and adults with stable CF lung disease.[260] In a study involving 35 CF patients from infancy to adults (range 0.5- 42 years, mean 15.3 years, median 15 years with interquartile ranges 8-20 years) with a wide spectrum of CF lung disease, MRI scores allocated were reproducible for a wide spectrum of disease and were equally reproducible at milder end of the disease spectrum. In addition, MRI scanning has the added advantage of being able to assess functional changes such as pulmonary perfusion[260] through the use of contrast during scanning which may allow differentiation between reversible and irreversible lung changes.

The use of hyperpolarised helium MRI may provide high resolution images of lung ventilation that correspond to ventilation inhomogeneity measured through gas washout methods. In a study of 4 CF patients (age 11.8 [SD 2.9] years with $FEV_1$% predicted 95[13]), preliminary results showed that ventilation abnormalities were observed more readily using hyperpolarised helium MRI compared to LCI or conventional lung function tests, hence suggesting the possibility of using such an imaging modality for detecting early CF lung disease.[261] Although more validation work is underway in the field of MRI and CT imaging, more research is still needed to correlate MRI findings with CT images and lung function, and to correlate changes seen on imaging with long term clinical outcomes, especially in infants and young children with early and mild disease where this form of imaging may be

particularly useful. However, before the use of such an imaging technique becomes common place, it is important to realise that its use will be limited by the lack of specialised facilities and the high costs involved for utilising this sort of imaging for routine clinical care.

## 7.6 FINAL CONCLUSIONS

NBS for CF allows early diagnosis and implementation of treatment to optimise nutrition and pulmonary health, potentially leading to a marked improvement in the prognosis of CF. In this study, despite early diagnosis and protocol-driven treatment in specialist centres, abnormal anthropometry and lung function were evident in many NBS CF infants by 3m of age. Increased ventilation inhomogeneity, hyperinflation and diminished airway function were observed in CF infants compared to contemporaneous HC. Contrary to my primary hypothesis of continual deterioration in lung function within the first year, most CF infants showed marked improvements in spirometric measures of pulmonary function with no further deterioration in measures of ventilation inhomogeneity and gas trapping. Improvement in growth profile was seen such that no anthropometric differences existed when compared with HC. The majority of NBS CF infants in this study revealed minor or no bronchial dilatation and air trapping on CT at a year. Minimal associations existed between CT changes and lung function with weak correlations reported between CT air trapping score with LCI and physiological "Trapped gas". The clinical significance of this association is not well understood and will require a longitudinal follow up before these findings can be interpreted appropriately. Hence secondary hypotheses have also been disproved through results arising from this study.

Parental attitudes to involvement in this study were positive, and both recruitment and retention of subjects were excellent,[191] providing important evidence for the design of future early therapeutic intervention trials aimed at minimising or preventing lung disease in young children with CF.

Treatment has normally been targeted at the downstream consequences of *CFTR* dysfunction, but there has been a shift to develop genotype class-specific therapies. It

seems likely that these novel therapies will be most effective in early stage disease, before irreversible airway damage has developed. It is therefore essential to understand the evolution of lung function in NBS CF infants given standard treatment, in order to determine objective outcomes or trial endpoints. Through this study, lung function changes observed were mild. Furthermore, CT scoring of mild changes was poorly reproducible with different scorers and at different times. Despite increasing interest in the use of chest CT either clinically or as a trial endpoint,[110] this cannot be recommended for NBS CF infants in the first year of life until refinement of CT scoring has been established and validated in this age group.

Choosing correct trial endpoints for different interventions is vitally important as the methodologies used should be tailored to individual trials and the interventions studied. Adequately powered intervention studies that use objective measures of lung function and structure in an unselected cohort of infants will therefore need to be much larger than previously thought. It may be safer to defer novel CF therapies with potential toxicity to the growing lung until beyond the first year of life when objective measures may be carried out more easily in the preschool years. Ongoing follow up of the current cohort through to school age will help address the important question of whether detailed assessments of lung function, structure and inflammation in the first year of life help to predict future outcome.

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# 9 APPENDICES

A1 Publications and Poster Presentations; Literature Review Tables

A2 Parent Information Leaflets for CF and Healthy Control Infants

A3 Portex Breathing Tests in Babies Leaflet

A4 Consent forms For ILFT for CF and Healthy Control Infants

A5 Test Study Questionnaires (Initial and Subsequent) for CF and Healthy Control Infants

A6 Lung Function Test for Infants Record Form

A7 Standardised Treatment Protocol for CF Infants

A8 Clinical Record Form for CF Infants

A9 Infection Control and Cleaning of Lung Function Equipment

A10 Chest CT Scanning and General Anaesthesia Protocols

A11 Bronchoscopy and Broncho-alveolar Lavage Protocol

A12 Brody II Scoring System

# A1 Publication and Poster Presentations

- **Is Chest Computed Tomography Useful in Newborn Screened Infants with Cystic Fibrosis at One Year of Age?**
  **Thia LP**/ Calder A, Stocks J, Bush A, Owens CM, Wallis C, Young C, Sullivan Y, Wade A, McEwan A and Brody AS on behalf of the London Cystic Fibrosis Collaboration (LCFC)

- **Evolution of Lung Function during the First Year of Life in Newborn Screened Cystic Fibrosis Infants**
  Nguyen TTD/ **Thia LP**, Hoo AF, Bush A, Aurora P, Wade A, Chudleigh J, Lum S, and Stocks J, on behalf of the London Cystic Fibrosis Collaboration (LCFC)

- **Recruiting infants with CF and controls to an observational study**
  Chudleigh J, Hoo AF, Ahmed D, Prasad A, Sheehan D, Francis J, Buckingham S, Cowlard J, Lambert C, **Thia LP**, Nguyen TTD, Stocks J, on behalf of the London Cystic Fibrosis Collaboration (LCFC)

- **Lung Function is abnormal in 3-month-old infants with cystic fibrosis diagnosed by newborn screening**
  Hoo AF, **Thia LP**, Nguyen TTD, Bush A, Chudleigh J, Lum S, Ahmed D,Balfour Lynn I, Carr SB, Chavasse RJ, Costeloe KL, Price J, Shankar A, Wallis C, Wyatt H, Wade A, Stocks J, on behalf of the London Cystic Fibrosis Collaboration (LCFC)

- **Evaluation and use of childhood lung function tests in cystic fibrosis**
  Stocks J, **Thia LP**, Sonnappa S

- **New reference equations to improve interpretation of infant lung function**
  Nguyen TTD, Hoo AF, Lum S, Wade A,**Thia LP**,Stocks J

- **High Resolution Computed Tomography (HRCT) in One Year Old CF Newborn Screened (NBS) Infants: Not a Useful Outcome Measure**
  **Thia LP**, Calder A, Owens C, Stocks J, Bush A, Wallis C, Brody A, on behalf of the London CF Collaboration

- **Early detection of lung disease in infants with cystic fibrosis diagnosed by newborn screening**
  **Thia LP**, Stocks J, Hoo AF, Chudleigh J, Prasad SA, Lum S, Bush A, and Wallis C on behalf of the London CF Collaboration

THORAX

# Is chest CT useful in newborn screened infants with cystic fibrosis at 1 year of age?

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OPEN ACCESS

ORIGINAL ARTICLE

# Is chest CT useful in newborn screened infants with cystic fibrosis at 1 year of age?

Lena P Thia,[1] Alistair Calder,[2] Janet Stocks,[1] Andrew Bush,[3] Catherine M Owens,[2] Colin Wallis,[1,4] Carolyn Young,[2] Yvonne Sullivan,[2] Angie Wade,[5] Angus McEwan,[6] Alan S Brody,[7] on behalf of the London Cystic Fibrosis Collaboration (LCFC)

## ABSTRACT

**Rationale** Sensitive outcome measures applicable in different centres to quantify and track early pulmonary abnormalities in infants with cystic fibrosis (CF) are needed both for clinical care and interventional trials. Chest CT has been advocated as such a measure yet there is no validated scoring system in infants.

**Objectives** The objectives of this study were to standardise CT data collection across multiple sites; ascertain the incidence of bronchial dilatation and air trapping in newborn screened (NBS) infants with CF at 1 year; and assess the reproducibility of Brody-II, the most widely used scoring system in children with CF, during infancy.

**Methods** A multicentre observational study of early pulmonary lung disease in NBS infants with CF at age 1 year using volume-controlled chest CT performed under general anaesthetic.

**Main results** 65 infants with NBS-diagnosed CF had chest CT in three centres. Small insignificant variations in lung recruitment manoeuvres but significant centre differences in radiation exposures were found. Despite experienced scorers and prior training, with the exception of air trapping, inter- and intraobserver agreement on Brody-II score was poor to fair (eg, interobserver total score mean (95% CI) $\kappa$ coefficient: 0.34 (0.20 to 0.49)). Only 7 (11%) infants had a total CT score $\geq$12 (ie, $\geq$5% maximum possible) by either scorer.

**Conclusions** In NBS infants with CF, CT changes were very mild at 1 year, and assessment of air trapping was the only reproducible outcome. CT is thus of questionable value in infants of this age, unless an improved scoring system for use in mild CF disease can be developed.

### Key messages

**What is the key question?**
Is chest CT a reliable surrogate outcome as a clinical tool or as an end-point in clinical trials in 1-year-old infants with cystic fibrosis (CF) diagnosed by newborn screening?

**What is the bottom line?**
No, because structural changes detected on chest CT were generally very mild and, with the exception of air trapping, inter and intraobserver agreements on CT scores were poor using the standard Brody-II scoring system.

**Why read on?**
Chest CT is of questionable value in infants of this age and thus should not be used routinely; development of an improved scoring system for use in mild CF disease is urgent.

## INTRODUCTION

Widespread newborn screening for cystic fibrosis (CF) has resulted in early diagnosis and the potential for early intervention before changes in lung function and structure become irreversible. Sensitive outcome measures to quantify and track early abnormalities in infants and young children are needed both for clinical care and interventional trials. However, early intervention studies are likely to be of considerable duration and involve treatments with possible side effects. Such studies should therefore not be undertaken without ensuring that any risk is justified by a reasonable likelihood of obtaining useful information.

CT of the chest has been advocated as a sensitive surrogate measure of early lung disease,[1–4] since bronchiectasis and gas trapping have been detected in newborn screened (NBS) infants with CF,[5–7] and a recent international seminar concluded that chest CT was a useful outcome for interventional trials in very young children with CF.[8] Despite increasing publications in this field,[5–7 9 10] the challenges in obtaining standardised chest CTs at consistent lung volumes[11] with acceptable radiation exposure in infants, and also identifying a reproducible scoring system, sensitive to very mild lung disease, which can quantify severity of changes in NBS infants with CF, have received relatively little attention. The Brody-II CT score[12] is the most widely used and validated scoring system in CF[2 4 13–16] which quantifies lung disease objectively in school-aged children with good interobserver agreement,[2 12] but its usefulness in young infants with mild disease has not been established. Thus it is difficult to know whether changes identified in this population represent disease, normal variation or experimental error.

A longitudinal observational study of lung function and structure in NBS infants with CF by the London CF Collaboration (LCFC)[17 18] in which volumetric CT scans were obtained at 1 year of age, provided the opportunity to explore these challenges. Before starting the study, discussions were held with international experts, including those from the Australian Respiratory Early Surveillance

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Team for CF (AREST-CF), in order to standardise data collection. In the absence of any validated scoring system for use in NBS infants with CF, the Brody-II system was selected. We hypothesised that significant changes would be detected by 1 year of age but that interobserver agreement using Brody-II would be lower in NBS infants with CF than in older children, owing to the greater proportion of subjects with no, or only subtle, abnormalities.[2 15]

The aims of this study were to (a) standardise CT data collection across multiple sites to achieve consistent data quality with an acceptable radiation exposure, (b) ascertain the incidence of bronchial dilatation and air trapping in NBS infants with CF at 1 year and (c) assess the reproducibility of Brody-II in such infants by measuring inter- and intraobserver agreement of scores.

## SUBJECTS AND METHODS

### Study subjects

NBS infants with CF born between 2009 and 2011 were referred by one of six specialist LCFC centres for this study.[17 18] Chest CT scanning under general anaesthesia (GA) was performed at three of these centres using standardised protocols at ~1 year of age as part of the study protocol. The study was approved by the North Thames multicentre research ethics committee (#09/HO71/314). Informed written parental consent was obtained (section 1, see online supplementary data).

### Protocol for controlled ventilation during GA

Infants were intubated and ventilated (section 2, see online supplementary data). Atelectasis was minimised by using slow inflations to a peak inspiratory pressure (PIP) of ~35 $cmH_2O$ while maintaining a positive expiratory pressure (PEEP) of 5 $cmH_2O$[19] before the scan. Inspiratory scans were obtained during a breath-hold at 25 $cmH_2O$ PIP, and expiratory scans at 0 $cmH_2O$. Initial adherence to protocols was variable across centres. Consequently, a team member monitored ventilation (see online supplementary table E3) using the $NICO_2$ respiratory monitor (Philips Respironics, USA).[20] (see online supplementary figure E3 and figure E4.)

### Thin-section CT scan protocol

CT scans were performed using multidetector CT units (see online supplementary table E1). A predetermined technique for volumetric CT image acquisition was used (see online supplementary table E2; section 3, see online supplementary data). Scanning ranges for inspiratory and expiratory scans were tailored for each infant. The planned radiation dose range for the entire scan was ≤2.0 mSv with a target of ~1.5 mSv (annual background radiation exposure in the UK ~2.5 mSv).[21–23]

### Scoring methodology

CT data collection was completed and scans anonymised before starting scoring. Studies were scored independently without clinical or laboratory information by two scorers (AB: 25 years' paediatric chest CT experience, 13 years' scoring CF lung disease; AC: 7 years' paediatric chest CT experience, 5 years' scoring CF lung disease) using Brody-II scores.[2 12] Using this scoring system, comprising five components, the maximum possible subscore is 72 for bronchial dilatation, 27 for air trapping and 243 for total CT; higher scores indicating more severe disease.[12] (see online supplementary figures E1 and E2)

The two scorers scored 12 training scans provided by AREST-CF, undertaken with similar protocols in young children with CF aged 1–4 years.[5–7] These 'training scans' were scored in two batches of six (section 7, see online supplementary data). Scoring of LCFC scans took place within 6 weeks of completing training; scores from both observers being analysed and compared by LPT who was not involved in scoring. LCFC scans with discrepant subscores were returned to both scorers (without details of prior scores allocated) for subsequent reassessment to investigate whether closer agreement might be achieved (section 7, see online supplementary data). A selection of LCFC scans was completely rescored after ~8 months to assess inter- and intraobserver agreement over time.

### Statistical analysis

Data were inspected for distribution (PASW Statistics V.18, Chicago, Illinois, USA) and summarised using number (percentage), mean (SD) or median (IQR) as appropriate. Agreement between observers was assessed using Cohen's κ statistic with linear weighting (MedCal for Windows, statistical software V.12.3.0, Mariakerke, Belgium). κ Coefficients were similar whether analysed as non-weighted (results not shown) or with linear weighting. κ Results were interpreted as 0–0.2: poor agreement; 0.21–0.4: fair agreement; 0.41–0.6: moderate agreement; 0.61–0.8: strong agreement; 0.81–1.0: excellent agreement.[24] Ventilatory pressures and radiation doses between the centres were compared using Kruskal–Wallis with post hoc comparison using Mann–Whitney U tests; adjusted for multiple comparisons so that the family-wise error rate remained at 0.05.

## RESULTS

### Patient population

The study was conducted between January 2009 and May 2012[17 18 25]; chest CT scans at 1 year were performed in 65 NBS LCFC infants. Table 1 summarises clinical details of the infants. At the time of chest CT, all infants were clinically well with no respiratory symptoms.

### Verification of adherence to protocols

PEEP during the recruitment inflations was slightly higher than intended (overall median (95% CI) PEEP 7.2 (5.4 to 8.8) $cmH_2O$, and was significantly higher in centre B than C ($p=0.012$; see online supplementary table E4). PIP during inflation manoeuvres and end-inspiratory breath-hold was close to protocol specifications, with no significant differences between centres.

### Radiation doses

Median effective radiation exposure across all centres was 1.5 (1.2 to 1.8) mSv, with centres A and B achieving median doses close to the target dose of 1.5 mSv; exposure was significantly higher at centre C (see online supplementary figure E5 and table E5; overall Kruskal–Wallis $p<0.0001$). Exposures of ≤1.5 mSv were achieved in 58% of infants; 79% received an effective dose of ≤2 mSv. Three infants in centre C received ≥3 mSv; two owing to suboptimal positioning.

### Training scan scoring

Interobserver agreement was, on average, fair for bronchial dilatation during training batch 1 ($\kappa=0.27$ (95% CI 0.08 to 0.46)) and, after a video conference to discuss discrepancies, moderate for training batch 2 ($\kappa=0.45$ (0.17 to 0.72)). During both training sessions greatest agreement was observed for air trapping ($\kappa=0.82$ (0.68 to 0.95) for training batch 1 and 0.79 (0.67 to 0.92) for batch 2) (see online supplementary table E6 and figure E6).

**Table 1** Clinical features of infants with NBS-diagnosed CF undergoing CT at 1 year of age

| Features | Value |
|---|---|
| N (% boys) | 65 (48) |
| Age at diagnosis, weeks* | 3.4 (3.0–4.4) |
| Pancreatic insufficiency, n (%) | 61 (94) |
| Meconium ileus, n (%) | 7 (11) |
| Delta F508†, n (%) | 58 (89) |
| Age at time of test, weeks | 52.7 (4.7) |
| *Somatic growth* | |
| Weight, z score‡ | 0.34 (0.10) |
| Length, z score‡ | 0.49 (0.97) |
| Body mass index, z score‡ | 0.09 (0.84) |
| *Before 1 year CT assessments* | |
| Respiratory symptoms, ever: | |
| Wheeze, physician-diagnosed | 22 (34) |
| Crackles, physician-diagnosed | 7 (11) |
| Bacterial growth on cough swab, ever§ | |
| *Pseudomonas aeruginosa*¶ | 20 (31) |
| Other significant bacterial growth** | 24 (37) |
| No growth†† | 21 (32) |

Results expressed as mean (SD) or n (%) unless otherwise stated.
*Median (IQR).
†Homozygous or heterozygous.
‡Calculated according to Cole *et al*.[26]
§Based on the presence of bacteria ever isolated in the first year.
¶Definition of colonisation according to Lee *et al*[27]; only 2/65 (3%) infants had any evidence of *Pseudomonas aeruginosa* (*PsA*) on bronchoalveolar lavage or cough swab within 5 days of the CT scan.
**Significant bacterial growth consisted of those who had *methicillin-sensitive* or *methicillin-resistant Staphylococcus aureus (MSSA or MRSA*, respectively), *Haemophilus influenza (HI), Stenotrophomonas maltophilia, Acromobacter xylosidans* or *Aspergillus fumigatus* with no previous growth of *PsA*.
††No bacterial growth consisted of those with isolation of coliforms and upper respiratory tract flora only.
CF, cystic fibrosis; NBS, newborn screened.

### LCFC scan scoring

The first round of scoring the LCFC scans (*initial LCFC,* n=65) started within 6 weeks of training and was completed within a month. Complete rescoring of a selected subset of LCFC scans (*rescoring LCFC;* n=22) occurred ~8 months after the initial scoring. As can be seen from table 2, changes were generally very mild, with only seven (11%) infants having a total CT score ≥12 (ie, ≥5% of maximum possible Brody score) according to scorer B, and only two (3%) according to scorer A.

#### Interobserver agreement between initial and rescoring LCFC rounds

Although discrepancies between scorers with respect to at least one subscore occurred in 50/65 scans, 90% of differences were between a score of 0 (normal), and 1 (minimal to mild disease) (table 2). There was fair agreement for bronchial dilatation and strong agreement for air trapping, both during initial scoring of all 65 LCFC scans and when rescoring (table 3). Scans selected for rescore were representative of those from the entire cohort for the number and severity of changes detected on CT (figure 1).

Scorer B identified more infants with CT changes and generally allocated higher scores than scorer A during initial scoring of LCFC scans, the reverse of that seen during training (see online supplementary figure E6). Scores for air trapping and total score were more similar between scorers during rescoring (figure 1). κ agreement between scorers was initially only fair for bronchial dilatation, with minimal improvement during rescoring, but agreement about the presence or absence of bronchial dilatation or air trapping was consistently achieved in >80% of the scans on initial and rescoring rounds (see online supplementary table E7).

#### Intraobserver agreement between study rounds

Intraobserver agreement after ~8 months was only fair for bronchial dilatation (scorer A: κ=0.24 (−0.13 to 0.60); B=0.35 (−0.06 to 0.76)) but strong for air trapping (A: κ=0.72 (0.59 to 0.85); B: κ=0.72 (0.55 to 0.88)). For total CT score, scorer A showed strong while scorer B showed moderate agreement (A: κ=0.66 (0.42 to 0.90); B: κ=0.51 (0.29 to 0.73)) (see online supplementary figure E7). Both scorers detected an identical proportion of changes when rescoring but those identified were not necessarily for the same infants. Challenges were faced in discriminating between very mild changes potentially attributable to bronchial dilatation or airtrapping and normal, even by those with considerable expertise, is illustrated in figure 2.

## DISCUSSION

This is the first study specifically to assess the reproducibility, and hence validity, of CT evaluation of lung disease in infants with CF. Despite the scoring being undertaken by experienced observers with prior training, with the exception of air trapping, the Brody-II score was not reproducible in this age range. The obvious interpretation of these results is that the mild nature of any CT changes at 1 year of age precluded reproducible evaluation of most parameters. A label of bronchial dilatation in the presence of very mild lung disease should therefore be applied cautiously, at least using current methods and definitions. These findings, together with the technical difficulties in standardising acquisition of CT scans across sites, suggest that the use of CT both clinically and as an endpoint in multicentre trials of infants remains extremely challenging.

### Strengths and limitations of the study

Standardised protocols for GA, scanning parameters and image acquisition were established to ensure consistent, reliable CT data were obtained between centres. This is the first study to monitor adherence to a specific CT ventilation protocol objectively. Use of both inspiratory and expiratory volumetric scans to evaluate lung disease (the first such study in NBS infants with CF at 1 year[28 29]) reduces the risk of missing subtle abnormalities, thereby increasing the likely accuracy of the reported changes.

We evaluated Brody-II in infants, as previously undertaken in older subjects with CF, by measuring inter- and intra-agreement of scores by two highly experienced scorers, who underwent training using scans from young children with CF immediately before scoring the LCFC scans in an attempt to ensure consistent interpretation.

The main limitation, as with similar studies, was the lack of normal CT scans for comparison owing to concerns about radiation exposure in healthy individuals. Since clinical CT scans in children with normal lungs (eg, screening for metastases) would not include expiratory images, even this group would not provide adequate controls. In addition, at the time of study, few training scans from 1-year-old NBS infants with CF were available. Owing to the time-consuming nature of the reproducibility studies, no other CT scoring system was used, but given that most use components which at least overlap with Brody-II, it is unlikely that the results would have been very different.

### Radiological evidence of structural lung disease

Although AREST-CF detected structural abnormalities in 81% of NBS infants with CF at a median age of 3.6 months,

**Table 2** Interobserver agreement for CT scores allocated to infants with NBS-diagnosed CF at 1 year of age during initial scoring of LCFC scans (n=65)

**(a) Bronchial dilatation (maximum possible score=72)**

| κ=0.21 (0.05; 0.37) | Scorer A | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| Scorer B | | | | | | |
| 0 | 48 | – | – | – | – | |
| 1 | 6 | – | – | – | – | – |
| 2 | 4 | 3 | 1 | – | – | – |
| 3 | 1 | – | – | – | – | – |
| 4 | 1 | – | – | – | – | – |
| 5 | – | 1 | – | – | – | – |

**(b) Air trapping (maximum possible score=27)**

| κ=0.66 (0.49; 0.83) | Scorer A | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 7 | 8 | 15 | 16 |
| Scorer B | | | | | | | | | | |
| 0 | 37 | – | 1 | – | – | – | – | – | – | – |
| 1 | 6 | 3 | – | – | – | – | – | – | – | – |
| 2 | 3 | – | 1 | 1 | – | – | – | – | – | – |
| 3 | 1 | 1 | 1 | 2 | – | – | – | – | – | – |
| 4 | – | – | – | – | 2 | – | – | – | – | – |
| 5 | – | – | – | – | 2 | – | – | – | – | – |
| 7 | – | – | – | – | – | 1 | – | – | – | – |
| 8 | 1 | – | – | 1 | – | – | – | – | – | – |
| 15 | – | – | – | – | – | – | – | – | 1 | – |
| 16 | – | – | – | – | – | – | – | – | – | – |

**(c) Total CT score (maximum possible score=243)**

| κ=0.34 (0.20 to 0.49) | Scorer A | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 12 | 13 | 14 | 17 | 19 | 25 | 30 |
| Scorer B | | | | | | | | | | | | | | | | | | |
| 0 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 7 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 5 | – | 2 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 3 | 1 | – | 2 | – | 1 | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | – | 4 | 1 | 1 | – | 1 | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 1 | – | 1 | – | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 1 | 1 | 1 | – | – | 1 | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 1 | – | – | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | – | – | – | – | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 10 | – | – | – | – | – | – | – | – | – | – | 1 | – | – | – | – | – | – | – |
| 12 | – | – | – | – | – | 1 | – | – | – | – | – | – | – | – | – | – | – | – |
| 13 | – | – | – | – | – | 2 | – | – | – | – | – | – | – | – | – | – | – | – |
| 14 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 17 | – | – | – | – | – | – | – | 1 | – | – | – | – | – | – | – | – | – | – |
| 19 | – | – | – | – | – | – | – | – | – | – | – | 1 | – | – | – | – | – | – |
| 25 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 30 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 1 | – |

Shaded cells across the diagonals within each table represent identical results by scorers A and B using the Brody-II scoring system. For air trapping scores >5 and total CT scores >12, only those for which any values were obtained are shown. Although scorer B identified more abnormalities on scans than scorer A as indicated by values generally falling below the shaded diagonal cells (17 (26%) vs 5 (7%) for bronchial dilatation; 27 (42%) vs 17 (26%) for air trapping, the severity of changes were generally very minor, with only seven (11%) and two (3%) of patients having a total CT score ≥12 or 5% of the total possible score). κ=κ coefficient (95% CI) as a measure of agreement of CT subscores and total scores allocated by scorer A and B. It can be seen that the majority of discrepancies for bronchodilatation occurred when changes were deemed to be very minor[(1–3)] by one scorer and absent [0] by the other.
LCFC, London Cystic Fibrosis Collaboration; NBS, newborn screened.

bronchial dilatation was only found in 11/57 (19%) at this age,[6] and remained low through the first 2 years of life (~8% at both 1 and 2 years of age) before increasing markedly to ~36% by 4 years.[7] In the most recent publication from this group, prevalence of bronchial dilatation in children with CF during the first 4 years of life was ~60%,[10] ~80% of whom had evidence of bronchial dilatation at some time during the first 3 years. Bronchiectasis as classically defined refers to irreversible dilatation due to damaged bronchi. The 'apparent improvement' in bronchiectasis reported in some of the AREST children with CF

**Table 3** κ Values between both scorers during the initial LCFC scoring round of the entire LCFC cohort, (n=65) and during initial and repeat scoring rounds of the subset of 22 scans

| | Initial scoring (entire cohort: n=65) | Initial scoring* (subset: n=22) | Rescoring* (n=22) |
|---|---|---|---|
| Bronchial dilatation | 0.21 (0.05 to 0.37) | 0.38 (0.01 to 0.76) | 0.24 (−0.27 to 0.75) |
| Air trapping | 0.66 (0.49 to 0.83) | 0.58 (0.37 to 0.79) | 0.80 (0.67 to 0.93) |
| Total CT scores | 0.34 (0.20 to 0.49) | 0.38 (0.13 to 0.62) | 0.67 (0.48 to 0.86) |

Results presented as mean (95% CI) linear weighted κ coefficient.
*Scans from 22/65 LCFC infants were selected, results of which are summarised both for initial values and after rescoring.
LCFC, London Cystic Fibrosis Collaboration.

might be associated with mild and borderline normal bronchi (see below). The AREST-CF studies also report more air trapping (67% at ∼4 months,[6] 62% at ∼1 year[7] and 69% at ∼3 years[10]) than in this study. These discrepancies may be partially explained by the fact that in contrast to the AREST-CF study, LCFC children were only studied when asymptomatic. Bronchial dilatation was significantly more likely (60.0% vs 10.2% in asymptomatic) and more severe in AREST infants with CF with respiratory symptoms at the time of CT.[6]

Use of different scoring systems makes direct comparisons difficult, particularly when attempting to quantify severity of changes. While changes could be identified on at least one Brody-II subscore in 34/65 (52%) of the LCFC infants, the magnitude of these changes was often trivial. Important changes (defined either by visual inspection and/or a total CT score ≥5% maximum possible) were only detected in 2% of infants by scorer A and 11% by scorer B (table 2).

### Comparing inter- and intraobserver agreement of CT scores with other studies

The interobserver agreement when using Brody-II in NBS infants with CF contrasts with previous studies in older subjects

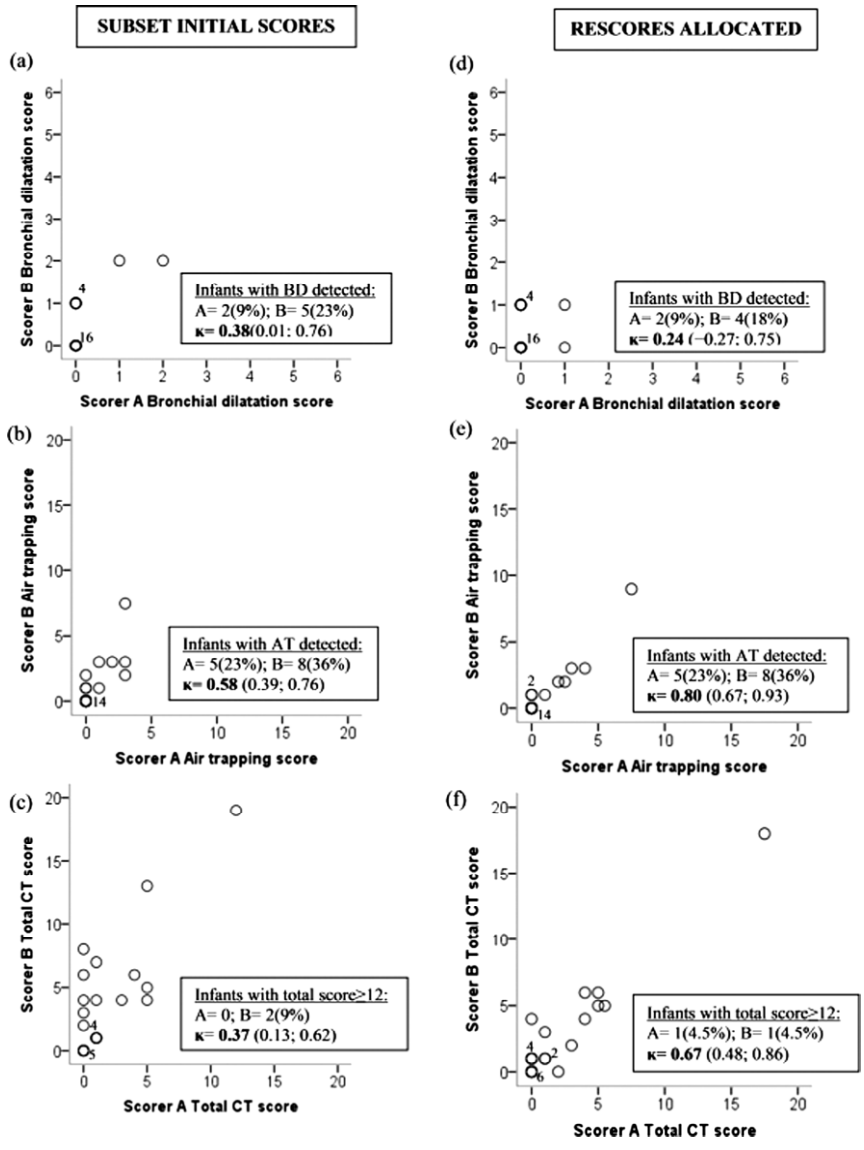


**Figure 1** Interobserver agreement between initial and rescoring London Cystic Fibrosis Collaboration (LCFC) rounds. CT scores were allocated by scorers A and B for the subset of 22 scans during initial and rescoring rounds. While all 22 pairs of results have been plotted, overlap of some data, particularly those with zero scores means that not all results can be identified individually. Bold circles represent data that overlaid each other, the number in brackets representing the number of infants with each combination of scores. During *initial scoring of the subset*, scores allocated by scorer B were generally higher than those by scorer A for bronchial dilatation and total scores (A and C). More consistent scores with good agreement were seen for air trapping (B). During *rescoring of this subset*, scores were more similar, although only fair agreement was again seen for bronchial dilatation (D), while good agreement was seen for air trapping and total scores (E and F). κ=κ coefficient (95% CI). AT, air trapping, BD, bronchial dilatation.

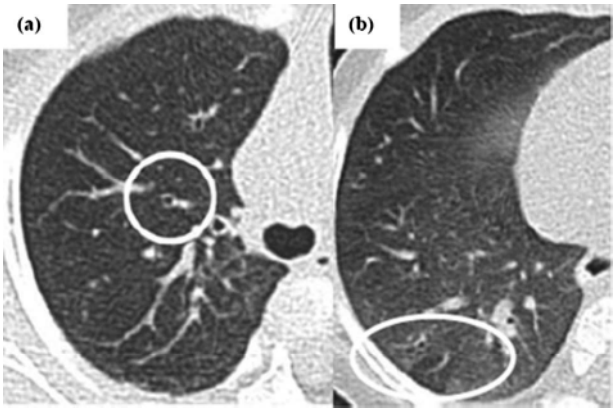


**Figure 2** Examples of CT images from infants with cystic fibrosis (CF) showing mild abnormalities in bronchial dilatation and air trapping leading to discrepancy in scoring. (A) An example of thin section CT of the left lung in an infant with CF taken at 1 year of age showing discrepancies in scoring bronchial dilatation (circled). This was scored as normal by scorer A, but mild by scorer B during the initial study round, whereas during the subsequent rescoring round ~ 8 months later, scorer A scored this as mild bronchial dilatation, while scorer B scored it as normal. (B) Subtle tiny areas of hyperlucency in some of the scattered secondary pulmonary lobules of the lower lobes in keeping with air trapping (ringed by oval). During the initial scoring round, scorer A scored this as mild air trapping while scorer B labelled it as no air trapping. During the rescoring round, both scorers allocated mild air trapping.

(including those in which scorers A and B participated, see online supplementary table E8). Previous studies have found that bronchial dilatation is the most reliably reproducible element when evaluating CF lung disease.[12 15 30] The relatively poor agreement in this study probably reflects the subtlety of changes seen. A single scorer scored all the AREST-CF scans with good intraobserver agreement after a 6–12-month interval[7] (see online supplementary table E8). Separate assessments for younger children in whom bronchial dilatation was infrequent and milder were not, however, reported. While use of a single dedicated observer to score all scans[6 7 9] might provide more consistent outcomes, such an approach is impractical in clinical practice and unlikely to be either generalisable or feasible in large multicentre trials. In the absence of measures of repeatability, the extent to which inter- and intraobserver variation contributes to the reported CT findings cannot be established.

### Definition of bronchial dilatation

Additional problems in interpreting CT scans relate to lack of international consensus on how to define bronchial dilatation, especially in infants. A bronchoarterial ratio (BAR) >1 as specified in Brody-II was used both in this study and CF-AREST. This speeds up evaluation, as judging whether the bronchus is bigger than the adjoining vessel can be assessed subjectively more easily than calculating a ratio. It has been suggested that a threshold of 0.76, rather than 1, should be applied in children,[31 32] but given the poor inter- and intraobserver agreement even when using BAR≥1 in infants with mild CF lung disease, it is unlikely that this would be effective. Furthermore, measuring changes in small bronchial luminal size to define bronchial dilatation may be beyond current CT spatial resolving ability. The accuracy of assessing BARs, especially in health, is also critically dependent on reliably achieving full lung inflations.[33]

### Technical challenges in acquiring standardised CTs

We experienced several challenges in performing thoracic CT in this age group. Despite clear protocols and briefing the anaesthetic and radiology teams across all centres, variability in the image acquisition parameters—namely, airway pressures and radiation doses delivered was seen. The greater variability in radiation doses in centre C might be due to their slightly different type of scanner (see online supplementary table E1) and/or the fact that it was not possible to organise a dedicated radiographer to perform procedures within that hospital, the latter being a problem likely to be found in clinical practice as well as multicentre trials. The presence of an investigator to monitor all procedures improved compliance, but is unlikely to be feasible in clinical practice or most clinical trials.

To date there is no consensus on the optimal method of acquiring CT scans in young children to ensure maximum information with minimal radiation exposure. After discussions with the AREST-CF team, we adopted their approach of obtaining end-inspiratory scans at 25 $cmH_2O$ PIP, and end-expiratory scans at 0 $cmH_2O$, together with recruitment manoeuvres to minimise procedure-related atelectasis. However, whereas we used a volumetric technique that images the entire lung volume, initial studies by AREST-CF consisted of three thin-slice scans during inspiration and expiration.[6 7 9] Limiting the dataset to three images, compared with ≥20 for the volumetric technique, severely limits the number of airways that can be evaluated. In addition, if bronchi were sampled and imaged at the point of bifurcation, this would overestimate the size of the bronchial lumen, potentially leading to overdetection of bronchial dilatation.

### Clinical implications

Results from this study suggest that both the acquisition and interpretation of CT scans need further evaluation before being applicable either as a research outcome measure or clinical tool in NBS infants with CF at least at 1 year of age. Based on the incidence of bronchial dilatation detected by both scorers in this study, between 190 and 850 infants per group would be required if a randomised trial such as the recent Ivacaftor trial[34] were to be extended to infants, in order to detect a reduction in bronchial dilatation of 50% with 90% power at a 5% significance level at 1 year of age; this number would rise further after accounting for those ineligible for such a trial or whose parents decline.[35] Suggestions that such a study would be feasible with only 100/group were based on the incidence of bronchiectasis at 4, not 1 year of age.[7] Since there is neither knowledge about the long-term clinical significance of mild changes detected in young infants with CF, nor any data to suggest that mild changes lead to alterations in clinical management or long-term clinical outcomes, it is questionable whether the risks of exposing young infants to additional ionising radiation outweigh the benefits. Indeed, as a result of this study, without specific clinical indications, chest CTs are no longer performed in NBS infants with CF at 1 year within the LCFC group.

## FUTURE DIRECTIONS

Before chest CT can be advocated for widespread use, especially in very young children, standardised CT scanning protocols, which demonstrably can be used in multiple centres, in combination with a reproducible scoring system with good intra- and interobserver agreement, are essential. Given the radiation burden and the expense of even limited, low-dose annual CT scans, it is essential to ensure that the information obtained is

useful; indeed there is a strong case for a randomised controlled study of whether CT improves outcome, analogous to the recent Australasian bronchoscopy study.[36] A more robust approach to CT scoring in infants with CF, in whom changes may be very mild, may be required; current relatively subjective methods could be augmented by publishing visual standards for comparison or by the more widespread use of formal airway measurements and quantitative assessment of air trapping.[37]

In conclusion, we do not believe that CT is ready for widespread clinical use or as a trial endpoint in the first year of life for NBS infants with CF. Until refinement of CT scoring has been established and validated, we recommend caution in reporting bronchial dilatation in NBS infants with CF, the incidence of which appears to be low in the first year of life.

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**Collaborators** London Cystic Fibrosis Collaboration (LCFC).

**Contributors** JS and AB were responsible for the conception and design of study; CMO, AC and ASB provided technical advice on imaging and scoring; AM provided anaesthetic advice. JS and LPT were responsible for supervision of the study and for research governance issues, including ethics committee approval. CY and YS supervised the CT imaging. LPT supervised and audited data collection and analyses. Infants with cystic fibrosis were recruited by the paediatric respiratory consultants participating in the London Cystic Fibrosis Collaboration, including AB and CW. LPT and AW performed statistical analyses; LPT, AC, ASB, AB and JS drafted the manuscript; all remaining authors revised and approved the manuscript for intellectual content before submission.

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# Evolution of lung function during the first year of life in newborn screened cystic fibrosis infants

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ORIGINAL ARTICLE

# Evolution of lung function during the first year of life in newborn screened cystic fibrosis infants

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## ABSTRACT

**Rationale** Newborn screening (NBS) for cystic fibrosis (CF) allows early intervention. Design of randomised controlled trials (RCT) is currently impeded by uncertainty regarding evolution of lung function, an important trial end point in such infants.

**Objective** To assess changes in pulmonary function during the first year of life in CF NBS infants.

**Methods** Observational longitudinal study. CF NBS infants and healthy controls were recruited between 2009 and 2011. Lung Clearance Index (LCI), plethysmographic lung volume (plethysmographic functional residual capacity ($FRC_{pleth}$)) and forced expired volume ($FEV_{0.5}$) were measured at 3 months and 1 year of age.

**Main results** Paired measurements were obtained from 72 CF infants and 44 controls. At 3 months, CF infants had significantly worse lung function for all tests. $FEV_{0.5}$ improved significantly (0.59 (95% CI 0.18 to 0.99) z-scores; $p<0.01$) in CF infants between 3 months and 1 year, and by 1 year, $FEV_{0.5}$ was only 0.52 (0.89 to 0.15) z-scores less than in controls. LCI and $FRC_{pleth}$ remained stable throughout the first year of life, being on average 0.8 z-scores higher in infants with CF. Pulmonary function at 1 year was predicted by that at 3 months. Among the 45 CF infants with entirely normal LCI and $FEV_{0.5}$ at 3 months, 80% remained so at 1 year, while 74% of those with early abnormalities remained abnormal at 1 year.

**Conclusions** This is the first study reporting improvements in $FEV_{0.5}$ over time in stable NBS CF infants treated with standard therapy. Milder changes in lung function occurred by 1 year than previously reported. Lung function at 3 months predicts a high-risk group, who should be considered for intensification of treatment and enrolment into RCTs.

### Key messages

**What is the key question?**
- Newborn screened cystic fibrosis (CF) infants have abnormal lung function by 3 months of age; how does this change during the first year of life?

**What is the bottom line?**
- Lung function remained stable or improved in newborn screened CF infants during the first year of life; deficits at 1 year were considerably smaller than previously documented in either screened or clinically diagnosed infants.

**Why read on?**
- This study, the largest of its kind and the only one with contemporaneous healthy controls, describes early lung development in newborn screened infants with CF; these data will inform the design of interventional trials in these children.

## INTRODUCTION

The major cause of morbidity and mortality in cystic fibrosis (CF) is pulmonary disease. Until recently, treatment has been targeted at the downstream consequences of *CFTR* dysfunction, such as bronchial infection, inflammation and mucus retention. A recent paradigm shift has, however, led to development of genotype class-specific therapies, such as $PTC_{124}$ to over-ride premature stop codons[1 2] and VX-770 for the class 3 mutation G551D.[3 4] It seems likely that these novel therapies will be most effective in early stage disease, before irreversible airway damage has developed. It is therefore essential to understand the evolution of lung function in newborn screened (NBS) CF infants given standard treatment, in order to determine optimal trial end-points and adequately power intervention studies.

CF infants diagnosed clinically have airflow obstruction at diagnosis, even in the absence of respiratory symptoms, signs or history of infection,[5] with no improvement in pulmonary function over the ensuing years despite specialist treatment.[6–8] CF NBS has been introduced in the hope that earlier diagnosis will lead to improved outcomes. Previous longitudinal studies investigating lung function in NBS CF infants reported progressive decline in the early years, despite specialist treatment.[9 10]

Following recent universal introduction of screening throughout the UK, we recruited a cohort of NBS infants with CF and healthy controls between 2009 and 2011. Disappointingly, only 56% of those with CF had normal pulmonary function tests (PFT) when assessed at 3 months.[11] The current manuscript describes follow-up PFTs at 1 year for this cohort. Our primary hypothesis was that lung function would deteriorate further between 3 months and 1 year of age. We also aimed to investigate the determinants of lung function at 1 year, and to

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collect data to assess feasibility of recruiting NBS CF infants to invasive studies[12] and inform future power calculations.

## METHODS

NBS CF infants born between January 2009 and July 2011 who were referred to the six specialist CF centres in the London CF Collaboration (LCFC) were eligible for recruitment.[11] Healthy controls were recruited contemporaneously from Homerton University Hospital, East London. Infants were ineligible if born <36 weeks gestation or had coexisting congenital abnormalities (see online supplementary data). The study was approved by the North Thames Multi-Centre Research Ethics Committee (#09/HO71/314). Informed written parental consent was obtained.

Participating centres prospectively completed Case Record Forms (CRF) at diagnosis and each subsequent clinic visit (see online supplementary data). CF infants were started on multivitamins and vitamin E, pancreatic enzyme replacement therapy where appropriate and, in accord with UK CF Trust guidelines, prophylactic flucloxacillin, according to a standardised treatment protocol (see online supplementary data).

### Infant PFTs

All infants were tested at Great Ormond Street Hospital/UCL Institute of Child Health at around 3 months and 1 year postnatal age. Infants were free of respiratory illness for at least 3 weeks before PFTs. Infants were weighed and examined prior to administering chloral hydrate orally or rectally (60–100 mg/kg). Weight and crown-heel length were expressed as z-scores to adjust for age and sex.[13] Heart rate and $SpO_2$ were monitored continuously throughout testing. Infant urine or maternal saliva samples were collected for cotinine assay to validate maternal report of smoking. PFTs were undertaken according to international guidelines.[14 15] Lung Clearance Index (LCI), a measure of ventilation inhomogeneity was measured by multiple breath washout (MBW), using mass spectrometry and customised software.[16] Plethysmographic Functional Residual Capacity ($FRC_{pleth}$) and forced expired volumes ($FEV_{0.5}$) and flows ($FEF_{75}$) from an inflation pressure of 30 cm $H_2O$ using the raised volume technique were measured using the Jaeger BabyBody device (CareFusion, San Diego, USA; V.4.65).[15] PFT results were electronically exported to a research database (Re-Base software, Re-Base, UK), which contained all relevant demographic and clinical details. PFT results were expressed as z-scores to adjust for body size, sex and age, using reference equations derived from healthy infants studied with identical equipment and protocols.[11 17–19] Abnormal PFTs were defined as results outside the 95% limits of normal: that is, >1.96 z-scores (>97.5th centile) for LCI and $FRC_{pleth}$ or <−1.96 z-scores (<2.5th centile) for $FEV_{0.5}$. Results were reported to the physicians responsible for the clinical care of each child, and subsequently discussed with parents.

### Statistical analysis

Data were inspected for distribution and calculation of descriptive statistics (PASW Statistics V.18, Chicago, Illinois, USA). Significance was taken as $p<0.05$. Lung function results at 3 months, at 1 year and changes between 3 months and 1 year were compared between groups using Student t-test. Multivariable linear regressions were used to investigate how lung function variables at 1 year, and change in lung function between 3 months and 1 year, varied according to potential determinants (background characteristics, clinical symptoms, antibiotic treatment and microbiological results, see online supplementary data for details). Model estimates and differences between groups are presented with 95% CIs. Multiple imputations were used to impute values for any failed PFTs at 3 months (see online supplementary data). Taking into account three primary outcomes (LCI, $FRC_{pleth}$ and $FEV_{0.5}$), a sample size of 72 infants with CF and 44 controls at 1 year (equivalent to 53/group if equal groups) allows detection of differences between groups equivalent to 0.66 z-scores at the 5% significance level with 84% power.[20–22]

## RESULTS

The screening, recruitment and follow-up of subjects are shown in figure 1. Paired measurements at 3 months and 1 year were obtained from 72 of 101 CF NBS infants, (90% of those tested at ~3 months of age). Inspection of CRFs and regular communication with consultants revealed excellent adherence to treatment protocols. Details of additional treatment are provided in the online supplementary data. Paired measurements were obtained from 44 contemporaneous controls (81% of those tested at 3 months). CF infants were born slightly earlier with lower birth weight than controls, but background characteristics were otherwise similar (table 1). There was no difference between groups regarding change in weight between birth and first PFTs at ~3 months (mean difference CF-controls: −0.14 (95% CI −0.56 to 0.29) weight z-scores).

For CF NBS infants, the median (IQR) age at diagnosis was 3.6 (3.0–4.4) weeks with 7 (10%) infants presenting with meconium ileus. Since inclusion of these infants did not affect the results (data not shown), they were included in the analysis. At 1 year PFTs, cough-swab cultures had been positive on at least one occasion for *Pseudomonas aeruginosa* in 25 (35%), and for other significant bacteria in 17 (24%).

### Anthropometry and lung function results

#### Comparison between infants with CF and healthy controls

Success in obtaining technically satisfactory PFTs were similar between groups, but varied by age and outcome, being lowest for $FRC_{pleth}$ at 3 months (76%) and highest for LCI (≥93% on both occasions; figure 1 and see online supplementary table E1). At 3 months, CF infants had significantly lower weight, height and body mass index (BMI); higher LCI and $FRC_{pleth}$; and lower $FEV_{0.5}$, forced vital capacity (FVC) and $FEF_{75}$ compared with controls (table 2). Significant increases in z-scores for somatic growth were observed in both groups between 3 and 12 months, but changes were significantly greater in CF infants, such that there were no between-group differences by 1 year. All PFT results remained stable in healthy infants during the first year of life, as did LCI, $FRC_{pleth}$ and FVC in those with CF. However, $FEV_{0.5}$ and $FEF_{75}$ z-scores improved between test occasions in CF infants (table 2, see online supplementary figure E1): differences in $FEF_{75}$ no longer being significant by 1 year when compared with controls. Although not one of the selected primary outcomes, there was a significant increase in gas trapping (as reflected by ΔFRC, ie, the within-subject difference in FRC assessed using plethysmography and MBW) in CF infants during the first year of life (table 2).

#### Determinants of lung function at 1 year

On linear univariable analysis, LCI, $FRC_{pleth}$ and $FEV_{0.5}$ z-scores at 1 year were significantly associated with CF status and 3 months PFT (see online supplementary table E3). Using multivariable linear regression, significant determinants of 1 year LCI z-score were: CF status (regression coefficient (95%

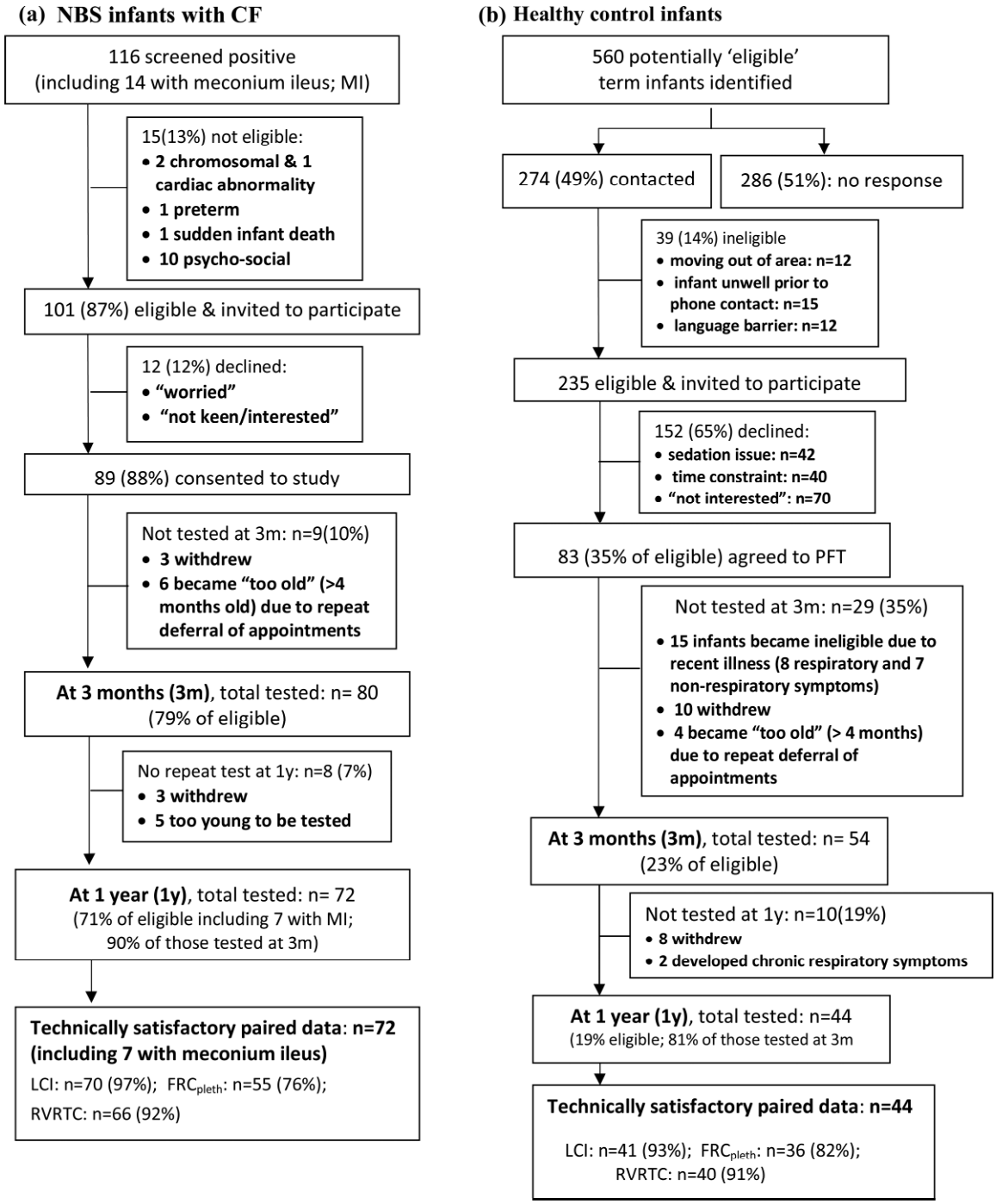


**Figure 1** Success rates for recruitment and achievement of technically acceptable infant pulmonary function data. NBS, newborn screened; PFTs, pulmonary function tests; LCI, lung clearance index; FRC, functional residual capacity; MBW, multiple breath inert gas washout; pleth, plethysmographic technique; RVRTC,Raised Volume Rapid Thoraco-abdominal Compression, from which forced expired flows and volumes were derived. See online supplementary table E1 for details of PFT success on each test occasion.

CI) 0.48 (0.04 to 0.93)), 3 month LCI (0.24 (0.07 to 0.41) per unit z-score), history of clinician-diagnosed wheeze (0.59 (0.05 to 1.12)) and change in weight z-score between birth and first PFT (−0.18 (−0.35 to −0.01) per unit z-score). For 1 year $FRC_{pleth}$ z-score, determinants were: 3 months $FRC_{pleth}$ (0.43 (0.27 to 0.59) per unit z-score), history of *PsA* infection (0.71 (0.24 to 1.17)) and change in weight z-score between 3 and 12 months (−0.20 (−0.41 to 0.0) per unit z-score change), whereas 1 year $FEV_{0.5}$ z-score was only significantly associated with 3 months $FEV_{0.5}$ on multivariable analysis (−0.18 (−0.35 to −0.01) per unit z-score).

### Relationship between PFT results at 3 months and 1 year

At 3 months of age, LCI, $FRC_{pleth}$ and $FEV_{0.5}$ were abnormal in 17% (12/71), 16% (9/57) and 26% (18/68) of CF infants, respectively. By 1 year, the percentage with abnormal $FEV_{0.5}$ had decreased to 9% (6/69) (mean difference (95% CI) −18% (−30% to −5%)) whereas those with abnormal LCI (18% (13/71)) and $FRC_{pleth}$ (16%(11/70)) remained virtually unchanged. Significant correlations were found between PFTs at 3 months and 1 year (figure 2 and see online supplementary table E2). Of the 52 infants in whom all three PFTs were technically successful on both occasions, abnormalities were observed in 33% (17/

**Table 1** Characteristics of CF and healthy controls infants with paired lung function at 3 months and 1 year

| | CF (n=72) | Controls (n=44) | Δ (95% CI) CF– controls |
|---|---|---|---|
| Male, n (%) | 34 (47) | 21 (48) | –1% (–19 to 18) |
| Gestational age, weeks | 39.1 (1.4) | 40.3 (1.1) | –1.1 (–1.6 to –0.6) |
| Birth weight, z-score* | –0.64 (0.84) | 0.12 (0.81) | –0.76 (–1.07 to –0.45) |
| Birth weight below 10th percentile*, n (%) | 13 (18) | 2 (5) | 14% (1 to 24) |
| White mother, n (%) | 61 (85) | 38 (86) | –2% (–14 to 13) |
| Maternal smoking during pregnancy, n (%) | 8 (11) | 3 (7) | 4% (–8 to 15) |
| Current maternal smoking†, n (%) | 9 (13) | 5 (11) | 1% (–13 to 13) |
| Maternal asthma, n (%) | 14 (19) | 8 (18) | 1% (–14 to 15) |
| Cystic fibrosis infants only | | | |
| Age at diagnosis, postnatal age (weeks) | 3.9 (1.7) | | |
| CFTR genotype (classes I–III)‡ | 59 (82%) | | |
| Presented with meconium ileus | 7 (10%) | | |
| Pancreatic sufficient | 5 (7%) | | |
| Respiratory symptoms ever prior to 1 year PFTs | | | |
| Wheeze, physician diagnosed | 24 (33%) | | |
| Crackles, physician diagnosed | 6 (8%) | | |
| Cough within 3 weeks of 1-year PFT | 15 (21%) | | |
| Bacterial growth on cough swab, ever§ prior to1 year PFTs | | | |
| *Pseudomonas aeruginosa, PsA*¶ | 25 (35%) | | |
| Other significant bacterial growth** | 17 (24%) | | |
| No growth†† | 30 (42%) | | |
| Additional treatment‡‡ prior to 1 year PFTs | | | |
| rhDNase | 6 (8%) | | |
| Intravenous antibiotics, number of courses | 0 (0; 3)§§ | | |
| GERD treatment | 38 (53%) | | |

Data shown as mean (SD) for continuous and n (%) for categorical variables unless otherwise stated.
*Calculated according to Cole *et al.*[13]
†Objectively validated by the analysis of cotinine levels.[23]
‡10% were classes IV–V and 8% unknown (or not classified).
§Swabs collected routinely in clinic at least every 2 months, prior to PFT and also when symptomatic.
¶Definition of colonisation according to Lee *et al.*[24] Only 1 infant had chronic *PsA.*
**Significant bacterial infection with no previous *PsA* ever included 12 (17%) with methicillin-sensitive *Staphylococcus Aureus,* 14 (19%) with *Haemophilus Influenzae,* 3 (4%) with *Stenotrophomonas maltophilia,* 2 (3%) with *Achromobacter xylosidans,* 3 (4%) with methicillin-resistant *Staphylococcus Aureus* and 2 (3%) with *Aspergillus fumigatus.*
††Included those with no growth, upper respiratory tract flora or isolated *E Coli* only.
‡‡In addition to the prophylactic flucloxacillin prescribed for all CF NBS infants from diagnosis.
§§Median (range).
Δ, mean difference between groups; CF, cystic fibrosis; GERD, Gastro-oesophageal reflux disease, n, number; NBS, newborn screened; PFT, pulmonary function test.

52) at first test, 10 (59%) of whom remained abnormal at follow-up. Of the 35 (67%) with entirely normal results at 3 months 25 (71%) remained so at 1-year.

### Identification of a high risk group of NBS CF infants

Based on results from LCI and $FEV_{0.5}$ which were the most feasible outcomes at 3 months (see online supplementary table E1), we attempted to delineate a subgroup of infants who would be at high risk of having abnormal lung function at 1 year, and who thus might be suitable candidates for an intervention study. Among the 64 CF infants in whom acceptable LCI and $FEV_{0.5}$ results were obtained on *both* occasions, abnormalities (elevated LCI *or* diminished $FEV_{0.5}$) were identified in 19 (30%) at ~3 months, of whom 14 (74%) remained abnormal at 1 year. Among CF infants with entirely normal PFTs at 3 months from these two tests (n=45), 36 (80%) remained so at 1 year (see online supplementary data for details). There were no significant differences at 1 year in $FEV_{0.5}$ (−0.36 (−0.9 to 0.17) z-scores) or LCI (0.46 (−0.13 to 1.05) z-scores) between CF infants with normal 3 months PFTs and healthy controls. By contrast, when compared with controls at 1 year, LCI was 1.33 (0.6 to 2.1) z-scores higher and $FEV_{0.5}$ −0.8 (−1.5 to −0.1) z-scores lower in those with abnormal PFTs by 3 months (see online supplementary table E3).

## DISCUSSION

Contrary to our hypotheses, forced expired flows and volumes improved by 1 year of age, with stability of other PFTs in NBS CF infants. This is the first time such improvement has been reported in an observational longitudinal study of NBS CF infants. The number of CF infants with abnormal LCI and $FRC_{pleth}$ at 1 year was similar to that at 3 months, while there was a significant reduction in those with abnormal $FEV_{0.5}$ during this period. Impaired lung function at 1 year was predicted by lung function at 3 months and associated with clinician-diagnosed wheeze (LCI), poor weight gain (LCI and $FRC_{pleth}$) and prior *P aeruginosa* ($FRC_{pleth}$).

### Strengths and limitations

The major strengths of this study are that longitudinal assessments of lung function were undertaken in a large cohort of NBS CF infants within a single location, results being directly compared with healthy controls. Attrition was minimal,[12] with no bias between those who did and did not complete the study. Selection of various PFTs enabled different aspects of pathophysiology to be assessed.[11] Appropriate reference equations for infant PFTs,[18 19] including LCI, which has now been shown to be dependent on body size during early life,[17] facilitated accurate interpretation of results. Limitations are that, in an

**Table 2** Comparison of anthropometry and pulmonary function at ~3 months and 1 year in CF NBS infants and healthy controls (HC)

| | 3 months | | | 1 year | | | Change over time (1 year–3 months) | | |
|---|---|---|---|---|---|---|---|---|---|
| | CF (n=72) | HC (n=44) | CF–HC* | CF (n=72) | HC (n=44) | CF–HC* | CF: Change 1 year –3 months† | HC: Change 1 year –3 months† | Difference in change‡ CF–HC |
| Age at test, weeks§ | 11.2 (2.3) | 12.1 (2.1) | –1.0 (–1.8 to –0.1) | 52.4 (5.3) | 53.7 (4.4) | –1.3 (–3.1 to 0.5) | 41.2 (37.3 to 43.1) | 41.6 (38.6 to 43.6) | –0.36 (–2.30 to 1.58) |
| Weight z-score¶ | –0.89 (1.03) | 0.01 (0.97) | **–0.90 (–1.27 to –0.52)** | 0.32 (0.90) | 0.55 (1.21) | –0.23 (–0.64 to 0.19) | **1.21 (1.02 to 1.40)** | **0.54 (0.28 to 0.80)** | **0.67 (0.35 to 0.99)** |
| Length z-score¶ | –0.21 (1.01) | 0.73 (0.92) | **–0.94 (–1.30 to –0.58)** | 0.47 (1.01) | 0.76 (1.20) | –0.28 (–0.71 to 0.15) | **0.68 (0.52 to 0.85)** | 0.03 (–0.19 to 0.25) | **0.66 (0.38 to 0.93)** |
| BMI z-score¶ | –1.08 (0.99) | –0.55 (0.96) | **–0.53 (–0.90 to –0.16)** | 0.08 (0.83) | 0.18 (1.12) | –0.10 (–0.49 to 0.29) | **1.15 (0.95 to 1.37)** | **0.72 (0.43 to 1.01)** | **0.44 (0.07 to 0.80)** |
| LCI z-score | 0.83 (1.32) | 0.36 (0.85) | **0.47 (0.06 to 0.87)** | 1.05 (1.23) | 0.25 (0.95) | **0.80 (0.40 to 1.21)** | 0.24 (–0.12 to 0.59) | –0.09 (–0.46 to 0.28) | 0.33 (–0.18 to 0.84) |
| $FRC_{pleth}$ z-score | 0.75 (1.07) | –0.01 (1.08) | **0.77 (0.32 to 1.22)** | 0.75 (1.14) | –0.05 (0.96) | **0.80 (0.40 to 1.20)** | –0.04 (–0.32 to 0.23) | –0.04 (–0.45 to 0.36) | 0.00 (–0.48 to 0.49) |
| ΔFRC z-scores (pleth – MBW) | 0.59 (0.96) | 0.22 (0.94) | 0.37 (–0.32 to 0.77) | 1.21 (0.86) | 0.46 (0.69) | **0.75 (0.44 to 1.06)** | **0.58 (0.26 to 0.89)** | 0.30 (–0.09 to 0.68) | 0.28 (–0.21 to 0.77) |
| FVC z-score | –0.50 (1.03) | 0.23 (0.67) | **–0.74 (–1.06 to –0.41)** | –0.43 (1.16) | 0.23 (0.94) | **–0.66 (–1.05 to –0.26)** | 0.06 (–0.18 to 0.29) | –0.02 (–0.31 to 0.28) | 0.08 (–0.29 to 0.45) |
| $FEV_{0.5}$ z-score | –1.23 (1.07) | –0.16 (0.76) | **–1.07 (–1.42 to –0.73)** | –0.41 (1.03) | 0.12 (0.92) | **–0.52 (–0.89 to –0.15)** | **0.83 (0.56 to 1.09)** | 0.24 (–0.07 to 0.56) | **0.59 (0.18 to 0.99)** |
| $FEF_{75}$ z-score | –0.76 (1.25) | –0.07 (0.96) | **–0.69 (–1.11 to –0.27)** | –0.09 (0.93) | 0.09 (0.91) | –0.18 (–0.54 to 0.18) | **0.84 (0.48 to 1.19)** | 0.20 (–0.17 to 0.58) | **0.63 (0.12 to 1.14)** |

Data shown as mean (SD) or mean difference (95% CI) between: *Groups, †Test occasions.
‡Change over time between groups (CF- HC); significant differences (p <at least 0.05) are shown in bold.
§Corrected for gestational age.
¶Calculated according to Cole *et al*.[13]
BMI, Body Mass Index; CF, cystic fibrosis; $FRC_{pleth}$, plethysmographic functional residual capacity; ΔFRC z-scores (pleth – MBW), difference between FRCpleth and $FRC_{MBW}$ z-scores as a measure of gas trapping; FVC, forced vital capacity; $FEV_{0.5}$, forced expired volume in 0.5 s; $FEF_{75}$, forced expired flow when 75% of FVC has been expired; LCI, Lung Clearance Index; MBW, multiple breath inert gas washout; NBS, newborn screened.

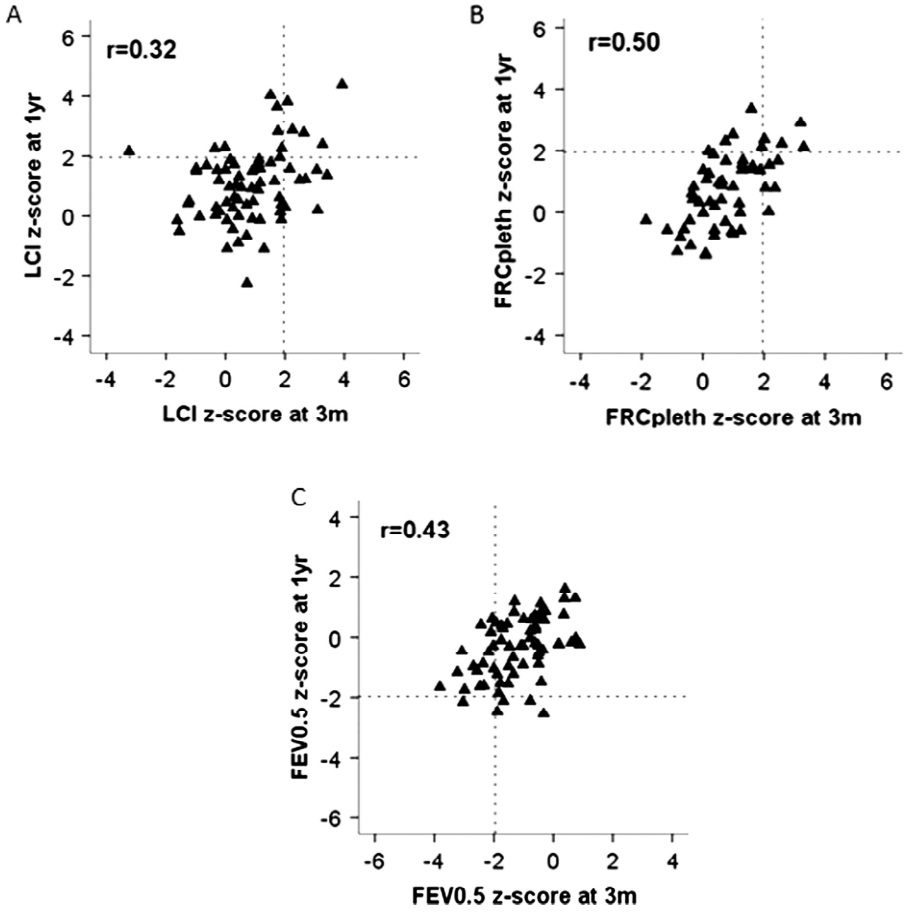


**Figure 2** Relationship between pulmonary function at 3 months and 1 year in newborn screened CF infants. The 95% limits of 'normal range' (97.5th centile for Lung Clearance Index (LCI) and functional residual capacity (FRC) and 2.5th centile for $FEV_{0.5}$) are represented by vertical dashed lines at 3 months (3m) and horizontal lines at 1 year (1yr). Those with normal pulmonary function tests on both occasions fall within the lower left quadrant for LCI and FRC, and upper right quadrant for $FEV_{0.5}$. Infants with abnormal LCI at 3 months but normal LCI at a year, lie within the lower right quadrant (A), while those with abnormal $FEV_{0.5}$ at 3 months which has normalised by 1 year are within the left upper quadrant of (C).

observational study such as this, we can only demonstrate association not causation of potential determinants of 1 year lung function. Computed tomography (CT) and broncho-alveolar lavage were performed at 1 year in CF infants, but not at 3 months, and are therefore not reported in this paper, which focusses on longitudinal changes. Furthermore, structural changes on CT at 1 year were very mild and poorly reproducible.[25]

### Interpretation of PFTs

As reported previously,[16] since the infant PFTs were selected to reflect a wide a range of lung pathology, the relatively poor correlations between the different primary outcomes on any one test occasion (see online supplementary table E2) was not surprising. While spirometry is known to be less sensitive than LCI for detection of mild lung disease in preschool children with CF,[8] during infancy $FEV_{0.5}$ has been shown to be a sensitive outcome in clinically diagnosed CF infants.[16] While this was also observed in this study of NBS infants at 3 months of age,[11] by 1 year far fewer NBS infants were identified by the raised volume technique than either plethysmography or LCI. This may reflect the mild nature of lung disease at 1 year in our NBS cohort when compared with those diagnosed clinically and the decreasing sensitivity of forced expiratory manoeuvres to mild lung disease as airway and chest-wall compliance decrease with increasing maturity.[26] By contrast with the lack of correlation between $FEV_{0.5}$ and other lung function outcomes on either test occasion, there were significant associations between LCI, $FRC_{pleth}$ and ΔFRC, all of which are thought to be sensitive measures of peripheral airway disease throughout childhood (see online supplementary table E2). Whatever the interpretation of these changes, as discussed below, they are in sharp contrast with those previously reported in CF infants. Consequently, when selecting outcome measures for intervention trials in NBS CF infants,[27] reliance should not be placed solely on the raised volume technique, since measures of LCI appear essential if mild abnormalities are to be detected. While hyperinflation and gas trapping also proved to be sensitive outcomes at 1 year, routine inclusion of these outcomes shortly after birth may be limited by equipment costs and increased failure rate of $FRC_{pleth}$ in young infants. With the exception of a significantly lower $FEV_{0.5}$ (mean (95% CI): −0.70 (−1.29 to −0.10) z-scores) in those who received additional antibiotics for symptoms or positive cough swab, there was no significant association between PFT outcomes and the infants' genotype, clinical status or any acute interventions prior to PFTs at 3 months.[11]

### Comparison with the literature

Results regarding evolution of early lung disease in those diagnosed by NBS have been conflicting (figure 3). The Australian Respiratory Early Surveillance team for CF (AREST-CF) have reported normal and reduced PFTs in such infants within the first 6 months of life,[9] with further rapid deterioration over the first year of life (mean $FEV_{0.5}$ being −2.4 z-scores by ~1 year of age).[10]

In the current study, lung function was abnormal by 3 months,[11] but stabilised or improved thereafter. As can be seen from figure 3, 1 year-lung function in the LCFC NBS cohort was significantly better than that in previous clinically diagnosed LCFC cohorts[6 16] or in the AREST-CF NBS cohort at similar age.[9 10] The reasons for the discrepancies between our results and those for AREST-CF are unclear. While the standardised protocol adhered to by the LCFC differs in some respects from that used by most centres in the USA, Australia and Europe (eg, use of

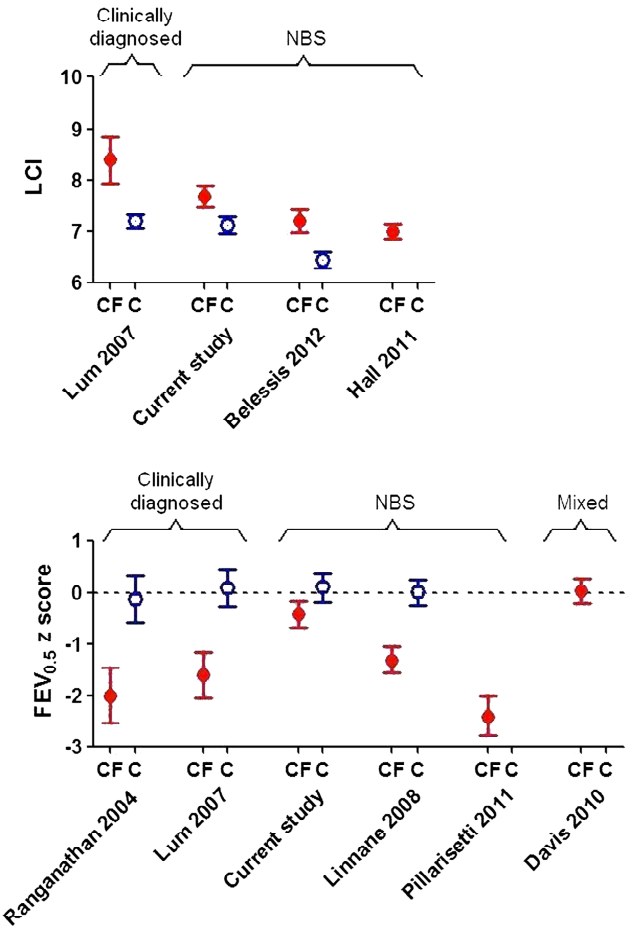


**Figure 3** Comparison of current lung function results in infants with cystic fibrosis (CF) and healthy controls (C) at ~1 year of age, with previously published results. Data expressed as mean (95% CI). To allow direct comparison with previously published studies, Lung Clearance Index is presented in absolute units, whereas $FEV_{0.5}$ is expressed as z-scores, based on different reference equations according to each author. The dashed horizontal line at 0 z-scores equates to 100% predicted based on a healthy population. Control data were not available in all studies. NBS, newborn screening.

flucloxacillin prophylaxis), the results should be a benchmark for other centres, and could serve as the basis for quality improvement.[28] Median age at first test in this study is younger than that in AREST-CF, which may reflect earlier diagnosis and implementation of treatment within the narrow geographical area of southeast England that we recruited from, thereby halting progression of any early lung disease. It is possible that infants recruited to AREST-CF were sicker, or deteriorated faster due to differences in modifier genes, environment or adherence to treatment, when compared with those in London. Most importantly, by contrast with the current study, AREST-CF data were not compared with contemporaneous controls, historical controls being used initially,[9] with subsequent results (obtained using higher inflation pressures[10]) being interpreted using reference data based on different equipment, which can bias interpretation.[19 29]

Improvements in lung function following treatment for acute exacerbations in infants with CF have been demonstrated, [30] but ours is the first study to document improvements in $FEV_{0.5}$ in infants treated with standard therapy, studied during periods of clinical stability. A recent exploratory study reported greater increases in $FEV_{0.5}$ over a 48-week period in 22 infants and young children treated with hypertonic saline compared with 23 randomised to isotonic saline (mean (95% CI) difference:38 (1 to 76) mL).[31] However, from the data presented, it is impossible to ascertain whether this reflected stability, improvement or simply less deterioration over time with active treatment, once effects of lung and somatic growth had been accounted for.

## Clinical implications

These results have implications for clinical practice and research. Although PFTs represent only one of the potential outcomes that can be used during early life,[27] with additional information gleaned from inflammatory markers and computerised tomography,[32] they represent the mainstay of clinical management and a major outcome in randomised controlled trials (RCTs) in children and adults. Since lung function tracks from late infancy into later life, accurate identification of early abnormalities is imperative. Furthermore, given the increasing number of centres undertaking 'clinical' infant PFTs,[33] the current study may facilitate more meaningful interpretation of results by providing vital evidence regarding the natural changes that can occur over time in healthy infants and those with lung disease, in the absence of any specific interventions.

We have shown that lung function and somatic growth during the first year of life are significantly better in infants diagnosed by NBS in the UK than in their counterparts who were clinically diagnosed a decade earlier[6 16] (figure 3). It is, however, of concern that despite early diagnosis and prompt treatment, LCI remains abnormal at 1 year (figure 3), albeit to a mild degree.[16] Further follow-up is required to establish the extent to which these changes predict later outcome. Nevertheless, in this study, normal lung function was sustained in at least 50% NBS CF infants to 1 year of age. The significant improvement in $FEV_{0.5}$ and stability of sensitive measures of distal airway function during early life when on 'standard therapy', and the relatively small deficits in lung function in CF NBS infants at 1 year also have important implications for design of future randomised intervention trials, which are essential to better define better standards of care in this age group. Despite considerable within-subject variability, the main predictor of lung function at 1 year was that at 3 months, allowing us to identify a 'high-risk' group who could potentially be targeted for future intervention trials.

Using data from this study, results from ~85 infants/arm would be required to detect relatively small differences in lung function (ie, equivalent to 0.5 z-scores) that might occur in response to an intervention if unselected NBS CF were recruited to such a trial. By contrast, were recruitment to such a RCT limited to a 'high-risk group' (ie, abnormal PFTs by 3 months, see online supplementary tables E3 and E4), a larger treatment effect would be expected, with only 22 infants/arm being required to detect a difference of 1 z-score (equivalent to ~9% for LCI), with 90% power. Such an approach could optimise recruitment since parents of infants with early PFT abnormalities would be more likely to consent, and also this approach would minimise exposure of children with potentially little to gain from therapy from unnecessary side effects.

In summary, we have shown that some measures of pulmonary function improve in the year following CF NBS diagnosis, and none deteriorate. Performing randomised intervention studies in an unselected cohort of infants using PFTs as an end point will, therefore, require large sample sizes due to the generally mild changes in lung function observed. Nonetheless, it is possible to identify CF infants with abnormal lung function by 3 months, who represent a high-risk group for persistent

abnormalities at 1 year, and who may benefit from additional treatment during the vital first few years of life.

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**Contributors** JS and AB were responsible for the conception and design of the study; JS is responsible for supervision of the study and together with JC, for research governance issues including ethics committee approval; A-FH provided technical training, supervision and audit of data collection and analyses; A-FH and JC set up the recruitment process. Infants with CF were recruited by the paediatric respiratory consultants participating in the LCFC, including AB and PA. TT-DN, LPT, A-FH, JC and SL recruited the healthy infants, undertook all lung function measurements and, together with JS, calculated and interpreted lung function results; TT-DN, LPT and AW performed statistical analyses; TT-DN, LPT, AB, PA and JS drafted the manuscript; all remaining authors revised and approved the manuscript for intellectual content before submission.

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**Competing interests** None.

**Patient consent** Obtained.

**Ethics approval** North Thames Multi-Centre Research Ethics Committee (#09/HO71/314).

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# High Resolution Computed Tomography in 1 Year Old CF Newborn Screened Infants: Not a Useful Outcome Measure

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## Introduction

- High Resolution Computed Tomography (HRCT) of the chest has been advocated as sensitive surrogate measure of early CF lung disease
- Bronchiectasis and gas trapping have been reported in CF Newborn Screened (NBS) infants in AREST-CF [1,2]
- Brody-II CT scores have been validated and used objectively to quantify CF lung disease in children but its use in infants is not yet established [3]

## Aim

To score HRCT scans from CF NBS infants using Brody II scoring system and assess inter-observer agreement

## Methods

- Prospective observational study of CF NBS infants recruited 2009 - 2011 across 6 London centers
- Chest HRCT under general anaesthesia at 1y of age at 3 sites using identical protocols
- Volumetric inspiratory and expiratory images obtained (Figures 1 & 2)
- After specialist training, anonymised scans scored independently by Dr Brody and experienced paediatric thoracic radiologist using Brody-II scoring system


Figure 1: Anaesthetist using a manometer to inflate infant's lungs to protocolised pressure

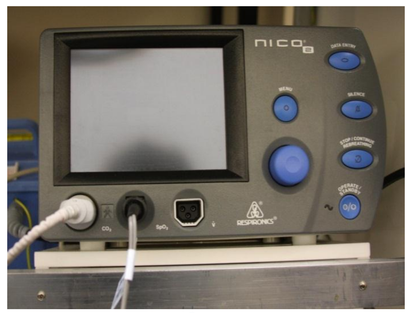


Figure 2: $NICO_2$ ® respiratory monitor used to record ventilatory pattern throughout procedure

## Results

### Background characteristics

- Scans obtained in **59** CF NBS infants at mean 52(SD:4) wks
- Mean age of diagnosis: 4 weeks (range: 1-12w)
- 85% CFTR genotype classes I-III
- 95% pancreatic insufficient; 5(8%) with meconium ileus
- Prophylactic flucloxacillin, pancreatic enzymes (if pancreatic insufficient) and multivitamins from diagnosis
- Managed by standardised treatment protocols

### CT Scores

- No CT changes were detected in 46% of infants by scorer A and in 8% by scorer B (Figure 3)
- Substantially discrepant scores were allocated to the different components
- Allocated scores suggested mild changes only

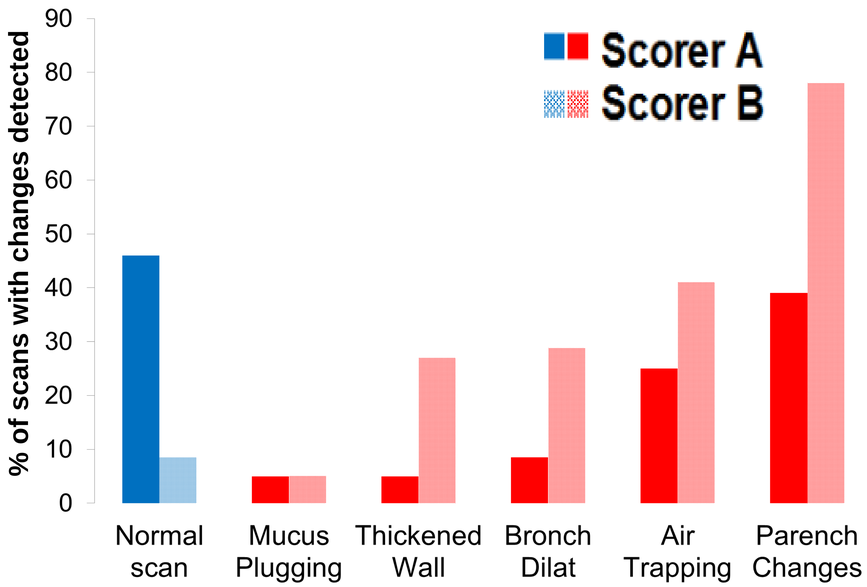


Figure 3: Percentage of scans where CT changes detected according to the two scorers

- The only sub-score with substantial agreement between scorers was air-trapping (Table 1)

| CT scores | Kappa coefficient |
|---|---|
| Bronchial Dilatation | 0.2 |
| Peribronchial Wall Thickening | 0.2 |
| Mucus Plugging | 0.3 |
| Parenchymal changes | 0.3 |
| Air trapping | 0.7 |

Table 1: Inter-rater agreement of CT scores

## Conclusions

- **Fewer CT changes (majority mild) were seen in current study of CF NBS infants than reported previously.**
- **Poor agreement was observed even between experienced and trained scorers.**
- **A new robust CT scoring system for evaluating mild early CF lung disease is needed before HRCT can be useful either clinically or as a trial endpoint in screened infants.**

## Acknowledgments & References

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# Early Detection of Lung Disease in infants with Cystic Fibrosis diagnosed by Newborn Screening

Lena Thia [1], Janet Stocks [1], Ah-Fong Hoo [1,2], Jane Chudleigh [2], Sooky Lum [1], Ammani Prasad [2], Andrew Bush [3], Colin Wallis [2] and on behalf of the LCFC

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Homerton University Hospital NHS Foundation Trust

## Introduction

- Newborn screening (NBS) for cystic fibrosis (CF) has been available in the UK since 2007.
- NBS offers the potential for early intervention in order to preserve lung function and nutritional status.
- It has been suggested that lung function is normal shortly after diagnosis in infants with CF detected by NBS[1]. However, relatively few infants were studied before 3m of age.

## Aim

To determine baseline lung function by ~3 months in NBS CF infants in comparison with prospective age-matched healthy controls

## Subjects and Methods

- **Infants with CF** were recruited from the London CF Collaboration comprising 6 tertiary CF centres –
  - Barts & the Royal London Hospital
  - Great Ormond Street Hospital
  - Royal Brompton Hospital
  - Lewisham University Hospital
  - King's College Hospital
  - St Helier's Hospital
- **Healthy controls** recruited from Homerton University Hospital.

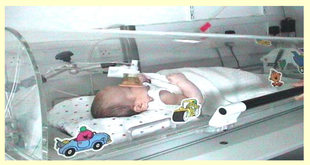
**Lung function tests** were undertaken by ~3 months postnatal age following chloral hydrate sedation (60–100 mg/kg) at the Great Ormond Street Hospital, using the

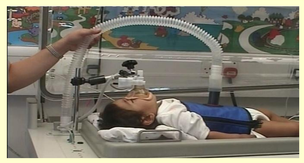
**a) Multiple Breath Washout (MBW):**

- Lung Clearance Index (LCI), $FRC_{MBW}$

**b) Plethysmography:** $FRC_{pleth}$

**c) Raised Volume Technique (RVRTC):** forced expired volumes and flows





## Results

### Recruitment

- 84 infants were screened positive for CF in the last 20mths.
- 8 not eligible for the study.
- 73 were approached and 53 recruited for the study (73%).
- 51 CF infants had baseline lung function at 3mths.
- 36 healthy controls had 3m lung function.

### Background Characteristics (mean [SD] or %)

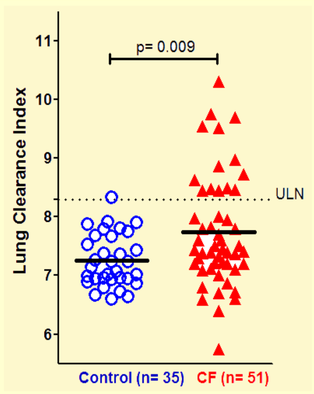
| | CF (n=51) | Controls (n=36) | Mean diff (95% CI) |
|---|---|---|---|
| Male, n (%) | 29 (57%) | 17 (47%) | 10% (–11%; 29%) |
| Gestation, w | 39.1 (1.7) | 40.0 (1.3) | –0.9 (–1.5; –0.3)** |
| Birth weight, z-score | –0.7 (1.1) | –0.2 (0.8) | –0.5 (–0.9; –0.1)* |
| Maternal smoking during pregnancy, n (%) | 8 (16%) | 4 (11%) | 5% (–11%; 19%) |

p values: * <0.05; ** <0.01, *** <0.0001

### Details at time of test ~3m (mean [SD])

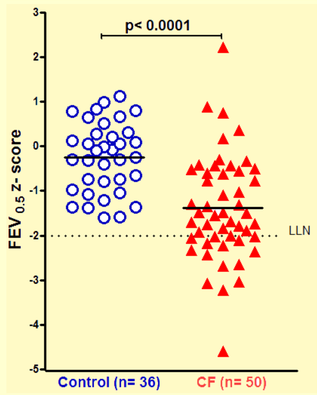
| | CF (n=51) | Controls (n=36) | Mean diff (95% CI) |
|---|---|---|---|
| Corrected age, w | 10.9 (2.3) | 12.4 (2.3) | –1.5 (–2.7; –0.5)** |
| Weight z-score | –0.8 (1.1) | 0.3 (1.2) | –1.1 (–1.7; –0.6)*** |
| Length z-score | –0.1(0.9) | 0.8 (0.9) | –0.9 (–1.3; –0.4)*** |

### Lung Clearance Index

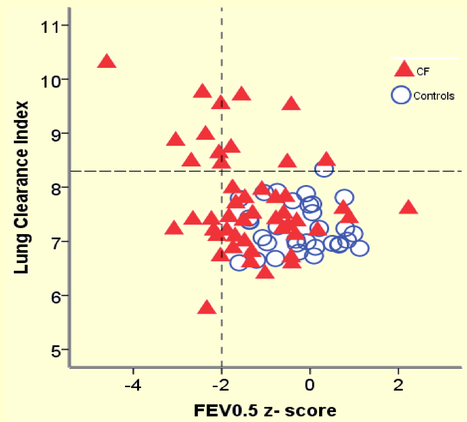



ULN: Upper limit of normal (=8.3 in healthy infants)

### $FEV_{0.5}$ z-score[2]

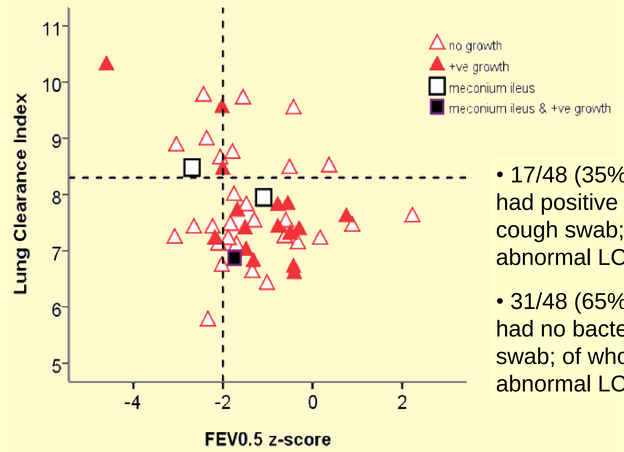



LLN: Lower limit of normal (–1.96 z-score)




- 20/50 (40%) of CF infants had either abnormal LCI or $FEV_{0.5}$ z-score.
- Only 8 infants had abnormal results identified by both methods.
- The difference in results demonstrated that these techniques measure different aspects of lung function [3].

### Lung function according to bacterial status in screened infants with CF




- 17/48 (35%) screened CF infants had positive bacterial growth on cough swab; of whom 7 (40%) have abnormal LCI or $FEV_{0.5}$ z-score.
- 31/48 (65%) screened CF infants had no bacterial growth on cough swab; of whom 20 (65%) had either abnormal LCI or $FEV_{0.5}$ z-score.

- There was no obvious pattern observed in association with prior clinical symptoms or genotypes.

## Conclusions

- Despite early diagnosis & treatment, lung function was abnormal by ~3 months in 40% of screened CF infants, $^2/_3$ of whom had no prior growth on cough swab.
- Incidence of abnormalities would have been under-estimated had either the MBW or RVRTC techniques been used in isolation.

**References**

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NACFC, Baltimore, USA 2010

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**Table A1-a: Table summarising studies that have assessed lung function and structure in infants and young children (age ≤2 years) with CF**

| Author | Type & Aim of study | Subjects | Methods & Equipment | Outcomes | Results and Authors' Conclusions |
|---|---|---|---|---|---|
| Castile[114] 2004 | Prospective Cross sectional Observational **Aim of study:** To compare trapped gas volumes in normal and CF infants | **Clinically diagnosed CF** n=29 age: 1-36m **Healthy controls** n=30 age: 1-34m | RTC, RVRTC (inflation= 30 $cmH_2O$) using custom built equipment, followed by plethysmograph and Nitrogen washout (FRC-$N_2$) | $FEF_{25-75}$, $FEF_{75}$ FVC TLC, RV, RV/TLC $V'max_{FRC}$ $FRC_{pleth}$ and FRC-$N_2$., | Significant differences in FEFV, FRC-$N_2$, $FRC_{pleth}$, RV and RV/TLC between CF and healthy controls. CF infants showed significant small amounts of trapped gas compared to healthy controls. Both CF and normal infants had evidence of hyperinflation and gas trapping. This may be due to glottic closure at low lung volumes during the measurement. **Conclusions**: Normal infants and CF infants have modest amounts of trapped gas. When assessed after the RVRTC manoeuvres, trapped gas measured as $FRC_{pleth}$-FRC-$N_2$ did not distinguish between minimally symptomatic CF infants and normal infants. |
| Ranganathan[60] 2004 (LCFC) | Prospective, Longitudinal Observational **Aim of study:** To determine if initial impairment in clinically diagnosed CF persists and asso. | **Clinically diagnosed CF** n= 34 1st test:17-43w 2nd test:48-69w **Healthy controls:** n=32 1st test: 6-9 w 2nd test:28-50w | RVRTC: from 30 $cmH_2O$ Custom built equipment (RASP). | $FEV_{0.5}$, FVC, $FEF_{75}$ z-scores | $FEV_{0.5}$ was significantly lower than healthy controls after diagnosis and 6 months later. 72% CF infants have abnormally low $FEV_{0.5}$ <1.64 z-scores (i.e <5th percentile). No improvement seen with growth. CF infants experienced a mean reduction in $FEV_{0.5}$ of 20% when compared with healthy controls. **Conclusions**: Airway function is diminished soon after diagnosis in CF infants and does not catch up during infancy and early childhood. |

***Footnote:*** *RTC raised tidal compression technique. $V'max_{FRC}$ maximal flow at functional residual capacity. RVRTC Raised Volume Rapid Thoraco-abdominal compression technique. FEFV (forced expiratory flows and volumes). FEV0.5 forced expiratory volume at 0.5 seconds. FVC Forced vital capacity. $FEF_{75}$ Forced expiratory flow when 75% of FVC expired. TLC total lung capacity. RV Residual Volume. $FRC_{pleth}$ Functional Residual Capacity measured through plethsmography. FRC-$N_2$ Functional Residual Capacity measured through nitrogen washout. RASP Respiratory Analysis Software program.*

| Author | Type & Aim of study | Subjects | Methods & Equipment | Outcomes | Results and Authors' Conclusions |
|---|---|---|---|---|---|
| Lum[83] 2007 (LCFC) | Prospective Cross sectional Observational **Aim of study:** To compare MBW and RVRTC tests in detecting abnormal lung function in clinically diagnosed infants with CF | **Clinically diagnosed CF** n= 39 age: 7.6-94.1w **Healthy controls** n= 21 age:15.3-7.9w | MBW (using mass spectrometer and $SF_6$ RVRTC (Jaeger Masterscreen) from 30cm $H_2O$ | LCI, $FEV_{0.5}$ and $FEF_{25-75}$ z-scores | Infants with CF had higher LCI and lower FEFV parameters compared with controls. **Conclusions:** Both MBW and RVRTC detected similar proportion of lung function abnormalities although they did not identify identical infants, so both techniques are required for early detection of lung disease. |
| Linnane[82] 2008 (AREST-CF) | Prospective Cross sectional Observational **Aim of study:** To measure lung function in infants and describe association with pulmonary infection and inflammation | **NBS and clinically diagnosed CF** n= 68 at 2 centres age: 6w- 2.5y **Historical Healthy controls** | RVRTC from 20 $cmH_2O$: Custom made equipment using reference equations from historical controls. | $FEV_{0.5,}$ FVC and $FEF_{75}$ z-scores | CF infants had reduced $FEV_{0.5}$ z score as a whole cohort, although no significant reduction in airway function was noted in infants < 6 months of age.<br><br>No association between diminished lung function and airway inflammation and infection.<br><br>**Conclusions**: Lung function is normal shortly after diagnosis by NBS but is diminished in older infants despite good nutrition and care in specialist centres. There may be a window of opportunity to intervene to maintain normal lung function as it appears to be normal within the first 6 months of life. |

***Footnote:*** *NBS Newborn screened. RVRTC Raised Volume Rapid Thoraco-abdominal compression technique. MBW Multiple Breath Washout. LCI Lung Clearance Index. $SF_6$ SulphurHexafluoride gas. FEV0.5 forced expiratory volume at 0.5 seconds. FVC Forced vital capacity. $FEF_{25-75:}$ Forced expiratory flow when 25-75% of FVC expired. $FEF_{75}$* Forced expiratory flow when 75% of FVC expired.

| Author | Type & Aim of study | Subjects | Methods & Equipment | Outcomes | Results and Authors' Conclusions |
|---|---|---|---|---|---|
| Pillarisetti[67]<br>2011<br>(AREST-CF) | Prospective Longitudinal Observational<br>**Aim of study:** To describe longitudinal change in lung function and its association with pulmonary infection and inflammation | **NBS CF** n=37<br>age: ≤ 2y<br>**Historical Healthy controls:** None | RVRTC from 30cm$H_2O$ Custom made equipment using reference equations from historical controls.<br>Measured at ~4m, ~1y, ~2y | $FEV_{0.5,}$ FVC and $FEF_{75}$ z-scores | Lung function impaired at all three test occasions and further deterioration with advancing age.<br><br>Significantly greater decline in $FEV_{0.5}$ z-scores occurred in those infected with S*taphylococcus aureus* or *Pseudomonas aeruginosa*.<br>**Conclusions**: Decline in lung function over time in clinically well CF infants is associated with neutrophilic airway inflammation and pulmonary infection with *Staphylococcus aureus* and *Pseudomonas aeruginosa* detected by surveillance BAL. |
| Hall[121]<br>2011<br>(AREST-CF) | Prospective Cross sectional Observational<br>**Aim of study:** To assess relationship between structural lung damage using chest CT and ventilation distribution | **NBS CF**<br>n=49<br>age:8.7-112.1w<br><br>**Healthy Controls:** None | MBW (Mainstream USFS: Exhalyzer; Ecomedics using $SF_6$) | LCI, $FRC_{MBW,}$ $M_1/M_0$ and $M_2/M_0$<br>**CT under GA:** CT scores (1 radiologist) – bronchiectasis and airtrapping recorded in binary function. If present, then extent | LCI and $M_1/M_0$ not significantly increased in infants with bronchiectasis or airtrapping.<br>$M_2/M_0$ significantly increased in presence of CT airtrapping (p= 0.049) but not with bronchiectasis (p=0.60).<br>Presence of:<br>• Bronchiectasis 13(27%)<br>• Airtrapping 24 (49%)<br>In early CF lung disease, there are weak associations between ventilation distribution reported as $M_2/M_0$ and CT airtrapping.<br>**Conclusions**: LCI cannot be used as surrogate marker for chest CT outcomes in CF NBS infants. LCI may detect early inflammation and infection but not onset of structural lung disease. |

***Footnote:*** *BAL Bronchoalveolar lavage. USFS Ultrasonic Flowmeter System. $SF_6$ Sulphur Hexafluoride 6 gas. $FRC_{MBW}$ Functional Residual Capacity measured through multiple breath washout technique. $M_1/M_0$ First moment ratio. $M_2/M_0$ Second moment ratio. $FEV_{0.5}$ forced expiratory volume at 0.5 seconds CT under GA Chest Computed Tomography under General Anaesthesia.*

| Author | Type & Aim of study | Subjects | Methods & Equipment | Outcomes | Results and Authors' Conclusions |
|---|---|---|---|---|---|
| Davis[62] 2010 | Prospective Longitudinal Observational **Aim of study:** To evaluate safety, feasibility and ability to detect abnormalities in serial pulmonary function tests in CF infants | **Clinically diagnosed CF (79%) and NBS or prenatal screened CF (21%)** n=100 at 10 centres age: 1.6-26.4m **Historical Healthy controls** | RVRTC from 30cm$H_2O$. (nSpire Infant Pulmonology Lab). Plethysmography | $FEV_{0.5}$, FVC, $FEF_{75}$ z-scores $FRC_{pleth,}$ RV, FRC/TLC, RV/TLC | FRC measurements (89%) were more feasible and with higher rate of technical acceptability across the centres than RVRTC (72%) Compared to historical controls, CF infants showed reduced forced expired flows but not volumes. Also had elevated $FRC_{pleth}$ and other fractional lung volumes. Impairment in lung function increased with advancing age. **Conclusions:** The feasibility of ILFT as a multicentre outcome is variable and depends on the experience of the centre. Potentially a large sample size is required to detect reasonable treatment effects. This precludes its use as a primary efficacy endpoint especially at inexperienced sites. |
| Kieninger[105] 2011 | Retrospective Longitudinal Observational **Aim of study:** To describe the longitudinal course of LCI from time of clinical diagnosis during infancy to school age. | **Clinically diagnosed CF** n=11 **Infant study:** (median age: 21.9w) **School age study:**(median age:9.7y) **Historical** | **Infant study:** *unsedated* using Exhalyzer , EcoMedics using $SF_6$ **School age study:** Exhalyzer EcoMedics using nitrogen washout) | LCI z-scores using reference data from healthy historical controls measured using similar protocols. | Elevated LCI was present during infancy in 7/11 cases, especially in those with later clinical diagnosis. Tracking of infancy LCI to school-age present in 4 of the most severe cases. **Conclusions:** MBW during natural sleep is feasible in infants. Tracking of LCI is seen from infancy to school age clinically diagnosed CF, especially in those with most severe disease. |

***Footnote:*** *$FEV_{0.5}$ Forced expiratory volume at 0.5 second. FVC Forced vital capacity. $FEF_{75}$ Forced expiratory flow when 75% of FVC expired. RV Residual volume, TLC Total lung capacity, $FRC_{pleth,}$ Functional residual capacity measured through plethysmography. LCI Lung clearance index. MBW Multiple breath washout test. NBS Newborn screening.*

**Table A1-b: Table summarising studies that have measured Lung Clearance Index in children ≥3 years with CF**

| Author | Type & Aim of study | Subjects | Methods & Equipment | Outcomes | Results and Authors' Conclusions |
|---|---|---|---|---|---|
| Gustafsson[63] 2003 | Prospective Cross sectional Observational **Aim of study:** To determine if ventilation distribution is impaired in early CF lung disease and abnormal more frequently than spirometry | **Clinically diagnosed CF** n= 43 age: 3-18 y **Healthy controls** n= 28 age: 4-19 y | MBW: Respiratory MS using $SF_6$ Spirometry: Jaeger Masterscreen + Body-plethysmograph | $FRC_{pleth}$, LCI and mixing ratio. $FEV_1$, $FEF_{75}$ % predicted | LCI and mixing ratio were significantly elevated in children with CF with majority of those with CF being above the upper limit of normality. CF with chronic bacterial airway infection had significantly worse lung function from all tests. **Conclusions:** Abnormal ventilation distribution exists in the majority of CF children, including those with normal spirometry. Findings suggest that destructive processes may start early and in the peripheral airways which may not be evident from conventional spirometry. |
| Aurora[85] 2005 (LCFC) | Prospective Cross sectional Observational **Aim of study:** To compare the relationship between LCI, $sR_{aw}$ and $FEV_{0.5}$ and $FEF_{25-75}$ in clinically diagnosed pre-school children with CF | **Clinically diagnosed CF** n= 30 age: 2-5y **Healthy controls** n= 30 age: 2-5y | MBW: Respiratory MS using $SF_6$ $sR_{aw}$ using Jaeger Masterscreen plethysmography Incentive spirometry using Jaeger spirometer | LCI, $zsR_{aw}$, $zFEV_{0.5}$ and $zFEF_{25-75.}$ | CF children had significantly higher LCI and $sR_{aw}$ and significantly lower $FEV_{0.5}$ than healthy controls. Those with *PsA* infection had significantly higher LCI. LCI was the most sensitive outcome for detecting abnormal lung function. **Conclusion:** MBW, plethysmography and spirometry were all feasible in preschool children. MBW detects abnormal lung function more readily than plethysmography or spirometry. |

***Footnote:*** *MBW Multiple Breath Washout. $FEV_1$ forced expiratory volume at 1 second. $FEF_{75}$ Forced expiratory flow when 75% of FVC expired. $FEF_{25-75:}$ Forced expiratory flow when 25-75% of FVC expired. Respiratory MS Respiratory Mass Spectrometer. $SF_6$ Sulphur Hexafluoride 6 gas. LCI Lung Clearance Index. $FRC_{pleth}$ Functional Residual Capacity measured through plethysmography. $sR_{aw}$ Specific airway resistance.*

| | | | | | |
|---|---|---|---|---|---|
| Kraemer[72] 2005 | Prospective Cross sectional Observational **Aim of study:** To investigate changes in lung volume, flow limitation and ventilation distribution in CF patients from age 6 to 20 years. | **Clinically diagnosed CF** n=142 age: 6-20y<br><br>No healthy controls | Plethysmography MBW using nitrogen washout<br><br>Spirometry | $FRC_{pleth,}$ sReff LCI and FRC-$_{N2,}$ $FRC_{pleth}$-FRC-$_{N2}$ (trapped gas) FVC, $FEV_1$ and $FEF_{50}$ | Highest progression of pulmonary dysfunction found in $FRC_{pleth}$, followed by LCI, trapped gas and then $FEF_{50}$.<br>Median age at onset of abnormal LCI, $FEF_{50}$, $FEV_1$, $FRC_{pleth}$ and trapped gas volume were 6.3y, 7.2y, 8.6y, 8.9y, 13.0y respectively<br>LCI and $FEF_{50}$ detected more abnormalities than $FEV_1$<br>All lung function parameters associated with onset of chronic *Pseudomonas aeruginosa* infection.<br>**Conclusions:** LCI predicts earlier in life and represented better functional progression than $FEV_1$. However no single functional predictor of CF progression exists. Onset of chronic *Pseudomonas aeruginosa*, genotype, hyperinflation, airway obstruction and ventilation inhomogeneities are important pathophysiologic processes that should be evaluated as determinants of lung progression in CF. |

***Footnote:*** *sReff Specific effective resistance. $FRC_{pleth}$ Function Residual Capacity measured through plethysmography. FRC-$_{N2}$ Functional Residual Capacity measured through nitrogen washout technique. $FRC_{pleth}$-FRC-$_{N2}$ measurement for trapped gas using the two different systems. LCI Lung clearance index. $FEV_1$ Forced expiratory volume at 1 second. FVC Forced vital capacity. $FEF_{50}$ Forced expiratory flow when 50% of FVC expired.*

| Author | Type & Aim of study | Subjects | Methods & Equipment | Outcomes | Results and Authors' Conclusions |
|---|---|---|---|---|---|
| Kraemer[76] 2006 | Prospective Longitudinal Observational<br>**Aim of study:**<br>1) To investigate whether or not hyperinflation and/ or trapped gas reflect functional deterioration during childhood.<br>2) Role of specific CFTR genotypes and *PsA* infection on rates of disease progression.<br>3) To demonstrate whether there is functional tracking over time of respiratory dysfunction. | **Clinically diagnosed CF**<br>n=152<br>age: 6-18 years<br>Analysis made according to 4 groups:<br>• Normal $FRC_{pleth}$ and LCI at entry (FN)<br>• Normal $FRC_{pleth}$ but high LCI (VIH)<br>• $FRC_{pleth}$ high, high LCI but normal trapped gas (PH)<br>• $FRC_{pleth}$, trapped gas and LCI high (PH&TG) | Plethysmography Spirometry [Jaeger BodyScreen then Jaeger Masterlab with electronic compensation]<br>MBW using nitrogen washout [Sensormedics 2200] | $FRC_{pleth;}$ sReff FVC, $FEV_1$ and $FEF_{50}$<br><br>LCI, $FRC\text{-}_{N2}$ and $FRC_{pleth}$-$FRC\text{-}_{N2}$ (trapped gas) | $FRC_{pleth}$ and trapped gas volume increase from age 6 to 18 years of age. Abnormal hyperinflation detected in 38% of subjects at age 6 which ↑ to 67% by 18y. Abnormal trapped gas volume increased from 15% to 54% during this period.<br>These abnormal lung function parameters appeared to track from early childhood to adulthood. However even in those with normal lung function initially or no hyperinflation but increased LCI i.e. FN and VIH groups, these groups also showed progression of $FRC_{pleth}$ and trapped gas.<br>Age related tracking of lung function parameters commences early in life and is significantly influenced by specific CFTR genotypes.<br>Group with chronic *Pseudomonas aeruginosa* had the most rapid deterioration while chronic *Staphylococcus aereus* had the slowest rate of progression. LCI was the most sensitive discriminator between the 3 types of infection examined. |

***Footnote:*** *sReff Specific effective resistance. $FRC_{pleth}$ Function Residual Capacity measured through plethysmography. $FRC\text{-}_{N2}$ Functional Residual Capacity measured through nitrogen washout technique. $FRC_{pleth}$-$FRC\text{-}_{N2}$ measurement for trapped gas using the two different systems. LCI Lung clearance index. $FEV_1$ Forced expiratory volume at 1 second. FVC Forced vital capacity. $FEF_{50}$ Forced expiratory flow when 50% of FVC expired.*

| Author | Type & Aim of study | Subjects | Methods & Equipment | Outcomes | Results and Authors' Conclusions |
|---|---|---|---|---|---|
| Gustafsson[64] 2008 | Retrospective Cross sectional Observational **Aim of study:** To determine correlation between LCI and spirometry with structural lung disease | **Clinically diagnosed CF** n= 44 age: 5-19y **No healthy controls** | MBW: Respiratory MS using $SF_6$ Spirometry: Jaeger Masterscreen Plethysmography: HRCT: Non-contiguous scanning in inspiration and 3-slice images during expiration | LCI, $zFEV_1$ and $zFEF_{75}$. **CT scoring:** Brody-II scoring by 1 experienced radiologist Composite and Component CT scores for different structural abnormalities | LCI was more sensitive in detecting abnormal lung function than $FEV_1$ or $FEF_{75.}$ LCI correlated better with CT scores than $FEV_1$ **Conclusion:** LCI is more sensitive than spirometry for detecting structural lung disease in CF. Normal LCI excludes CT abnormalities. Abnormal LCI with normal scan suggests that LCI may be even more sensitive than CT for detecting lung involvement in CF. |
| Fuchs[93] 2008 | Prospective Cross sectional Observational **Aim of study:** To assess feasibility of using sidestream USFS prototype device in CF children and adults. | **Clinically diagnosed CF** n= 26 age: 7-19y **Healthy controls** n= 22 age: 5-18y | MBW: Side stream USFS (EasyOne Pro, NDD) using $SF_6$ Spirometry: Jaeger Masterscreen plethysmograph | LCI, $FRC_{MBW}$, $zFEV_1$ Chest radiograph: Crispin Norman score | LCI similar to that obtained through mass spectrometry. It is more sensitive than spirometry. LCI correlated with CN score while $FEV_1$ did not. **Conclusions:** Sidestream ultrasonic MBW is a valid and simple alternative to mass spectrometry for assessing ventilation inhomogeneity in children. |

***Footnote:*** *Respiratory MS Respiratory mass spectrometer. USFS Ultrasonic Flowmeter System. $SF_6$ Sulphur Hexafluoride 6 gas. $FEV_1$ Forced expiratory volume at 1 second. $FEF_{75.}$ Forced expiratory flow when 75% of FVC expired. LCI Lung clearance index. $FRC_{MBW}$ Functional Residual capacity measured through multiple gas washout technique. CN Crispin Norman CXR. score. LCI Lung clearance Index*

| Author | Type & Aim of study | Subjects | Methods & Equipment | Outcomes | Results and Authors' Conclusions |
|---|---|---|---|---|---|
| Horsley[90] 2008 | Prospective Cross sectional Observational<br>**Aim of study:**<br>1. To investigate the feasibility of obtaining LCI using modified Innocor device in healthy and CF children and adults.<br>2. To assess how LCI changes with age and the reproducibility in healthy volunteers.<br>3. To use adapted Innocor to measure FRC and LCI in normal and CF subjects and compare LCI to spirometry. | **Clinically diagnosed CF**<br>Adults: n= 33<br>age: 17-49y<br><br>**Healthy controls**<br>Adults: n= 49 non-smokers<br>age: 19-58y<br>Child: n=13<br>age: 6-16y | Modified Innocor device (Innovision, Denmark) using $SF_6$ (0.2%)<br>Spirometry using ECCS predicted values for $FEV_1$ in adults and Rosenthal for children (≤ 16y) | LCI and FRC<br><br>$FEV_1$ | • Both Innocor and MS had↓signal:noise ratio as concentration ↓ but Innocor signal quality remained superior to MS.<br>• Slower rise time with Innocor (150ms) vs MS (60ms); may not be usable in infants and younger children with faster resp rate.<br>• LCI showed high reproducibility within and between visits for healthy and CF adults and healthy children.<br>• No relationship between age (≥16y) and LCI. LCI restricted to a narrow range in healthy controls.<br>• LCI in healthy adults was significantly different from CF. LCI detected more CF adults with abnormality compared to $FEV_{1.}$<br>• LCI is highly sensitive: 97% in detecting CF compared to 70% in $FEV_1$.<br><br>**Conclusion:** First adult study showing LCI more sensitive than $FEV_1$ in detecting lung abnormalities and that it is reproducible with little change over height and age, using a commercially available robust compact apparatus. |

***Footnote:*** *$SF_6$ Sulphur Hexafluoride 6 gas. $FEV_1$ Forced expiratory volume at 1 second. LCI Lung clearance index MS Mass Spectrometer. ms milliseconds.*

| Author | Type & Aim of study | Subjects | Methods & Equipment | Outcomes | Results and Authors' Conclusions |
|---|---|---|---|---|---|
| Ellemunter[117] 2010 | Prospective Cross sectional Observational **Aim of study:** To investigate the diagnostic accuracy of LCI in comparison to CT in CF patients with early lung disease (normal $FEV_1$>80% pred) | **NBS (13), Mec ileus (4), Family history (4), Clinically diagnosed (13)** n= 34 age: 6-26 y **No healthy controls** | MBW: Side stream USFS system (EasyOne Pro, NDD) using $SF_6$ Spirometry: Jaeger Masterscreen Plethysmograph CT scan: Ultra low dose volumetric thin section multi-detector CT. | LCI, $zFRC_{MBW}$, $zFEV_1$ *Inspiratory* images during a single deep inspiration. *No expiratory images.* **CT scoring:** Bhalla scores (2 independent experienced radiologists). | MBW revealed abnormal results in majority of patients (76.5%). Using Bhalla scores, 76.5% of patients have abnormal findings. Good concordance between LCI and CT scores (82.3%). No correlation seen between $FEV_1$ and CT total or subscores. Sensitivity and specificity of LCI were high (88% and 63% respectively) when compared to CT. **Conclusion:** LCI can be used as a surrogate marker for detecting early structural CF lung disease and hence minimise the need for CT scans and reduce radiation burden. More longitudinal data required for evolution of CT changes. |
| Owens[107] 2010 (LCFC) | Prospective Cross sectional Observational **Aim of study:** To assess whether LCI is as effective as HRCT in identifying pulmonary abnormalities and to explore its relationship with other lung function | **Clinically diagnosed CF** n= 60 age: 6-10y n= 57 (lung function and CT) **Healthy controls** n= 54 age: 5.5-10y (lung function only) | MBW: Respiratory MS using $SF_6$ Spirometry+$sR_{aw}$ (Jaeger Masterscreen plethysmograph HRCT: Volumetric *inspiratory* and 3 slices on *expiration* | LCI, $zFRC_{MBW}$ $zFEV_1$, zFVC, $zFEF_{25-75}$, $sR_{aw}$, $zFRC_{pleth}$, zRV, zTLC **CT scoring:** Composite and component CT scores using Brody II scored by 2 experienced radiologists. | CF children had significantly impaired lung function compared to healthy controls in all lung function parameters. LCI was the most sensitive parameter, detecting abnormalities in 85% of CF with strongest correlation with total CT scores and sub-scores. Scans were classified as abnormal in 84% of children with CF. Both scorers had good agreement in total CT scores and subscores. **Conclusion:** LCI and HRCT have similar sensitivity**.** Good correlation between LCI, total scores and subscores; best concordance in severe lung disease. |

| Author | Type & Aim of study | Subjects | Methods & Equipment | Outcomes | Results and Authors' Conclusions |
|---|---|---|---|---|---|
| Aurora[102] 2011 (LCFC) | Prospective Longitudinal Observational<br>**Aim of study:** To determine if preschool spirometry and LCI predict subsequent abnormal lung function at early school age | **Clinically diagnosed CF** n= 48 Tested at 3-5y with follow-up test at 6-10y<br>**Healthy controls** n=45 Tested at 3-5y with follow-up at 6-10y | MBW: Respiratory MS using $SF_6$ Incentive spirometry | LCI and $FEV_1$ z-score | Majority of preschool CF children have abnormal LCI even when spirometry normal. LCI more sensitive in detecting abnormalities.<br>Normal preschool LCI associated with normal school-age lung function. Preschool LCI had a high PPV (94%) and NPV (62%) for predicting abnormal school-age result<br>**Conclusion:** Abnormal preschool LCI predicts subsequent lung function abnormalities. MBW may be a useful research and clinical outcome. |

***Footnote:*** *MBW Multiple Breath Washout. MS Mass Spectrometer. $SF_6$ Sulphur Hexafluoride 6 gas. LCI Lung Clearance Index.. $FEV_1$ Forced expiratory volume at 1 second. PPV Positive Predictive Value. NPV Negative Predictive Value.*

**Table A1-c: Table summarising studies using ILFT and/or LCI as outcome measures in interventional trials involving infants and older children with CF**

| Author | Type & Aim of study | Subjects | Methods & Equipment | Outcomes | Results and Authors' Conclusions |
|---|---|---|---|---|---|
| Berge[113] 2003 | Prospective<br>Interventional, Open-label, Randomised cross-over<br>Nebulised DNase vs 0.9% saline<br>**Aim of study:**<br>To examine the feasibility and sensitivity of clinical endpoints to assess effects of inhaled DNase in CF infants. | **Clinically diagnosed CF**<br>n=9<br>mean age: 1.4 ± 0.16y | 2-3 weeks cross over trial with DNase or 0.9% saline once daily<br>Jaeger Baby-Body | Oxygen saturations, respiratory and pulse rate<br>Daily symptom scores<br>$V'_{max}FRC$ and $FRC_{pleth}$ | Significant improvement in $V'_{max}FRC$ seen in infants while on DNAse with no significant improvement while on normal saline.<br>No significant improvement in $FRC_{pleth}$ whether on DNAse or normal saline.<br>No significant difference observed in oxygen saturations or clinical symptoms between infants on DNAse or normal saline.<br>**Conclusion:** Objective assessment of the effects of DNase is feasible in infants with CF who have few or no respiratory symptoms. Results warrant a larger randomized placebo-controlled trial. |
| Amin[120] 2010 | Prospective<br>Interventional<br>Cross over trial<br>Hypertonic (HS) vs isotonic(IS) saline<br>**Aim of study:**<br>The ability of LCI to detect a treatment response to hypertonic saline in CF patients with normal spirometry. | **Clinically diagnosed CF**<br>n= 17<br>mean age: 10.5 (4.3-16.7) y<br>**No healthy controls** | 12w cross over with 7% HS or 0.9% IS twice daily for 4w followed by 4w washout then 4w other treatment.<br>MBW-MS($SF_6$)<br>Spirometry (Viasys Cardinal Health) | **Primary outcome:**<br>LCI<br>**Secondary outcomes:** $FEV_1$, FVC, $FEF_{25-75}$ z-scores using Stanojevic reference equations and CFQR scores. | LCI, spirometry and CFQR at baseline between HS and IS groups were similar.<br>LCI significantly lower after 4w of HS inhalation compared with IS but no difference detected by spirometry and CFQR domains.<br>**Conclusion**: LCI but not spirometry can detect treatment effect from hypertonic saline inhalation in CF patients with mild disease. |

***Footnote****: w weeks. $V'_{max}FRC$ Maximal flow at functional residual capacity. $FRC_{pleth}$ Functional Residual Capacity measured through plethysmography. CFQR Cystic Fibrosis Questionnaire-Revised. LCI Lung Clearance Index. $FEV_1$ Forced expiratory volume at 1 second. FVC Forced Vital capacity. $FEF_{25-75}$ Forced expiratory flow when 25-75% of FVC expired. MBW-MS (SF6) Multiple Breath Washout Technique using Respiratory Mass Spectrometer and Sulphur Hexafluoride 6 Gas.* $FEF_{25-75}$ z - *Forced expiratory flow when 25-75% of FVC expired.*

| Author | Type & Aim of study | Subjects | Methods & Equipment | Outcomes | Results and Authors' Conclusions |
|---|---|---|---|---|---|
| Amin[119] 2011 | Prospective Interventional Cross over trial Dornase vs placebo **Aim of study:** To determine if LCI can detect treatment response to dornase alfa in paediatric CF patients with normal spirometry | **Clinically diagnosed CF** n= 17 age: 3.5-17.1y **Historical healthy controls** 28 healthy Canadian children; age 10-13 years | 12 w cross over trial with 2.4ml dornase alfa or placebo once daily for 4w, followed by 4w of washout then 4w of the other treatment. MBW-MS ($SF_6$) Spirometry (Viasys Cardinal Health USA) | **Primary outcome:** LCI **Secondary outcomes:** $FEV_1$, FVC, $FEF_{25-75}$, z-scores using Stanojevic reference equations and CFQR scores | LCI in CF worse than historical controls. All CF had abnormal LCI at baseline while only 18% had abnormal $FEF_{25-75.}$ Significant improvement in LCI compared with placebo after 4 weeks treatment with dornase alfa. FEV1, FVC and CFQR scores not significantly different between treatment and placebo groups. $FEF_{25-75}$ significantly better with dornase treatment group. Strongest correlation seen between LCI and $FEF_{25-75.}$ **Conclusion:** Dornase alfa significantly improved LCI, suggesting it may be a sensitive and responsive outcome measure with the ability to identify treatment responders. |

***Footnote**: w weeks. CFQR Cystic Fibrosis Questionnaire-Revised. LCI Lung Clearance Index. $FEV_1$ Forced expiratory volume at 1 second .FVC Forced Vital capacity. $FEF_{25-75}$ Forced expiratory flow when 25-75% of FVC expired. MBW-MS (SF6) Multiple Breath Washout Technique using Respiratory Mass Spectrometer and Sulphur Hexafluoride 6 Gas.*

## A2 Parent Information Leaflets

- For CF Infants
- For Healthy Control Infants

## A3 Breathing Tests in Babies Leaflet

## A4 Consent Forms for ILFT for CF and Healthy Control Infants

***Parent Information Sheet***

**Early detection of lung disease in infants with CF diagnosed by newborn screening**
**NREC Number: _09H071314**

**Thank you for taking the time to read this information document.**

**PART 1: Essential elements of the study**

We would like to invite you to take part in a research study 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 are approaching you as a parent of a baby that has recently been diagnosed with CF.*

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 baby. Please take time to read the information in this document, which is six pages long, 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 information and consent form to keep

**What is the purpose of this study?**

Newborn screening for CF is undertaken routinely the UK. This means that we can identify babies who have CF very early, often while they are still healthy. However, there will be no benefits to newborn screening unless we develop suitable treatments which will minimise lung damage during the critical first two years of life when the lungs are growing and developing very quickly.

Worsening lung disease is a major problem in CF. The changes which happen in the lung can begin very early in life, often before the child develops obvious problems. Early lung disease of CF begins at the edges of the lungs and can only be detected by using special tests. We know that some of these tests are good at detecting problems but as yet we do not know if they are good enough to pick up very early lung disease. We also need to know if these tests can show us whether the lungs are getting better, staying the same or getting worse. We hope to answer these questions with our research project by undertaking three tests during the first year, which are explained below. We will also ask you to fill out a questionnaire about your experience of taking part in this study.

**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 participate, 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 baby receives at the hospital in any way. Many of the tests included in this study are part of our routine care at Great Ormond Street Hospital and so your baby will be having them anyway. If you decide not to take part in the main study we would like you to

consider whether you would be happy for us to use the results of the routine tests you have during normal clinic visits as these will also help us to understand more about CF in babies diagnosed by newborn screening.

**What are the three tests in the study?**

The tests we will be doing for the study are describe below but you will also be given some separate leaflets which explain each test in more detail.

***1. Breathing tests***

Breathing tests in babies 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 (GOSH). We will arrange the timing of these visits to suit your family and all travel expenses will be refunded.

Older children and adults do breathing tests by taking deep breaths and blowing through special equipment. Babies are unable to cooperate in this way and therefore need to be asleep for the tests to be done. Babies do not always sleep for long during the day, so we give them a mild sleeping syrup called chloral hydrate to help them fall asleep. This medicine is often used in children and has been used for breathing tests in babies for over 25 years. Your baby will be able to feed before the tests and eat/drink normally as soon as they wake up. The tests do not upset the babies, who usually sleep through the whole process. It usually only takes about an hour to do all the tests but it is difficult to predict how quickly the baby will fall asleep, so it is best to allow about three hours for the visit. During the visit a research doctor will examine the baby and ask a few health questions.

While the measurements are being made we will collect a urine sample from your baby. This will be sent to the laboratory to examine it for a substance which is produced when inflammation is broken down in the lungs and also for a substance which indicates passive exposure to smoke. This urine sample will be stored securely for six years and at the end of that time destroyed. The reason for storing extra sample is that in future we may want to look at the sample again for some further testing. You can be informed of any extended analysis if you wish.

We will carry out the tests on two separate occasions about 9 months apart. We use four types of measurements to see how your baby's lungs are developing and growing

***a) Breathing Patterns using Electrical impedance tomography (EIT) and Structured Light Plethysmography (SLP)***

Most of the lung function tests we make require babies to be fast asleep, which is why we need to give some sedative syrup before we start. However, some new approaches are now being developed that could make it easier to obtain similar results without sedation. This would mean we could offer these assessments to far more babies with breathing problems and in hospitals without the special facilities available at GOS. The first technique simply involves placing small sticky pads around the baby's chest. This allows us to record tiny changes which occur as the baby breathes in and out and tells us whether the air breathed in is being spread evenly over the lungs, or whether there are some parts of the lung which are getting less air (for example due to some obstruction of the airway). The pads will remain on the baby's chest throughout the testing session.

The second approach (SLP) is even more straightforward and simply involves projecting a light grid onto the baby's chest and recording the movements of this grid as they breathe with two overhead cameras. Several sets of measurements will be taken, each lasting around 1 minute.

The technique is entirely painless and non-invasive and does not require anything to be connected to your child. There is no radiation involved.

***b) Multiple breath washout test***

This test is designed to find out how evenly your child breathes. The baby 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 baby breathes this mixture for a few minutes so that it mixes through the lungs. After a few minutes the gas mixture is swapped for normal air and we measure how quickly the baby breathes (or "washes") out the $SF_6$ from the lungs. Babies with normal lungs quickly wash out SF6 whereas babies with early lung disease take longer to clear the gas from their lungs.

***c) Lung volumes***

The third test measures how big the lungs are (lung volume). In order to do this the baby lies in a special cot (which looks a bit like a large incubator) and breathes air through a face mask. The cot has a transparent hood which is closed for 2-3 minutes so that we can record the tiny pressure changes that occur while your baby is breathing quietly.

***d) Forced expiration***

This test measures how quickly your baby can breathe out. An inflatable jacket (like a small life jacket) is placed around the baby's chest and tummy. Once the baby has taken a breath in we inflate the jacket. This gives a gentle squeeze to the chest and encourages air to be breathed out quickly. Babies with lung disease cannot blow out as much air or as quickly as babies with normal lungs.

2. **Bronchoscopy and Broncho-alveolar lavage**

Lung disease in CF is caused by infections that damage the lungs. Sometimes infections occur without any obvious signs, such as cough or breathing problems. Older children and adults often produce sputum (phlegm) when they have infections and this is sent to the laboratory to find out what is causing the infection. Babies and young children cannot cough out sputum. Instead we take mucus samples from the lungs during a procedure called a bronchoscopy and send them to the laboratory. If we find infection we change the baby's treatment to fight the infection.

Bronchoscopy is widely used in children and adults. Many CF centres (including GOSH) already use it routinely in babies they look after. For this study, one research bronchoscopy will be performed at around about 12 months of age, and at a time when your baby is well. This will not be extra to the routine bronchoscopy your baby would be having as a routine part of his/her care at GOSH.

The test will generally be performed at your specialist CF centre by your CF consultant. All of the consultants in the specialist centres involved in this study are experienced in the use of this technique in babies. The bronchoscope, which is like a flexible telescope, is passed through the baby's mouth or nose into his/her lungs while they are under a light general anaesthetic. We take the sample by putting a small amount of saline in to the lungs through the bronchoscope and sucking it back, together with some mucus (this is called broncho-alveolar lavage). The sample is sent to the hospital microbiology lab to find out what, if any, infection is in the lungs.

Infection in the lungs also causes inflammation and some of the sample collected will be sent to our research lab to look at how much inflammation is present in the lungs. We will use the opportunity of the anaesthetic to take a blood test, which we do routinely each year on all children with CF as part of their annual review. For this study we would like to ask your

permission to take an extra 3ml (less than a teaspoon) of blood to look for any signs of inflammation in the lungs.

Any remaining samples from the broncho-alveolar lavage and blood test will be stored securely for 6 years and at the end of that time destroyed. The reason for storing extra samples is that in future we may want to look at them again for some further testing. You can be informed of any extended analysis if you wish.

### *3. Computed Tomography Scan (CT scan)*

CT scans are being used more and more to find out how CF is affecting the lungs of children. For this study just one CT scan will be performed when your baby is about one year of age. This is the only extra test your baby will have which is different from their routine care at GOSH.

The CT scan will usually be done at your own specialist CF centre during the same anaesthetic that is needed for the bronchoscopy, to ensure that your baby lies quietly during the scan. CT scans are a specialised type of x-ray which allows us to look at the structure of the lungs in detail. Although this type of scan involves a higher dose of radiation than a normal chest x-ray, the results are far more informative, especially in the presence of early lung disease. New scanning techniques mean that we can get excellent pictures without exposing the child to high doses of radiation. The technique used in this study only exposes the child to a level of radiation which is about half of what we all receive each year from background sources.

**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 beginning and once at the end of the study.

**Are there any other tests or measurements?**
Any other measurements or results of tests which will be used for this study are all part of your baby's routine care at your CF centre.

**What will we have to do if we take part?**
Whether or not you take part in this study, we will see your baby regularly in clinic (just as is the case normally), but in addition your baby will have three other visits to have the tests described above. At GOSH all but the CT scan are part of our routine care and as this is done on the same day as the bronchoscopy, there will be no additional visits.

*On the first and second visits* (at around 3 months and one year of age ) we will invite you to Great Ormond St Hospital to measure your child's lung function. Each visit will take about 3 hours in total. You can take your child home when he/she is fully awake.

*The third visit* will take place in your own specialist CF centre, about two weeks after the second lung function test. At this visit your child will have an anaesthetic for the bronchoscopy and CT scan described earlier. The procedure itself takes no longer than 30-45 minutes but you should expect to be in the hospital for 6-8 hours (to allow for examination, preparation for the anaesthetic and waking up time). In addition to this information sheet you will receive the standard hospital information about how to prepare your child for an anaesthetic and what time to come to the hospital. You will be asked to sign another consent form for these tests as is normal hospital procedure.

**Are there any risks or discomfort for my baby?**
*Breathing tests:* Since we can only perform the breathing tests while babies are sleeping quietly, we make sure that they remain comfortable at all times. The only time when they might object is

while they are being given the spoonful of syrup (which has a slightly bitter taste), and when we are measuring them to see how long they are. While the medicine is wearing off, which may take a few hours, you baby may remain a little sleepy and need to be watched carefully to ensure they do not tumble if they have already started to walk

*Bronchoscopy*: Although any general anaesthetic is associated with a slight risk (such as reaction to the anaesthetic medication), all bronchoscopies for this study will be undertaken by expert paediatric anaesthetists and consultant respiratory paediatricians. Under such conditions, risks are considered to be extremely low. Some children develop a slight fever for 24 hours following the test, especially if there are signs of a respiratory infection at the time of the bronchoscopy. This can be treated with medication such as paracetamol, but rarely occurs if the bronchoscopy is timed for when the child is well.

*CT scan:* Since this will be undertaken under the same anaesthetic as the bronchoscopy, there will be no additional discomfort for the child. The scan does involve some radiation exposure, but this will be kept very low by selection of appropriate techniques performed by specialists.
You will be given the name and telephone number of a doctor or nurse whom you can contact at any time should you have any concerns once you are back home after the tests.

**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 baby does not have an obvious cough or other symptoms). The two most common bugs seen in young children with CF are Staphylococcus aureus (SA) and Pseudomonas aeruginosa (PA). It is very important that these 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 baby 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 baby 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 baby will also have the opportunity to have specialised assessments such as infant lung function (breathing) tests and CT scans. 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 baby'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 baby to be identified.

**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 every 2 months when cough swabs would be taken, and lung function tests and bronchoscopy would be scheduled during the first year of life as is current routine practice at GOSH. Your child would not have the CT scan at one year of age as part of the study.

**What will happen when the study stops?**
Once your child is one year 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 2013).
- 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?**
Great Ormond Street Hospital Special Trustees/ Children's Charity 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 Research Ethics Committee at UCL, Institute of Child Health and GOS Hospital for Children who consider that it is addressing an important question regarding treatment of infants 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 (*contact number*).

In the extremely unlikely event that something does go wrong and you or your baby 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 *(add details)* 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:

If you would like further information before this time, you can either telephone or email

a) Dr XXX (Responsible clinician @ CF centre)

b) Xxx (Responsible research nurse)

c) Xxx (one of the principle investigators)

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

***Parent Information Sheet***

**Early detection of lung disease in infants with CF diagnosed by newborn screening**
**NREC Number: _09H071314**

**Thank you for taking the time to read this information document.**

**PART 1: Essential elements of the study**

We would like to invite you to take part in a research study 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 are approaching you as a parent of a healthy baby.*

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 baby. Please take time to read the information in this document. Ask us if there is anything that is not clear or if you would like more information. Once you have decided whether you would like your child to take part you will be given a copy of this information and a signed consent form to keep.

**What is the purpose of this study?**
Newborn screening for CF is now undertaken routinely the UK. This means that we can identify babies who have CF very early, often while they are still healthy. However, there will be no benefits to newborn screening unless we develop suitable treatments to minimise lung damage during the critical first years of life when the lungs are growing and developing very quickly.

In order to detect changes in babies with lung disease, we need to understand how the lung grows and develops in healthy babies. That is why we are asking for your help as we would like to measure breathing patterns in your baby at around 3 months of age and again at around one year of age. The information we obtain will help improve the treatment of children born with CF.

**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 care in any way. We are sending you this information sheet to read so you 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 baby 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 baby is around 3 months and one year of age to measure his/her lung function. Each visit will take about 3 hours in total. You can take your child home as soon as he/she is fully awake.

***A) Breathing Tests:***
The breathing tests are described below but we are also sending you a separate leaflet which shows some photos of the tests. Breathing tests in babies 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 will be refunded. You can stay with your child all the time that they are being tested.

Older children and adults do breathing tests by taking deep breaths and blowing through special equipment. Babies are unable to co-operate in this way and therefore need to be asleep for the tests to be done. Babies do not always sleep for long during the day, so we give them a mild sleeping syrup called chloral hydrate to help them fall asleep. This medicine is often used in children and has been used for breathing tests in babies for over 25 years. Your baby will be able to feed before the tests and eat/drink normally as soon as they wake up. The tests do not upset the babies, who usually sleep through the whole process. It usually only takes about an hour to do all the tests but it is difficult to predict how quickly the baby will fall asleep, so it is best to allow about three hours for the visit. During the visit a research doctor will examine the baby and ask a few health questions.

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

***a) Breathing Patterns using Electrical impedance tomography (EIT) and Structured Light Plethysmography (SLP)***

Most of the lung function tests we make require babies to be fast asleep, which is why we need to give some sedative syrup before we start. However, some new approaches are now being developed that could make it easier to obtain similar results without sedation. This would mean we could offer these assessments to far more babies with breathing problems and in hospitals without the special facilities available at GOS. The first technique simply involves placing small sticky pads around the baby's chest. This allows us to record tiny changes which occur as the baby breathes in and out and tells us whether the air breathed in is being spread evenly over the lungs, or whether there are some parts of the lung which are getting less air (for example due to some obstruction of the airway). The pads will remain on the baby's chest throughout the testing session.

The second approach (SLP) is even more straightforward and simply involves projecting a light grid onto the baby's chest and recording the movements of this grid with two overhead cameras as they breathe. Several sets of measurements will be taken, each lasting approximately 1 minute. The technique is entirely painless and non-invasive and does not require anything to be connected to your child. There is no radiation involved.

***b) Multiple breath washout test***

This test is designed to find out how evenly your child breathes. The baby 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 baby breathes this mixture for a few minutes so that it mixes through the lungs. After a few minutes the gas mixture is swapped for normal air and we measure how quickly the baby breathes (or "washes") out the $SF_6$ from the lungs. Babies with normal lungs quickly wash out $SF_6$ whereas babies with early lung disease take longer to clear the gas from their lungs.

***c) Lung volumes***

The third test measures how big the lungs are (lung volume). In order to do this the baby lies in a special cot (which looks a bit like a large incubator) and breathes air through a face mask. The cot has a transparent hood which is closed for 2-3 minutes so that we can record the tiny pressure changes that occur while your baby is breathing quietly.

***d) Forced expiration***

This last breathing test measures how quickly your baby can breathe out. An inflatable jacket (like a small life jacket) is placed around the baby's chest and tummy. Once the baby has taken a breath in we inflate the jacket. This gives a gentle squeeze to the chest and encourages air to be breathed out quickly. Babies with lung disease cannot blow out as much air or as quickly as babies with normal lungs.

**B) Other investigations:**

While you are at the laboratory, we will also weigh and measure your baby, and ask you a few questions about your family and your baby'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 beginning and once at the end of the study.

While the measurements are being made we will collect a ***urine sample*** from your baby. This will be sent to the laboratory to examine it for a substance which is produced when inflammation is broken down in the lungs and also for a substance which indicates passive exposure to tobacco smoke. This urine sample will be stored securely for six years and then destroyed. The reason for storing the extra urine is in case we need to look at the sample again in the future. You can be informed of any extended analysis if you wish.

**Are there any risks or discomfort for my baby?**

*Breathing tests:* Since we can only perform the breathing tests while babies are sleeping quietly, we make sure that they remain comfortable at all times. The only time when they might object is while they are being given the spoonful of syrup (which has a slightly bitter taste), and when we are measuring them to see how tall they are. While the medicine is wearing off, which may take a few hours, you baby may remain a little sleepy and need to be watched carefully to ensure they do not tumble if they have already started to walk

**<u>PART 2</u>: 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 baby does not have an obvious cough or other symptoms). It is very important that these infections are detected and treated rapidly, using tests like those described for 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 baby, but the information we obtain will help improve the treatment of children born 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 only used in a way that will not allow you or your baby to be identified.

**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 Research Ethics Committee at UCL, Institute of Child Health and GOS Hospital for Children who consider that it is addressing an important question

regarding treatment of infants with CF and that there will be minimal risk to you or your child if you participate.

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

- All members of the infant lung function team will be happy to explain the tests and answer your questions. You may phone us at Great Ormond Street Hospital on **020 7405 9200 (ext. 5454),** or if you cannot get through on this line, then contact our secretary, Jana Varma (Portex Unit) on **020 7905 2382.**
- Dr Ah-Fong Hoo and Dr Jane Chudleigh, who will be organising the tests can also contacted via the above telephone numbers.

**Thank you for your time.**

# Breathing tests for babies

## Information for families

**Great Ormond Street Hospital for Children NHS Trust and the Institute of Child Health**

**For many years, a research team of doctors and nurses from this hospital have been measuring breathing patterns in babies in order to help prevent and treat chest problems in infants and young children.**

**This leaflet is designed to explain simply and clearly the work that we do – assessing the way babies' lungs work.**

*A sleeping baby breathing through a flow sensor*

## Why do we need to measure babies' lungs and breathing?

Breathing problems are very common in babies and young children, for example:

- Some babies and young children are prone to chest infections or wheezy episodes which may continue through infancy and into adult life.
- Premature and other small babies sometimes have under-developed lungs and they may need to be given oxygen or assisted ventilation. Occasionally, they continue to have breathing problems when they are older.
- Very rarely, babies are born with serious lung problems such as diaphragmatic hernia or cystic fibrosis.

Breathing tests done soon after birth can help us to understand more about how these problems arise and how we can treat them.

## We need your help in this work

To find out more about breathing problems in sick babies, we need to measure breathing patterns in as many healthy infants as possible. This is why we have a special baby testing room at GOS Hospital.

A member of our research team may contact you to ask if you would like to be involved in this work.

## What do breathing tests involve?

Breathing tests are carried out while your baby sleeps and are not at all painful or upsetting. Young babies usually fall asleep after a feed, but we usually need to give older babies a spoonful of sedative syrup that helps them to sleep for about an hour. We then gently place a small mask over their mouth and nose. This is attached to a meter (or sensor) which monitors your baby's breathing, and the results are displayed on a computer screen.

We measure the amount of air that your baby breathes, how fast the air goes in and out, and how much effort your baby is making.

One of the important pieces of information that we need is how much air the lung holds at the end of each breath, and how rapidly your baby can remove waste gases that the body produces (e.g. carbon dioxide) from his/her lungs. We can do this by giving your baby a special air mixture to breathe for 1-2 minutes. This mixture has the same amount of oxygen that your baby normally breathes and is completely harmless.

You may know that breathing tests in older children and adults involve taking a deep breath in, and then breathing out as fast as possible.

We obviously cannot ask a baby to do this, but we are able to do the same test by giving him/her some help. We encourage your baby to take a deep breath in by providing extra air through the facemask.

In order to help him/her to breathe out quickly, a small jacket (rather like a miniature life jacket) is secured around the chest and inflated when your baby has taken a breath in.

This test has been performed many times by the research team, and at other centres around the world with no problems. Babies usually stay sound asleep.

For some tests, we may place your baby in a special cot with a Perspex hood, which looks rather like an incubator so that we can measure how big your baby's lungs are.

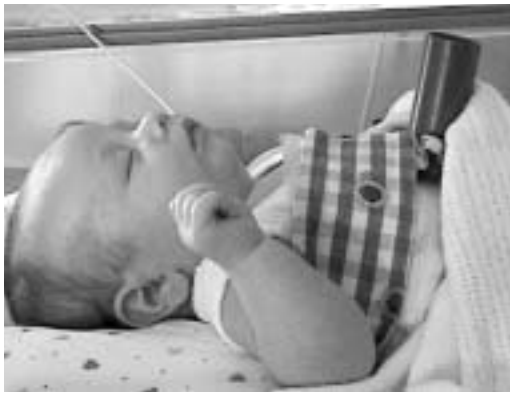
## What do we find out from these tests?

The infant lung function tests are used to measure how much effort your baby needs to make to breathe, and how big their lungs and breathing tubes (airways) are.

The lungs contain a huge network of branching tubes, which look rather like two upside-down trees. The air can get in and out easily if the airways are wide and the lung tissue is stretchy.

When a baby has narrow airways or stiff lungs, they have to work much harder to breathe.

*Babies usually sleep through all these measurements and begin to wake towards the end of the test or as soon as it has finished.*



*A baby wearing the jacket*

## Questions you may have

### Will the test hurt my baby?

No! The test does not involve any needles or painful procedures. We need your baby to be sleeping quietly before we can make any measurement, and so we try to make him/her as comfortable and relaxed as possible.

### How long will the test take?

The time of the test can be rather variable, depending upon when your baby falls asleep. We try to fit in around your normal routine as far as possible. If your baby wakes during the test, they can be fed or changed, which can take a little time. Most tests are completed within two hours.

### Can I come and watch?

Yes. We like parents to be actively involved in the tests and to ask questions. You know your baby best, and so your help is very useful.

### What happens afterward?

After the test, we measure your baby's length and weight, and ask a few questions that are relevant to the tests (e.g. family history of asthma). We then take you and your baby back to the ward, or offer transport if you have come from home.

### Does my baby have to take part?

No. Taking part is your decision whether your baby has breathing problems or is a 'healthy control'. Your baby will receive all the care they need whether or not you take part.

**Where can I get more information?**
**Members of the Infant Lung Function Team will be happy to answer any questions you may have. You can contact us at the Lab on 020 7405 9200 ext 5454, or our secretary (Portex Unit) on 020 7905 2382.**

Great Ormond Street Hospital for Children NHS Trust
Great Ormond Street
London WC1N 3JH

Tel: 020 7405 9200

Compiled by the Cardiorespiratory Department


Ref: PIG 2002/801

**Great Ormond Street Hospital for Children NHS Trust and Institute of Child Health Research Ethics Committee REC Number: _09H071314**

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

# Early detection of lung disease in infants with CF diagnosed by newborn screening:

## 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 the Head of the Research and Development Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH or if urgent, by telephone on 0207 905 2179.

**Please initial boxes**

| | | |
|---|---|---|
| 1. | I confirm that I have read and understand the information sheet dated 18/03/2009 (version 2) 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 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 regulatory authorities or from the NHS 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 GP being informed of my child's participation in the study. | |
| 5. | I agree for my child to take part in the above study. | |

| _____ | _____ | _____ |
|---|---|---|
| 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.

## A5 Test Study Questionnaires

- Initial and subsequent test study questionnaires for CF Infants

- Initial and subsequent test study questionnaires for Healthy Control Infants

## A6 Lung Function Test Record Form for Infants

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Subject No:** | 5 | 3 | | | | **Hospital Number:**_____ | |
| **Subject ID:** | | | | | | **NHS number:**_____ | |
| **Date** | | | | | | | |

# Questionnaire for GOSH CF referral

## Background Information

**Baby's surname:**

**First name:**

**Date of Birth** | | | | | | |

**Birth weight** | | | • | | | kg

**EDD** | | | | | | |

**Gestational age** | | | w | + | | d

**Sex** Male | Female

**Date of test 1** | | | | | | |

**Date of test 2** | | | | | | |

**Mother's first name**

**Mother's last name**

**Mother's DOB**

**Mother's email**

**Father's first name**

**Father's last name**

**Father's DOB**

**Father's email**

**Child's address**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Telephone number** | | | | | | | | | | | **Home** |
| ***or*** | | | | | | | | | | | **Mum work** |
| ***or*** | | | | | | | | | | | **Mum mobile** |
| ***or*** | | | | | | | | | | | **Dad work** |
| ***or*** | | | | | | | | | | | **Dad mobile** |
| ***or*** | | | | | | | | | | | **Other** |

**Social History:**

| **Does the child's natural mother have parental responsibility?** | **Yes** | **No** | **Not sure** |
|---|---|---|---|

**Mother's most recent job (title/description, state if self-employed):**

**(For later coding)**

**Father's current job (title/description, state if self-employed):**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Subject No:** | 5 | 3 | | | | **Hospital Number:_____** | |
| **Subject ID:** | | | | | | **NHS number:_____** | |
| **Date** | | | | | | | |

**(For later coding)** | | | |

**Years mother spent in full time education after the age of 16:** |

| **Mother's ethnic origin** | | **Father's ethnic origin** | |
|---|---|---|---|
| White/British | | White/British | |
| White/Irish | | White/Irish | |
| Other White | | Other White | |
| Black-Caribbean | | Black-Caribbean | |
| Black-African | | Black-African | |
| Black-other | | Black-other | |
| White-Black-Caribbean | | White-Black-Caribbean | |
| White-Black-African | | White-Black-African | |
| White-Asian | | White-Asian | |
| Other mixed | | Other mixed | |
| Pakistani | | Pakistani | |
| Bangladeshi | | Bangladeshi | |
| Indian | | Indian | |
| Chinese | | Chinese | |
| Other Asian | | Other Asian | |
| Other | | Other | |

| | | | | | |
|---|---|---|---|---|---|
| **Number of siblings:** | | | | | |
| **Number of older siblings:** | | | | | |
| **Day care:** | No | Yes, Nursery | Yes, Child Minder | **Age when started (months)** | |

| | | | |
|---|---|---|---|
| **Recruitment centre** | | **Date of recruitment** | |
| **Referring LCFC Consultant** | | **Date of referral** | |
| **GOSH Consultant** | | | |

**Local Paediatrican Name:** **GP's name:**

**Address:** **Practice address:**

**Telephone no:** **Practice tel no:**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Subject No:** | 5 | 3 | | | | **Hospital Number:_____** | |
| **Subject ID:** | | | | | | **NHS number:_____** | |
| **Date** | | | | | | | |

**Date of Diagnosis** | | | | | | |

| **Genotype (if and when known)** | **Mutation one** | **Mutation two** |
|---|---|---|
| | | |

**Presentation**

| **Mode(s) of Presentation** | | **Sweat Test Result** | | **Repeat Sweat Test Result** | |
|---|---|---|---|---|---|
| Asymptomatic | | **Positive** | | **Positive** | |
| Meconium ileus | | **Negative** | | **Negative** | |
| Failure to thrive/malabsorption | | **Borderline** | | **Borderline** | |
| Recurrent chest infections | | **Not Done** | | **Not Done** | |
| Recurrent wheezy episodes | | | | | |
| Prolonged jaundice | | | | | |
| Biochemical abnormalities | | **$Cl^-$** | | **$Cl^-$** | |
| Rectal Prolapse | | **$Na^{2+}$** | | **$Na^{2+}$** | |
| Antenatal bowel pathology | | **Osmol** | | **Osmol** | |
| Family history | | | | | |
| Screening | | | | | |

| **Recorded Diagnosis/Diagnoses, including CF and any Congenital Abnormalities:** | |
|---|---|

| **Significant neonatal history (if admitted for special care, document reason)** |
|---|
| |

**Duration Exclusively breastfed (weeks)**

| | | | |
|---|---|---|---|
| **Number of respiratory admissions before diagnosis:** | | | |
| **Number of respiratory admissions between diagnosis and before first RFTs:** | | | |
| **URTI** | | **LRI** | |

| **Has a doctor diagnosed upper airway obstruction in your child?** | **Yes** | **No** | **Not sure** |
|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Subject No:** | 5 | 3 | | | | **Hospital Number:_____** | |
| **Subject ID:** | | | | | | **NHS number:_____** | |
| **Date** | | | | | | | |

**Family medical History:**

**Does anyone in your family have cystic fibrosis?**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| None | | Mother | | Brother | | Grandfather | | Niece | |
| | | Father | | Half-sibling | | Aunt | | Nephew | |
| | | Sister | | Grandmother | | Uncle | | Cousin | |

**Family history of atopy: Have any of the people below been diagnosed with the following by a <u>doctor</u>?**

| | Mother | Father | Sister | Brother | Half-sibling |
|---|---|---|---|---|---|
| **Asthma** | | | | | |
| **Wheezing** | | | | | |
| **Eczema** | | | | | |
| **Hay Fever** | | | | | |

| | |
|---|---|
| **Additional information:** | |

| | |
|---|---|
| **Source of information:** | |

## Are there any reasons for exclusion from study?

| | | | |
|---|---|---|---|
| **History of apnoeic episode** | | **Neonatal lung disease** | |
| **Upper airway pathology** | | **Heart, lung, renal disease** | |
| **Failure to thrive** | | **Parental psycho-social reasons** | |
| **Lack of Understanding** | | | |

| | | |
|---|---|---|
| **Does your child have any other disease congenital or acquired?** | **Yes** | **No** |

| | |
|---|---|
| **If so, what is the other problem?** | |

| Study number | 5 | 3 | | | | Test occasion: 01 | Date | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

# Questionnaire for GOSH CF Referral

## Information From Parent at First Visit Only

**Baby's name:** **Date of Birth**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Time of arrival at test site** | | | • | | | |
| **(oral) chloral sedation: Yes / No** | **Dose of sedation given:** | | **mg/ kg** | | | |
| **Time of administration of sedation** | | | • | | | |
| **Time of sleep** | | | • | | | |
| **Time of test commencement** | | | • | | | |
| **Time of leaving test site** | | | • | | | |
| **Number of sleep epochs required to complete test:** | | | | | | |

| | | | |
|---|---|---|---|
| **Barometric Pressure** | mbar | **Face mask: type /size** | |
| **Temperature** | **C** | **PNT Size (MBW)** | |
| **Humidity** | **%** | **PNT Size (Jaeger)** | |

| | | | |
|---|---|---|---|
| **Operators** | | | |

| Test | MBW | Tidal | Pleth | Crs | RTC | RVRTC | EIT | Other |
|---|---|---|---|---|---|---|---|---|
| **Order** | | | | | | | | |
| **Data acceptable?** | | | | | | | | |

| | |
|---|---|
| **Physical examination at time of test:** | **Performed by:** |

**Wheezes** **Yes / No** **Crackles** **Yes / No**

**Pre sedation**

**Respiratory rate** bpm **$SaO_2$** % **Mean HR**

**Post sedation**

**Respiratory rate** bpm **$SaO_2$** % **Mean HR**

| | | |
|---|---|---|
| **Remainder of clinical examination normal:** | **Yes** | **No – comment:** |

**Anthropometry:** **Weight** • **kg**

**Crown-heel length** • **cm** **OFC** • **Cm**

| | | | |
|---|---|---|---|
| **Whether the child has any atopic disorder?** | Yes | No | Not known |
| **Whether the child has developed eczema?** | Yes | No | Not known |
| **Respiratory problems other than CF?** | No | Not known | Yes (details?) |

| Study number | 5 | 3 | | | | Test occasion: 01 | Date | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

| Non-respiratory medical problems? | No | Not known |
|---|---|---|
| If Yes, please give details: | | |

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

## Hospital admissions since birth, the following information is required for each:

Date of admission; reason for admission; hospital name; date of discharge; whether in-patient treatment for a respiratory infection included I/V antibiotics; Duration and type of any ventilation.

| Date Admitted / Discharged | | Reason and hospital name | Ventilation (Date/duration) (Mode/Modes used) | IV/Inhaled Antibiotics (for chest) |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

## Intermittent antibiotic therapy

**For each parameter record number of courses and name of drug (if applicable) received since diagnosis:**

| Date | Reason for course (respiratory/nonrespiratory) | Location (Home/Hosp/Both) | Route (Oral/IV/Inhaled) | Total (Number) |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |

| Whether the child has had bronchiolitis? | Yes | No | Not known |
|---|---|---|---|
| Number of admissions for bronchiolitis since birth? | | | |

| Number of admissions for respiratory illnesses (excl above) since birth? | |
|---|---|

| Any operations since birth: | |
|---|---|

| Whether the child has ever needed mechanical ventilation since birth? | | | Yes | No | Not known |
|---|---|---|---|---|---|
| Date ventilation started: | | No. of days ventilated | | | |

**Study number** | 5 | 3 | | | | **Test occasion: 01** **Date** | | | | | | |

**Medications Occasion 1: Tick all current medications**

| Pulmonary | Yes | No |
|---|---|---|
| Antibiotics – oral (not quinolones) | | |
| Antibiotics – inhaled | | |
| Corticosteroids | | |
| Bronchodilators (specify): | | |
| Mucolytics | | |
| Oxygen | | |
| | | |

| Nutritional | Yes | No |
|---|---|---|
| Pancreatic enzymes | | |
| $H_2$ Blockers | | |
| Proton Pump Inhibitors | | |
| Motility agents | | |
| Vitamin supplements | | |

| | | |
|---|---|---|
| **Has your child ever been prescribed a bronchodilator ?** | **Yes** | **No** |

| | | |
|---|---|---|
| **Has your child had a bronchodilator in the last 12 hours?** | **Yes** | **No** |

**Hours since bronchodilator given** **hrs**

| | |
|---|---|
| **Has your child had a cold in the last 3 weeks?** | **Yes / No** |

**URTI in last 3 weeks**

- **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** | **With physio only** | **Not just with physio, but not daily** | **Daily** |

| | | | |
|---|---|---|---|
| **Wheeze** | **Yes** | **No** | **Don't Know** |

**Physiotherapy given?** **not at all** **Once a day** **times a day**

**No. of hours since last physio session:** **hrs**

**Smoking History**

**Mother's smoking habit: How many cigarettes a day did you smoke during your pregnancy?**

Not at all
Yes — Number of cigarettes per day
Unknown — If gave up, when? (Weeks)

**Does mother smoke now?** **No** **Yes** cigarettes a day

**Does mother's partner smoke now?** **No** **Yes** cigarettes a day

| **Study number** | 5 | 3 | | | | **Test occasion: 01** | **Date** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

**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?**

| | | |
|---|---|---|
| **Has urine been collected?** | **Yes** | **No** |
| **Has saliva been collected?** | **Yes** | **No** |

| **Cough Swab taken?** | **Yes** | **No** | **Date** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|

**Microbiology from 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 | |

| **Specimen** **(BAL/Cough Swab/Sputum)** | **Date** | | | | | | **Recorded Cultures** **(See list – include no growth)** |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

**Date and result of CXR:**

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| | | | | | | |

| Study number | 5 | 3 | | | | Test occasion: 0 | Date | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

# Questionnaire for GOSH CF Referral

## Information From Parent at Subsequent Visit Only

**Baby's name:** **Date of Birth**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Time of arrival at test site** | | | • | | | |
| **(oral) chloral sedation: Yes / No** | **Dose of sedation given:** | | | **mg/ kg** | | |
| **Time of administration of sedation** | | | • | | | |
| **Time of sleep** | | | • | | | |
| **Time of test commencement** | | | • | | | |
| **Time of leaving test site** | | | • | | | |
| **Number of sleep epochs required to complete test:** | | | | | | |

| | | | |
|---|---|---|---|
| **Barometric Pressure** | mbar | **Face mask: type /size** | **RB / 1, 2 (circle)** |
| **Temperature** | **C** | **PNT Size (MBW)** | **Fleisch 0** |
| **Humidity** | **%** | **PNT Size (Jaeger)** | **S** |

| | | | |
|---|---|---|---|
| **Operators** | | | |

| Test | MBW | Tidal | Pleth | Crs | RTC | RVRTC | EIT | CT | Bronch | Other |
|---|---|---|---|---|---|---|---|---|---|---|
| Order | | | | | | | | | | |
| Data acceptable? | | | | | | | | | | |

| | |
|---|---|
| **Physical examination at time of test:** | **Performed by:** |

**Wheezes** **Yes / No** **Crackles** **Yes / No**

**Pre sedation**

**Respiratory rate** bpm **$SaO_2$** % **Mean HR**

**Post sedation**

**Respiratory rate** bpm **$SaO_2$** % **Mean HR**

| | | |
|---|---|---|
| **Remainder of clinical examination normal:** | **Yes** | **No – comment:** |

**Anthropometry:** **Weight** • **kg**

**Crown-heel length** • **cm** **OFC** • **Cm**

| | | | |
|---|---|---|---|
| **pH study performed since previous visit** | **Yes** | **No** | **If Yes – date & result** |

| **Study number** | 5 | 3 | | | | **Test occasion: 0** | **Date** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BAL performed since previous visit** | **Yes** | **No** | **If Yes – date & result** | | | | | | | | | |
| **Date and result of CXR:** | **Yes** | **No** | **If Yes – date & result** | | | | | | | | | |
| | **Yes** | **No** | **If Yes – date & result** | | | | | | | | | |
| **If CT/BAL booked state date** | **Yes** | **No** | **If Yes – date & result** | | | | | | | | | |
| | | | | | | | | | | | | |

| | | | |
|---|---|---|---|
| **Whether the child has any atopic disorder?** | Yes | No | Not known |
| **Whether a doctor has ever diagnosed asthma?** | Yes | No | Not known |
| **Whether the child has developed eczema?** | Yes | No | Not known |
| **Whether the child has developed hay fever?** | Yes | No | Not known |

| | | | |
|---|---|---|---|
| **Respiratory problems other than CF?** | No | Not known | Yes (Details): |
| **Non-respiratory medical problems?** | | No | Not known |
| If Yes, please give details: | | | |

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

## Hospital admissions since birth, the following information is required for each:

Date of admission; reason for admission; hospital name; date of discharge; whether in-patient treatment for a respiratory infection included I/V antibiotics; Duration and type of any ventilation.

| **Admitted / Discharged** | | **Reason and hospital name** | **Ventilation (Date/duration) (Mode/Modes used)** | **IV/Inhaled Antibiotics (for chest)** |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

**Intermittent antibiotic therapy**

**For each parameter record number of courses and name of drug (if applicable) received since diagnosis:**

| **Date** | **Reason for course (respiratory/nonrespiratory)** | **Location (Home/Hosp/Both)** | **Route (Oral/IV/Inhaled)** | **Total (Number)** |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |

| Study number | 5 | 3 | | | | Test occasion: 0 | Date | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

| Whether the child has had bronchiolitis since last LFT? | Yes | No | Not known |
|---|---|---|---|
| Number of admissions for bronchiolitis since last LFT? | | | |

| Number of admissions for respiratory illnesses (excl above) since last LFT? | |
|---|---|

| Any operations since last LFT: | |
|---|---|

| Whether the child has needed mechanical ventilation since last LFT? | | | Yes | No | Not known |
|---|---|---|---|---|---|
| Date ventilation started: | | No. of days ventilated | | | |

**Medications Occasion 1: Tick all current medications**

| Pulmonary | Yes | No |
|---|---|---|
| Antibiotics – oral (not quinolones) | | |
| Antibiotics – inhaled | | |
| Corticosteroids | | |
| Bronchodilators (specify): | | |
| Mucolytics | | |
| Oxygen | | |
| | | |

| Nutritional | Yes | No |
|---|---|---|
| Pancreatic enzymes | | |
| $H_2$ Blockers | | |
| Proton Pump Inhibitors | | |
| Motility agents | | |
| Vitamin supplements | | |

| Has your child been prescribed a bronchodilator since last LFT ? | Yes | No |
|---|---|---|

| Has your child had a bronchodilator in the last 12 hours? | Yes | No |
|---|---|---|

**Hours since bronchodilator given** hrs

**Has your child had a cold in the last 3 weeks?**

**URTI in last 3 weeks**

- ☐ **No**
- ☐ **Yes but asymptomatic for** ☐ days
- ☐ **Yes and still symptomatic**

**Study number** 5 3 | **Test occasion: 0** | **Date**

**How often has your child coughed and has he/she wheezed in the last 7 days?**

| | | | | |
|---|---|---|---|---|
| **Cough** | **None** | **With physio only** | **Not just with physio, but not daily** | **Daily** |
| **Wheeze** | **Yes** | **No** | **Don't Know** | |

**Physiotherapy given?** ☐ **not at all** ☐ **once a day** ☐ **times a day**

**No. of hours since last physio session:** ☐ **hrs**

**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?**

**Has urine been collected?** **Yes** / **No**

**Has saliva been collected?** **Yes** / **No**

**Cough Swab taken?** **Yes** / **No** **Date**

| **Microbiology from 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 | |
| Candida | Klebsiella | No growth / Normal flora |

| **Specimen** **(BAL/Cough Swab/Sputum)** | **Date** | **Recorded Cultures** **(See list – include no growth)** |
|---|---|---|
| | | |
| | | |
| | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Subject No:** | 5 | 3 | | | | **Hospital Number:**_____ | |
| **Subject ID:** | | | | | | **NHS number:**_____ | |
| **Date** | | | | | | | |

# Questionnaire for GOSH Healthy Control

## Background Information

**Baby's surname:**

**First name:**

**Date of Birth**

**Birth weight** kg

**EDD**

**Gestational age** w + d

**Sex** Male | Female

**Date of test 1**

**Test 1 ID**

**Date of test 2**

**Mother's first name**

**Mother's last name**

**Mother's DOB**

**Mother's email**

**Father's first name**

**Father's last name**

**Father's DOB**

**Father's email**

**Child's address**

**Telephone number** **Home**

***or*** **Mum work**

***or*** **Mum mobile**

***or*** **Dad work**

***or*** **Dad mobile**

**Social History:**

| **Does the child's natural mother have parental responsbility?** | **Yes** | **No** | **Not sure** |
|---|---|---|---|

**Mother's most recent job (title/description- state if self-employed):**

**(For later coding)**

**Father's current job (title/description- state if self-employed):**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Subject No:** | 5 | 3 | | | | **Hospital Number:_____** | |
| **Subject ID:** | | | | | | **NHS number:_____** | |
| **Date** | | | | | | | |

**(For later coding)** | | | |

**Years mother spent in full time education after the age of 16:** |

| **Mother's ethnic origin** | | **Father's ethnic origin** | |
|---|---|---|---|
| White/British | | White/British | |
| White/Irish | | White/Irish | |
| Other White | | Other White | |
| Black-Caribbean | | Black-Caribbean | |
| Black-African | | Black-African | |
| Black-other | | Black-other | |
| White-Black-Caribbean | | White-Black-Caribbean | |
| White-Black-African | | White-Black-African | |
| White-Asian | | White-Asian | |
| Other mixed | | Other mixed | |
| Pakistani | | Pakistani | |
| Bangladeshi | | Bangladeshi | |
| Indian | | Indian | |
| Chinese | | Chinese | |
| Other Asian | | Other Asian | |
| Other | | Other | |

| | | | | | |
|---|---|---|---|---|---|
| **Number of siblings:** | | | | | |
| **Number of older siblings:** | | | | | |
| **Day care:** | No | Yes, Creche | Yes, Child Minder | **Age when started (months)** | |

**GP's name:**

**Practice address:**

**Practice tel no:**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Subject No:** | 5 | 3 | | | | **Hospital Number:_____** | |
| **Subject ID:** | | | | | | **NHS number:_____** | |
| **Date** | | | | | | | |

| **Significant neonatal history (if admitted for special care document reason)** |
|---|
| |

**Duration Exclusively breastfed**

**Family medical History:**

**Does anyone in your family have cystic fibrosis?**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| None | | Mother | | Brother | | Grandfather | | Niece | |
| | | Father | | Half-sibling | | Aunt | | Nephew | |
| | | Sister | | Grandmother | | Uncle | | Cousin | |

**Family history of atopy: Have any of the people below been diagnosed with the following by a <u>doctor</u>?**

| | Mother | Father | Sister | Brother | Half-sibling |
|---|---|---|---|---|---|
| **Asthma** | | | | | |
| **Wheezing** | | | | | |
| **Eczema** | | | | | |
| **Hay Fever** | | | | | |

| **Additional information:** | |
|---|---|

| **Source of information:** | |
|---|---|

## Are there any reasons for exclusion from study?

| | | | |
|---|---|---|---|
| **History of apnoeic episode** | | **Neonatal lung disease** | |
| **Upper airway pathology** | | **Heart, lung, renal disease** | |
| **Failure to thrive** | | **Parental psycho-social reasons** | |
| **Lack of Understanding** | | **History of bronchiolitis** | |

| **Does your child have any other disease congenital or acquired?** | **Yes** | **No** |
|---|---|---|

| **If so, what is the other problem?** | |
|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Subject No:** | 5 | 3 | | | | **Hospital Number:_____** | |
| **Subject ID:** | | | | | | **NHS number:_____** | |
| **Date** | | | | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study No** | 5 | 3 | | | | **Test occasion: 01** | **Date** | | | | | | |
| **Hospital No** | | | | | | | | | | | | | |

# Questionnaire for GOSH Healthy Controls

## Information From Parent at First Visit Only

**Baby's surname:** **Date of Birth**

**First name:**

| | | | | | |
|---|---|---|---|---|---|
| **Time of arrival at test site** | | | • | | |
| **Sedation preparation used** | | | | | |
| **Dose of sedation given** | **mg/kg** | | | | |
| **Time of administration of sedation** | | | • | | |
| **Time of sleep** | | | • | | |
| **Time of test commencement** | | | • | | |
| **Time of leaving test site** | | | • | | |
| **Number of sleep epochs required to complete test:** | | | | | |

| | | | |
|---|---|---|---|
| **Barometric Pressure** | mbar | **Face mask: type /size** | |
| **Temperature** | **C** | **PNT Size (MBW)** | |
| **Humidity** | **%** | **PNT Size (Jaeger)** | |

| | | | |
|---|---|---|---|
| **Operators** | | | |

| **Test** | **MBW** | **Tidal** | **Pleth** | **Crs** | **RTC** | **RV-RTC** | **EIT** | **Other** |
|---|---|---|---|---|---|---|---|---|
| **Order** | | | | | | | | |
| **Data acceptable?** | | | | | | | | |

| | |
|---|---|
| **Physical examination at time of test:** | **Performed by:** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Wheezes** | **Yes / No** | | **Crackles** | **Yes / No** | | | | |
| **Pre sedation** | | | | | | | | |
| **Respiratory rate** | | bpm | **$SaO_2$** | | % | **Mean HR** | | |
| **Post sedation** | | | | | | | | |
| **Respiratory rate** | | bpm | **$SaO_2$** | | % | **Mean HR** | | |

| | | |
|---|---|---|
| **Remainder of clinical examination normal:** | **Yes** | **No – comment:** |

## Anthropometry

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Weight** | | • | | kg | **Crown-heel length** | | • | | cm | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study No** | 5 | 3 | | | | **Test occasion: 01** | **Date** | | | | | | |
| **Hospital No** | | | | | | | | | | | | | |

**OFC** | | | • | | Cm

| | | | |
|---|---|---|---|
| **Whether the child has any atopic disorder?** | Yes | No | Not known |
| **Whether the child has developed eczema?** | Yes | No | Not known |

| | | | | |
|---|---|---|---|---|
| **Any operations since birth:** | | | | |
| **Whether the child has needed mechanical ventilation?** | | | **Yes** | **No** | **Not known** |
| **Date ventilation started:** | | **No. of days ventilated** | | |

**Has your child had a cold in the last 3 weeks?**

**URTI in last 3 weeks**

| | |
|---|---|
| | **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?**

| | Occasion 1 | | | |
|---|---|---|---|---|
| **Cough** | **None** | **With physio only** | **Not just with physio, but not daily** | **Daily** |

| | | | |
|---|---|---|---|
| **Wheeze** | **Yes** | **No** | **Don't Know** |

**Smoking History**

**Mother's smoking during pregnancy: How many cigarettes a day did you smoke during your pregnancy?**

| | | | |
|---|---|---|---|
| Not at all | | | |
| Yes | | Number of cigarettes per day | |
| Unknown | | If gave up, when? (Weeks) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **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** |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study No** | 5 | 3 | | | | **Test occasion: 01** | **Date** | | | | | | |
| **Hospital No** | | | | | | | | | | | | | |

**If yes - Who?**

| | | |
|---|---|---|
| **Has urine been collected?** | **Yes** | **No** |
| **Has saliva been collected?** | **Yes** | **No** |

| Study number | 5 | 3 | | | | Test occasion: 0 | Date | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

# Questionnaire for GOSH Healthy Control

## Information From Parent at visit 2 and subsequent visits

**Baby's surname:** **Date of Birth**

**First name:**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Time of arrival at test site** | | | • | | | |
| **Sedation preparation used** | | | | | | |
| **Dose of sedation given** | **mg/kg** | | | | | |
| **Time of administration of sedation** | | | • | | | |
| **Time of sleep** | | | • | | | |
| **Time of test commencement** | | | • | | | |
| **Time of leaving test site** | | | • | | | |
| **Number of sleep epochs required to complete test:** | | | | | | |

| | | | |
|---|---|---|---|
| **Barometric Pressure** | mbar | **Face mask: type /size** | |
| **Temperature** | **C** | **PNT Size (MBW)** | |
| **Humidity** | **%** | **PNT Size (Jaeger)** | |

| | | | |
|---|---|---|---|
| **Operators** | | | |

| Test | MBW | Tidal | Pleth | Crs | RTC | RV-RTC | EIT | Other |
|---|---|---|---|---|---|---|---|---|
| **Data acceptable?** | | | | | | | | |
| **Order** | | | | | | | | |

| | |
|---|---|
| **Physical examination at time of test:** | **Performed by:** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Wheezes** | **Yes / No** | | | **Crackles** | **Yes / No** | | | | | |
| **Pre sedation** | | | | | | | | | | |
| **Respiratory rate** | | | bpm | **$SaO_2$** | | | % | **Mean HR** | | | |
| **Post sedation** | | | | | | | | | | |
| **Respiratory rate** | | | bpm | **$SaO_2$** | | | % | **Mean HR** | | | |

| | | |
|---|---|---|
| **Remainder of clinical examination normal:** | **Yes** | **No – comment:** |

**Anthropometry**

| **Study number** | 5 | 3 | | | | **Test occasion: 0** | **Date** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Weight** | | | • | | | kg | **Crown-heel length** | | • | | cm |
| **OFC** | | | • | | Cm | | | | | | |

| | | | |
|---|---|---|---|
| **Whether the child has any atopic disorder?** | Yes | No | Not known |
| **Whether a doctor has ever diagnosed asthma?** | Yes | No | Not known |
| **Whether the child has developed eczema?** | Yes | No | Not known |
| **Whether the child has developed hay fever?** | Yes | No | Not known |

| | | | |
|---|---|---|---|
| **Respiratory problems developed since last test?** | No | Not known | Yes: |
| **Non-respiratory medical problems?** | | No | Not known |
| If yes, please give details: | | | |

## Hospital admissions since last LFT, the following information is required for each:

Date of admission; reason for admission; hospital name; date of discharge; whether in-patient treatment for a respiratory infection included I/V antibiotics; Duration and type of any ventilation.

| **Date admitted / Discharged** | | **Reason and hospital name** | **Ventilation (Date/duration) (Mode/Modes used)** |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

| | | | |
|---|---|---|---|
| **Whether the child has had bronchiolitis?** | **Yes** | **No** | **Not known** |
| **Number of admissions for bronchiolitis since last test?** | | | |

| | |
|---|---|
| **Number of admissions for respiratory illnesses (excl above) since last test?** | |

| | |
|---|---|
| **Any operations since last test:** | |

| Study number | 5 | 3 | | | | Test occasion: 0 | Date | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

| Whether the child has needed mechanical ventilation? | | | Yes | No | Not known |
|---|---|---|---|---|---|
| Date ventilation started: | | No. of days ventilated | | | |

**Medications Occasion 1: Tick all current medications**

| Pulmonary | Yes | No |
|---|---|---|
| Antibiotics – oral | | |
| Corticosteroids | | |
| Bronchodilators (specify): | | |
| | | |

| Has your child ever been prescribed a bronchodilator ? | Yes | No |
|---|---|---|

| Has your child had a bronchodilator in the last 12 hours? | Yes | No |
|---|---|---|

| Hours since bronchodilator given | Occ 1 |
|---|---|
| | |

**Has your child had a cold in the last 3 weeks?**

**URTI in last 3 weeks**

| | |
|---|---|
| | 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?**

**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?**

| | | |
|---|---|---|
| **Has urine been collected?** | **Yes** | **No** |
| **Has saliva been collected?** | **Yes** | **No** |

Lung Function Lab, Level 6, Cardiac Wing
Tel: 020 7405 9200 extension 5454 / 0404
Direct Line: 020 7905 2382 (Secretary)

Great Ormond Street Hospital for Children NHS Trust

# LUNG FUNCTION TESTS FOR INFANTS

**GOS Hospital No**:

**Referring Consultant**: Dr.

**Referring Hospital**:

**Child's name**:

**Referring Hospital number**:

**DOB**: male / female

**Test date**: **Study no**: (test: ) **Time of arrival**: hrs

Weight (kg) ☐☐ • ☐☐ Crown-Heel length ☐☐ • ☐

## Physical Examination

**Clinician name**: …….…… **Signature**:……

Wheeze: ☐ Yes ☐ No Crackles: ☐ Yes ☐ No

Was overall physical examination normal? ☐ Yes ☐ No

Comments:

Cough swab taken? ☐ Yes ☐ No *Comments:*

**Sedation:** Chloral Hydrate …….. mg given orally at …….. hrs

Any observed adverse effects from sedation No ☐ Yes ☐

*Comments*:

*Pre-sedation*: **oxygen saturation**: …….. % **RR**: ……. bpm **Heart rate**: …….. bpm

*Post sedation*: **oxygen saturation**: …….. % **RR**: ……. bpm **Heart rate**: …….. bpm

### On Completion of Lung Function Test

(a) Is infant fully arousable / responsive? Yes ☐ No ☐

*Comments*:

(b) Taken a Feed / Drink? Yes ☐ No ☐

*Comments*:

Time of departure: ……. hrs

Lung function tests performed by: …… / ……

Present at tests: Yes ☐ No ☐ parents / relative

Post test phone call made by: Date & Time:

*Comments*:

## A7 Standardised Treatment Protocol for CF Infants

## A8 Clinical Record Form for CF Infants

## A9 Infection Control and Cleaning Protocol for Equipment

**APPENDIX 2: ANTIBIOTIC PROTOCOL:**
**EARLY DETECTION OF LUNG DISEASE IN NEWBORN SCREENED INFANTS WITH CF**

## A. Definition of chest exacerbation

**Background:** Many studies have followed the definition of an exacerbation first used in the Genentech DNase study [1]. Their protocol-defined exacerbation was confirmed when the patient had 4 or more of the following 11 criteria:

1. adverse change in sputum production (volume, colour, consistency);
2. new or increased haemoptysis;
3. increased cough;
4. increased dyspnoea;
5. malaise, fatigue and lethargy;
6. fever > 38ºC;
7. anorexia or weight loss;
8. sinus pain,
9. tenderness or discharge;
10. $FEV_1$ or forced vital capacity (FVC) drop of 10% or more from previous recording;
11. adverse changes in chest sounds on auscultation (crackles, wheeze); chest radiographic changes.

However, many clinicians and investigators feel that in practice this definition of an exacerbation is too strict, as most clinicians would not wait for 4 criteria to be satisfied before instituting therapy. For example, an increase in purulent sputum accompanied by a fall in $FEV_1$ of over 10% would almost always lead to a course of antibiotics on the assumption the patient had a chest exacerbation (even though only two criteria were satisfied for the study definition). Furthermore, many patients would be treated with intravenous antibiotics if they felt 'not quite right' and had a big event like an exam coming up for which they really needed to be well.

A newer definition has been derived from North American ESCF data, which for patients 6 years or older has suggested 3 out of 4 of

1. decreased $FEV_1$,
2. increased cough frequency,
3. new crackles and
4. haemoptysis [2].

This is, however, still unsuitable for the infants in our study as lung function will only be measured intermittently (3 and 12 months when the child is clinically stable) and haemoptysis almost never occurs in this age group. Indeed defining an exacerbation is far more difficult in 0-2 year olds as we have no access to spontaneously expectorated sputum or regular lung function, which form the main clinical criteria in older children, and there is in any case no validated definition in this age group even from centres where regular infant lung function is available.

Rather than come up with yet another definition, for the purposes of this collaborative study, it has been decided to use the pragmatic definition of **new courses of antibiotics** determined by the treating clinician, as a substitute, as used in CF WISE [3]. The actual reasons for the antibiotic course will be recorded prospectively. Any new course (oral or intravenous) of either hospital or home-administered antibiotic will be recorded as a single event, and a change of drug once antibiotic sensitivities are known does not count as a new antibiotic event [3]. Routine 3-monthly intravenous antibiotics will not count as a new course and in any case are unlikely to be implemented in under two year olds (currently, no participating centre has an infant this young on this regime).

**Conclusion**: **New courses of antibiotics rather than a protocol-defined exacerbation will be used as an outcome** (excluding those given prophylactically for increased home infection exposure etc). For primary care prescribing of antibiotics, the data extracted from the primary care databases will enable matching of antibiotic prescription to corresponding event read code, i.e. LRTI, thereby enabling identification of new courses of antibiotics from those prescribed as prophylaxis. For hospital prescribed courses, this would be captured in the study CRF.

1. Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsey BW, Rosenstein BJ, Smith AL, Wohl ME for the Pulmozyme Study Group. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. *N Engl J Med* 1994 331:637–42.
2. Rabin HR, Butler SM, Wohl ME, Geller DE, Colin AA, Schidlow DV, Johnson CA, Konstan MW, Regelmann WE; Epidemiologic Study of Cystic Fibrosis. Pulmonary exacerbations in cystic fibrosis. *Pediatr Pulmonol* 2004;37:400–6.
3. Balfour-Lynn IM, Lees B, Hall P, Phillips G, Khan M, Flather M, Elborn JS, on behalf of the CF WISE (Withdrawal of Inhaled Steroids Evaluation) Investigators. Multicenter randomized controlled trial of withdrawal of inhaled corticosteroids in cystic fibrosis. Am J Resp Crit Care Med 2006;173:1356-62.

**B. Treatment protocol for infants with CF diagnosed by NBS**

**1. Cough swabs**

All infants in the study to have cough swabs done at all clinic visits, and as a minimum of 2-3 monthly using a standard protocol for collection, storage and analysis of samples.[1]

**2. Oral flucloxacillin prophylaxis dose**

| | |
|---|---|
| 3 to < 5 kg | 125 mg bd |
| 5 to < 9 kg | 175 mg bd |
| 9-15 kg (~1-2 y) | 250 mg bd |

*Based on therapeutic dose given twice daily to achieve MIC for Staphylococcus Aureus with each dose.*

**3. Pseudomonas aeruginosa (PsA)**

**a. First growth**

Cough swabs to be done at monthly intervals while on treatment.

***Well child*** (clinical judgment), home therapy:[2]

- Oral Ciprofloxacin 15mg/kg bd for 3 weeks, PLUS
- Nebulised Colistin 1 mu bd for 3 months

***Unwell child*** (clinical judgment), hospital therapy -
*The choice of the initial IV antibiotics will be independent of sensitivities and if necessary tailored once sensitivities are known*[3;4]

- IV tobramycin 10 mg/kg once daily for 2 weeks (trough level 23 hours after 2nd dose, must be < 1 mg/l), PLUS
- IV ceftazidime 50 mg/kg three times a day

- Also start nebulised Colistin 1 mu bd for 3 months, (initiated in hospital as appropriate).

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

***Well child***

- Give a further 3 weeks Ciprofloxacin 15mg/kg bd for 3 weeks

***Unwell child***

- IV Tobramycin and Ceftazidime for 2 weeks then further 3 months nebulised colistin (doses as above).

- OR If IV antibiotics already given at 1st isolation, can give 3 weeks ciprofloxacin and further 3 months nebulised colistin (if 2nd IVAB course inappropriate).

**c. Regrowth at end of 3 months nebulised colistin course**

- Admit for 2 weeks of IV antibiotics (tobramycin and ceftazidime) and 3 further months nebulised Colistin (1 mu bd) or TOBI (300mg bd).

**d. Regrowth after IVs and at least 6 months of nebulised colistin**

- Try 28 days nebulised TOBI ™ [5] and then continuous nebulised colistin 1 mu bd 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 3a ie first growth.

**f. Chronic Pseudomonas Infection**

Defined for analysis purposes by the Leeds criteria:[6]

| | |
|---|---|
| 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 |

**4. Staphylococcus aureus**

**a. First growth**

***Well child*** (clinical judgment), home therapy:

- 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 yrs 5ml TDS Augmentin 250/62
  for 2 (minimum) to 4 weeks (clinical judgment)

***Unwell child*** (clinical judgement), hospital therapy:

- Tobramycin 10 mg/kg once daily (trough level 23 hours after 2nd dose, must be < 1 mg/l), for 2 weeks, PLUS
- Teicoplanin 10 mg/kg 12 hrly for 3 doses then 6mg/kg once daily for 2 weeks total

**b. Re-growth after more than 6 months from first growth**

- Treat as for 4a ie first growth.

**c. Re-growth less than 6 months from first growth**

- Oral flucloxacillin 50mg/kg bd for 28 days

**d. Further re-growth within 6 months**

- Two oral anti-staphylococcal antibiotics (clinical judgment) for 28 days.

**5. Haemophilus influenzae**

**a. First growth**

***Well child*** (clinical judgement), home therapy:

- 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 yrs 5ml TDS Augmentin 250/62
  for 2 (minimum) to 4 weeks (clinical judgment)

***Unwell child*** (clinical judgement), hospital therapy:

- IV tobramycin 10 mg/kg once daily for 2 weeks (trough level 23 hours after 2nd dose, must be < 1 mg/l), PLUS
- IV ceftazidime 50 mg/kg three times a day

**b. Re-growth after more than 6 months from first growth**

- Treat as for 5a ie first growth

**c. Re-growth less than 6 months from first growth**

- 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 yrs 5ml TDS Augmentin 250/62
  for 2 (minimum) to 4 weeks (clinical judgment)

**d. Further re-growth within 6 months**

- Clarithromycin 62.5-125mg daily for 14-28 days. In practice this is unlikely to arise during the study

**6. Other growths**

- Well child (clinical judgment), home therapy: Oral antibiotic (clinical judgment) for 2 (minimum) to 4 weeks

- Unwell child (clinical judgment), hospital therapy: 2 IV antibiotics (clinical judgment) for 2 weeks

**7. Viral URTI (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 yrs 5ml TDS Augmentin 250/62
  for 2 (minimum) to 4 weeks (clinical judgment)

  Cough swab, treat as per protocol for any organism cultured.

**8. Respiratory exacerbation with unknown organism, unwell child (clinical judgment)**

Depending on severity of exacerbation:

- 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 yrs 5ml TDS Augmentin 250/62
  for 2 (minimum) to 4 weeks (clinical judgment)

  OR

- IV tobramycin 10 mg/kg once daily for 2 weeks (trough level 23 hours after 2nd dose, must be < 1 mg/l), PLUS
- IV ceftazidime 50 mg/kg three times a day

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

**Data recording must pick up use of all additional drugs.**

**References**

(1) Equi AC, Pike SE, Davies J, Bush A. Use of cough swabs in a cystic fibrosis clinic. Arch Dis Child 2001; 85(5):438-439.

(2) Frederiksen B, Koch C, Hoiby N. Antibiotic treatment of initial colonization with Pseudomonas aeruginosa postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. Pediatr Pulmonol 1997; 23(5):330-335.

(3) Davies G, McShane D, Davies JC, Bush A. Multiresistant Pseudomonas aeruginosa in a pediatric cystic fibrosis center: natural history and implications for segregation. Pediatr Pulmonol 2003; 35(4):253-256.

(4) Smith AL, Fiel SB, Mayer-Hamblett N, Ramsey B, Burns JL. Susceptibility testing of Pseudomonas aeruginosa isolates and clinical response to parenteral antibiotic administration: lack of association in cystic fibrosis. Chest 2003; 123(5):1495-1502.

(5) Gibson RL, Emerson J, McNamara S, Burns JL, Rosenfeld M, Yunker A et al. Significant microbiological effect of inhaled tobramycin in young children with cystic fibrosis. Am J Respir Crit Care Med 2003; 167(6):841-849.

(6) Lee TW, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic Pseudomonas aeruginosa infection in cystic fibrosis patients. J Cyst Fibros 2003; 2:29-34.

# Early Detection of lung disease in newborn screened infants with cystic fibrosis

## Case Record Form

| | |
|---|---|
| **Patient Initials:** | __ __ |
| **Research ID:** | |
| **CF Centre:** | |

The London Collaborative Cystic Fibrosis Group:
Barts & the Royal London Hospitals, Great Ormond Street Hospital for Children, Lewisham Hospital, Kings College Hospital, St Helier Hosptal & The Royal Brompton & Harefield NHS Trust

For any queries regarding the study please contact:
Lena Thia
The Portex Respiratory Unit
Guilford Street, London WC1N 1EH
Tel: 020 7905 2226
Fax: 020 7829 8634
j.chudleigh@ich.ucl.ac.uk

**Early Detection of lung disease in newborn screened infants with cystic fibrosis**

SUBJECT INITIALS | RESEARCH ID

## CLINICAL DETAILS

**Date of birth** ☐☐ ☐☐ ☐☐ **Sex:** M ☐ F ☐

**Date of sweat test:** ☐☐ ☐☐ ☐☐

**Sweat chloride results:** ☐☐☐ ☐☐☐

**Genotype:** ☐ / ☐ **Date:** ☐☐ ☐☐ ☐☐

**Pancreatic function:** **Sufficient** ☐ **Insufficient** ☐

**Mode of presentation:** Antenatal ☐ Newborn screening ☐ Meconium Ileus ☐

Other *(specify)*_____

## RECRUITMENT CHECK LIST

*Prior to recruiting a patient to the study please check the following:*

| | YES | NO |
|---|---|---|
| Does the patient fulfil the eligibility criteria for the study? | ☐ | ☐ |
| Have the child's parent(s) / legal guardian given written informed consent for their child to participate in the study? | ☐ | ☐ |

**Early Detection of lung disease in newborn screened infants with cystic fibrosis – Clinic visit**

SUBJECT INITIALS | RESEARCH ID

***Following completion please fax to Jane Chudleigh: 020 7829 8634***

**VISIT DATE**: | | | | | |

**Height**: | | | • | | cm **Weight**: | | • | | kg **OFC**: | | • | | cm

## RECENT HISTORY *(Since previous visit to Specialist Centre)*

**Hospital attendance / admissions**

*Date* *Reason*

| | Yes | No | |
|---|---|---|---|
| **Viral URTI since last visit** | ☐ | ☐ | **dates** *(approx)*: _____ _____ |
| **Cough since last visit:** | ☐ | ☐ | |
| **Wheeze since last visit** | ☐ | ☐ | **(Parental report / Clinician diagnosed) Please circle** |

Please ensure that any **new and or short term courses of** medications taken since the last visit are entered on concurrent medication form.

## INVESTIGATIONS: Cough swabs since last visit

Cough swab today: Y / N

| DATE: | GROWTH: |
|---|---|
| 1. | None / Normal flora / Ps A / StaphA / Haemoph / Other _____ |
| 2. | None / Normal flora / Ps A / StaphA / Haemoph / Other _____ |
| 3. | None / Normal flora / Ps A / StaphA / Haemoph / Other _____ |

## CURRENT STATUS

**Cough details:** dry / wet

daytime / nocturnal / with physiotherapy

| | | YES | NO |
|---|---|---|---|
| **Chest:** | Cough | ☐ | ☐ |
| | Wheeze | ☐ | ☐ |

## CLINICAL EXAMINATION

**Non respiratory complications:** *(specify)*

| | | YES | NO |
|---|---|---|---|
| **Chest:** | Wheeze | ☐ | ☐ |
| | Crackles | ☐ | ☐ |
| | Tachypnoea | ☐ | ☐ |

Suspected GOR ☐

Confirmed GOR ☐

GA since last visit ☐ reason: _____

Other: _____

Comments:

## CURRENT TREATMENT /TREATMENT CHANGES

Please ensure that **ALL** medications are **correct** on the concurrent medication form and **enter** any changes / new medications.

| **Current:** | YES | NO | **Other Current:** | **Changes:** |
|---|---|---|---|---|
| Creon | ☐ | ☐ | | |
| Multivitamins | ☐ | ☐ | | |
| Vitamin E | ☐ | ☐ | | |

**COMPLETED BY:** **SIGNATURE:** **DATE:**

# Early Detection of lung disease in newborn screened infants with cystic fibrosis

SUBJECT INITIALS

TRIAL NUMBER

## CONCURRENT MEDICATIONS FORM

***Please ensure that ALL medications (including short term courses between visits) and changes to medication are correctly entered on this form.***

| **Drug** (Generic name) | **Dose** | **Units** | **Freq** | **Route** | **Date Started** | **Date Stopped** | | | **Indication** (CF & non CF) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | **Date (dd/mm/yyyy)** | **Date (dd/mm/yyyy)** | **or** | **Cont. Post-trial Tick** | |
| e.g. ranitidine | 150 | mg | bd | oral | 10/11/2001 | 27/12/2001 | | | ulcer |
| 1. | | | | | | | | | |
| 2. | | | | | | | | | |
| 3. | | | | | | | | | |
| 4. | | | | | | | | | |
| 5. | | | | | | | | | |
| 6. | | | | | | | | | |
| 7. | | | | | | | | | |
| 8. | | | | | | | | | |
| 9. | | | | | | | | | |
| 10. | | | | | | | | | |
| 11. | | | | | | | | | |
| 12. | | | | | | | | | |

# Early Detection of lung disease in newborn screened infants with cystic fibrosis

SUBJECT INITIALS

TRIAL NUMBER

## CONCURRENT MEDICATIONS FORM

***Please ensure that ALL medications (including short term courses between visits) and changes to medication are correctly entered on this form.***

| Drug (Generic name) | Dose | Units | Freq | Route | Date Started | Date Stopped | | | Indication (CF & non CF) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Date (dd/mm/yyyy) | Date (dd/mm/yyyy) | or | Cont. Post-trial Tick | |
| e.g. ranitidine | 150 | mg | bd | oral | 10/11/2001 | 27/12/2001 | | | ulcer |
| 13. | | | | | | | | | |
| 14. | | | | | | | | | |
| 15. | | | | | | | | | |
| 16. | | | | | | | | | |
| 17. | | | | | | | | | |
| 18. | | | | | | | | | |
| 19 | | | | | | | | | |
| 20. | | | | | | | | | |
| 21.. | | | | | | | | | |
| 22. | | | | | | | | | |
| 23. | | | | | | | | | |
| 24. | | | | | | | | | |

**Early Detection of lung disease in newborn screened infants with cystic fibrosis – Bronchoscopy results**

SUBJECT INITIALS | RESEARCH ID

DATE OF BRONCHOSCOPY ☐☐ ☐☐ ☐☐

**FLUSH SAMPLE**

**Growth:** -ve / +ve (details) _____

**INITIALS OF:**

**Bronch Operator _____**

**Bronch Assistant _____**

## RESULTS

| | | Pooled BAL Sample YES | NO | Cfu/L |
|---|---|---|---|---|
| **Bacteriology:** | No growth | ☐ | ☐ | _____ |
| | Normal flora | ☐ | ☐ | _____ |
| | Pseudomonas aeruginosa | ☐ | ☐ | _____ |
| | Staphylococcus aureus | ☐ | ☐ | _____ |
| | Haemophilus influenzae | ☐ | ☐ | _____ |
| | NTM | ☐ | ☐ | _____ *Specify*_____ |
| | Other | ☐ | ☐ | _____ *Specify*_____ |

**Virology:** +ve / -ve Details of +ve culture: _____

**Fungal:** +ve / -ve Aspergillus / Candida / Other _____

**Cytology:**

**Macrophages % count:** **Neutrophils % count:**

**Eosinophils % count:** **Lipid laden macrophages**: Yes / No

Mild / Moderate / Severe

**Bronchoscopy secretion quantification:** ***(please circle)*** 1 2 3 4 5 6

*(Explanation of grading overleaf)*

**Cough swab result:**

## COMPLICATIONS

**During procedure:**

**Following procedure** *(within 24 hours)***:** ↑ temperature / ↑ cough

other **:**_____

Secretion Quantification at bronchoscopy:

BS Grade 1 = Nil secretions

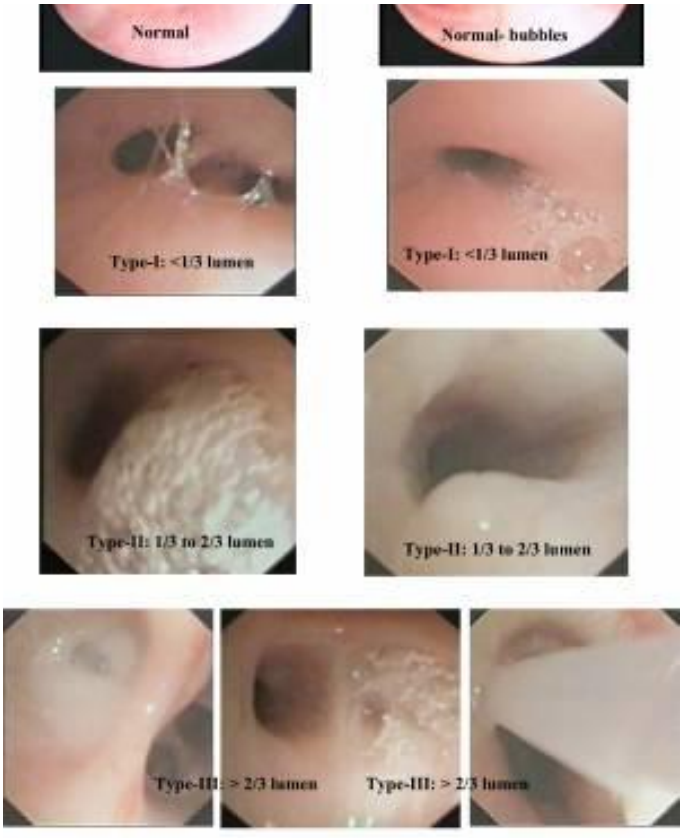
BS Grade 2 = Near dry = Bubbles only in < half total number of bronchi involved

BS Grade 3 = Minimal = Bubbles found in > half total number of bronchi involved or Secretion type-I in < half total number of bronchi involved

BS Grade 4 = Mild = Secretion type-I, > half total number of bronchi involved or Secretion type-II, < half total number of bronchi involved

BS Grade 5 = Mod = Secretion type-II, > half total number of bronchi involved or Secretion type-III, < half total number of bronchi involved

BS Grade 6 = Large = Secretion type-III, > half total number of bronchi involved




*Chang et al Cough quality in children: a comparison of subjective vs. Bronchoscopic findings. Respir Res 2005; 6:3*

**Early Detection of lung disease in newborn screened infants with cystic fibrosis – CT results**

SUBJECT INITIALS | RESEARCH ID

DATE OF CT | | | | | |

CT: REFUSED / NOT DONE _____

*(please circle as appropriate and specify reason if not done)*

## ADVERSE EVENTS

**During procedure:**

**Following procedure:**

SUBJECT INITIAL | TRIAL NUMBER

# STUDY SUMMARY

| | YES | NO |
|---|---|---|
| Have all the sections of this booklet been completed? | ☐ | ☐ |
| Did the patient complete the study? | ☐ | ☐ |

If **NO**, please tick the primary reason for withdrawal (*tick one box only*).

- ☐ Non-attendance
- ☐ Patient withdrew, give reason _____
- ☐ Other, please specify: _____

_____

Date of withdrawal: ☐☐ ☐☐ ☐☐
Day Month Year

Investigators comments: _____

_____

_____

Investigator's signature _____ Date _____

# CLEANING POLICY

1. Remove PNT from its housing and support arm/or connecting tubings.
2. Disassemble the PNT into its individual components.

- ***Jaeger (J) PNT***
  - Affix a 'nipple' onto the metal balloon control inlet of the Jaeger shutter to prevent moisture from getting inside the balloon during the cleaning/disinfectant process.

3. If urgently required for subsequent use (i.e. within 1 hr)

  - Clean PNT components with hot soapy water
  - Soft brush wire mesh/screens
  - Rinse under tap water, and then
  - Soak ALL PNT components in alcohol for 10 minutes
  - Wipe over the transducer housing and support arm (Jaeger) with alcowipes.

If there are no further studies for the day requiring use of these PNTs, then cleaning strategy may follow the protocol as given for the Fleisch PNT (see below).

*Please note changes to cleaning of Fleisch PNTs*

- ***Fleisch PNT (NOT to be soaked in alcohol) plus white connectors***

  - Rinse the flow tube and connectors with water
  - Soak PNT and connectors in Terralin solution 0.5% (disinfectant) for 1 hr* (effective for MRSA)

    * If Tuberculosis is suspected, equipment will need to be soaked for 4 hrs

  - Rinse the flow tube and connectors under tap water, then
  - Rinse the flow tube with distilled/sterile water
  - Remove water from the interior of flow tube using compressed air at a pressure up to 3 bar
  - Dry flow tube in the compressed air jet or in normal room air

**Note: Terralin (0.5%) and Milton (1/80; i.e. 125 ppm) solution when diluted for use is active for 24 hrs.**

| Other Equipment | Cleaning Strategy |
|---|---|
| Putty | Disposable |
| Face mask | Heat treated decontamination by HSDU |
| Inflatable bladders | Washed in hot soapy water |
| Squeeze jackets | Washed in hot soapy water |
| Large-bore 3-way tap of barrel | Wipe down with alcohol |

**Tubing: PNT to transducers**

*CF study:*

- Dispose and replace after each study

*Non-CF study:*

- Rinse/syringe through with soapy water
- soak in Milton (1:80) for 30 min
- Dry tubing using compressed air (immediately)

| | |
|---|---|
| Mouth pieces, connectors for Spirometry | Wash in hot soapy water and soak in Milton |
| Y-piece or T-piece connector (green) | Dispose and replace after each test |
| Corrugated tubing for bias flow | Dispose and replace after each test |
| Plethysmograph | Clean surfaces with hot soapy water and dry |
| Mattress | As above |
| Sheets and Linen | Send to laundry. Fresh linen for each subject |
| Toys | Wash in hot soapy water |

## A10 Chest CT Scanning and General Anaesthesia Protocols

- Old Guidelines
- New Amended Guidelines

## A11 Bronchoscopy and Broncho-alveolar Lavage Protocol

## A12 Brody II scoring system and scoring sheet

## Scanning parameters used in the study

| | **Topogram** | **Inspiratory Spiral** | **Expiratory Spiral** |
|---|---|---|---|
| Tube voltage (kVp) | 80 | 100 | 100 |
| Tube reference current (mAs) | 20 | 17 | 20 |
| CTDIvol (mGy) | | 0.57 | 0.67 |
| Detector collimation | | 64 x 0.6mm | |
| Tube rotation time | | 0.5 seconds | |
| Scan Pitch | | 1 | 1 |
| Coverage | ~ 256 mm | ~140 mm | ~ 30mm less than inspiratory range |
| Scan slice width | | 1mm | |
| Reconstructed slice thickness | | 1mm | |
| Reconstructed algorithm | | • 1st reconstruction- B60 sharp kernel<br>• 2nd reconstruction- mediastinum setting | • lung parenchyma setting. |
| Post processing | | 2mm coronal reconstruction on B60 lung setting | |

# ANAESTHETIST GUIDELINES (OLD PROTOCOL)

**Revised 30th April 2010**

## INTRODUCTION

- Unless contra-indicated, induction of anaesthesia will generally be gaseous using oxygen and nitrous oxide and sevoflurane.

- Atracurium (0.5mg/kg) will be administered IV as a muscle relaxant, paralysis being maintained throughout the CT and BAL procedures.

- The child will be intubated with an appropriately sized endotracheal tube to ensure minimal leak at 25 $cmH_2O$ and sufficient calibre to pass a 2.8mm bronchoscope.

- Anaesthesia will be maintained for the CT scan with sevoflurane oxygen and air ($FIO_2$ 0.3) and the patient ventilated to maintain an appropriate end tidal $CO_2$ (4.5-5kPa) with the addition of positive end expiratory pressure (5 $cmH_20$).

- Baseline ventilatory pattern via anaesthetic machine: pressure controlled IPPV,
    - Respiratory rate 20bpm
    - I:E ratio 1:2
    - VT 8-10ml/kg
    - PEEP: 5 $cmH_2O$

# PROCEDURE

- The anaesthetist will ensure **patient breath-hold on full inspiration at 25 $cmH_2O$** (while Topogram/scout is performed) until instructed to release by radiographer, i.e. **'FINISHED'**.

- Anaesthetist will perform **ten deep slow inflations** to **30 $cmH_2O$** with a **PEEP of 5 $cmH_2O$** Anaesthetist will count down from 10 to 1 and then say **GO** as he/she performs the final inflation (on the count of 1) to 25$cmH_2O$ prior to spiral inspiration acquisition.

- During the scan, the child's lungs will be held in inspiration for 6-10 s at 25 $cmH_2O$, until radiographer instructs **'FINISHED'**.

- Bag released to allow **passive expiration to relaxed end expiratory volume (NO PEEP)**

- **Once lung deflation complete; Anaesthetist instructs radiographer 'GO'** (by which time CT settings will have been adjusted for expiratory scan). Nb The subsequent 6 second delay before scan commences should ensure completely stable end expiratory level attained with no subsequent volume drift

- Radiographer will inform anaesthetist when complete and **normal ventilatory support can resume**.

**Research Project: Structural Changes in infants diagnosed with Cystic Fibrosis by Newborn Screening**

# ANAESTHETIST'S_RADIOGRAPHER'S GUIDELINES

**(Read in conjunction with CT Scan protocol) Revised 11th Nov 2010 (NEW)**

## INTRODUCTION

- Unless contra-indicated, induction of anaesthesia will generally be gaseous using oxygen and nitrous oxide and sevoflurane.

- Atracurium (0.5mg/kg) administered IV as a muscle relaxant, paralysis being maintained throughout the CT and BAL.

- The child will be intubated with an appropriately sized endotracheal tube to ensure minimal leak at 35 $cmH_2O$ and sufficient calibre to pass a 2.8mm bronchoscope.

- Anaesthesia will be maintained for the CT scan with sevoflurane oxygen and air ($FiO_2$ 0.3) and patient ventilated to maintain appropriate end tidal $CO_2$ (4.5-5kPa) with 5 $cmH_20$ PEEP, using **handheld pressure gauge/manometer. (essential equipment to take to CT– do not rely on ventilator settings)**

- During initial mask bagging, there is a tendency for air to enter stomach which may distort images. Pass NG tube and apply suction to reduce any gastric distension PRIOR to initial topogram.

- Baseline ventilatory pattern via anaesthetic machine: pressure controlled IPPV,
    - Respiratory rate 20 bpm
    - I:E ratio 1:2
    - VT 8-10ml/kg
    - PEEP: 5 $cmH_2O$

# PROCEDURE

- Radiographer will adjust scan parameters and once ready for topogram will say **'READY FOR TOPOGRAM'.**

- The anaesthetist will then ensure **patient breath-hold on full inspiration at 25 cm$H_2O$** and say **'GO FOR TOPOGRAM'** until instructed to release by radiographer who will say **'FINISHED'**.

- Radiographer will adjust scan parameters for inspiratory and expiratory acquisitions. Once ready, radiographer will say **'START INFLATIONS for INSPIRATORY SCAN'.**

- Anaesthetist will then perform
  - **6 deep slow inflations** to **35 cm$H_2O$** with a **PEEP of 6 cm$H_2O$** to reverse any anaesthetic related atelectasis (anaesthetist will count up from **1 to 6**), followed by
  - **4 deep slow inflations** to **25cm$H_2O$** with a **PEEP of 5 cm$H_2O$** to provide standard lung volume history (anaesthetist will count down from **4 to 1** and then **say GO** at the final inflation (on the count of 1) **to 25cm$H_2O$.**

- During the inspiratory scan, the child's lungs will be held in inspiration for **~6s at 25 cm$H_2O$,** until radiographer instructs **'FINISHED INSPIRATORY SCAN'**.

- Anaesthetist will then release BAG completely to allow **passive expiration to relaxed end expiratory volume (ZERO PEEP).**

- Once lung deflation complete**;** Anaesthetist instructs radiographer by saying **'GO FOR EXPIRATION'** *(In-built 6s delay before scan commences ensures stable end expiratory level)*
- Radiographer will inform anaesthetist when complete and **normal ventilatory support can resume**.

# Research Project: Structural Changes in infants diagnosed with Cystic Fibrosis by Newborn Screening

# CT Scan under General Anaesthetics Protocol

## *To be read in conjunction with Anaesthetist_Radiographer guidelines v.11th November 2010 (NEW)*

## *Amendment to volume history prior to CT, 11th November 2010*

**Rationale for amendment:**

Following the first 22 CT scans that were performed, there were concerns about anaesthetic related atelectasis in dependent lung regions in some scans. In order to minimise this, both the Americans and Australians have found it necessary to perform the initial lung inflations to 35-40cm$H_2O$ with 5-6 cm$H_2O$ PEEP, to preclude/reverse any atelectasis rather than the 30 cm$H_2O$ that we have used to date.
Hence for subsequent CT scans, we will use higher inflation pressures for the first 6 inflations (35-40cm $H_20$), followed by the remaining 4 inflations to 25cm $H_20$. This will not however influence the procedure during the actual CT scans in any way

**The DAY before CT scan**

- Senior LCFC representative at each centre to ensure that anaesthetist, ODP, and radiographer responsible for the procedure realise that this is a RESEARCH CT with special protocol that must be adhered to from the point of anaesthetics, ventilatory pattern and scanning parameters.
- To read the respective research protocols (anaesthetics and scanning protocols) prior to the day of the procedure.
- Ensure that intercom fully functional in CT suite

**Preparation before patient's arrival** ( At least 15 minutes before patient's arrival)

- Anaesthetist, radiographer, ODP and senior member of the LCFC meet to discuss execution of the research protocol and to clarify instructions/ communication about acquiring topogram, inspiratory spiral and expiratory scans.
- Ensure that handheld manometer gauge and anaesthetic circuit set up as per research protocol and working. (fresh circuit per subject)
- Ensure that intercom between CT scan and control room is working and at adequate volume. It is VITAL that anaesthetist and radiographer can hear each other clearly, as communication MUST be verbally expressed and **not through automated CT machine**.

**Topogram/Scout** (Planning)

**Anaesthetist will ensure patient breath-hold on full inspiration 25cm$H_2O$ until instructed to release by radiographer to mimic circumstances during inspiratory scan**

Include from top of apices to costo-phrenic angle.

| | |
|---|---|
| kVp | 80 |
| mAs | 20 |
| coverage | 256 mm |

nb Expiration Topogram/Scout not carried out to avoid increase in radiation dose.

**Plan both Inspiration and Expiration ranges so that they follow each other.**

**Inspiration – Spiral Acquisition**

**Anaesthetist will ensure patient breath-hold on full inspiration at 25 cm$H_2O$ for up to 10s and until instructed to release by radiographer (see accompanying Guidelines for precise wording)**

| | |
|---|---|
| kVp | 100 |
| Ref mAs | 17 |
| CTDIvol | 0.57mGy |
| Collimation | 32 x 0.6mm (64 with flying focal spot technology) |
| Tube rotation time | 0.5 seconds |
| Coverage | 140mm (should not be necessary to exceed this length) |
| Pitch (table feed) | 1 (19.2mm) |
| Dose modulation | Care dose 4D used |
| Scan slice width | 1mm |
| Recon slice thickness | 1mm |
| Recon algorithm | B30 medium-soft kernel on mediastinum setting, B60 sharp kernel on lung parenchyma setting |
| Post processing | 2mm coronal reconstruction on B60 lung setting |

- **Radiographer will notify Anaesthetist on completion of scan.**
- **Do not reconstruct scan data at this stage.**
- **Anaesthetist will cease ventilation and expiratory acquisition will occur immediately following passive deflation to stable end expiratory plateau.**
- **Anaesthetist will notify Radiographer when to start scanning for expiration at ZERO PEEP.**
- **A 6-second scan start delay is factored in to ensure full deflation.**

**Expiration - Spiral Acquisition**

| | |
|---|---|
| Scan Start delay | 6 second |
| kVp | 100 |

| | |
|---|---|
| Ref mAs | 20 |
| CTDIvol | 0.67mGy |
| Collimation | 32 x 0.6mm (64 with flying focal spot technology) |
| Tube rotation time | 0.5 seconds |
| Coverage | Around 30mm less than Inspiration range |
| Pitch (table feed) | 1 (19.2mm) |
| Dose modulation | Care dose 4D used |
| Scan slice width | 1mm |
| Recon slice thickness | 1mm |
| Recon algorithm | B60 sharp kernel on lung parenchyma setting |
| Post processing | 2mm coronal reconstruction on B60 lung setting |

**Once CT completed, resume normal ventilation via hand bagging/ventilator, before proceeding to BAL.**

**Clinical CT Reports:** Each centre will prepare a standard clinical report to be forwarded asap to the child's referring consultant.
GOSH will send reports together with the anonymised CT data on a CD to Lewisham, Kings College Hospital and Epsom St Helier's where appropriate

**CT scoring for research study:**

- For the purposes of the study, all CT images will be viewed and scored on a Leonardo Console (Siemens Erlangen) at GOSH by two experienced paediatric radiologists, masked to the patient's clinical details.
- These radiologists will assess, modify and score the CT scans according to a validated CT scoring system adapted for CF lung disease.(Brody 2006, Brody 2007). Additional scoring using approach adopted by the Australian AREST CF group may also be undertaken, still under discussion
- Post processing will include high resolution algorithm reconstruction on lung parenchymal windows for the presence and extent of bronchiectasis, bronchial wall thickening, and air trapping using the modified BRODY score (Brody 2006, Brody 2007 – see below)

**Funding:**

Funding has been made available for CT and bronchoscopy under GA by the London Collaboration of Research Networks (LCRN) or CF Trust and each department will be reimbursed individually following the satisfactory completion of tests.

## Protocol for samples collected at bronchoscopy - GOSH

### i) INFLAMMATORY MARKERS – SAMPLES TO Somers Clinical Research Facility (CRF)

***CRF lab assistant (extn 6934) must be informed beforehand so that they are expecting the samples (research team member). Also please inform them when the child is called to theatre for the bronchoscopy.***

**BAL**

- Research BAL sample should be divided into 2 universal samples and handed to Research Team member.
- The BAL samples for inflammatory marker assessment will be labelled with the child's study number, date and time of the sample, type of sample [one labelled **WHOLE BAL** and the other labelled **SPUN BAL**].
- They will be placed in a specimen bag with additional labels, stating child's study number and date of sample.
- They will be taken to the CRF by Research Team member.
- **WHOLE BAL** is stored in a freezer at –80°C at the CRF.
- **SPUN BAL** sample will be centrifuged for **10 minutes at 3500rpm at 4°C**.
- The supernatant will be aspirated into aliquots of 0.5ml and will be stored at –80°C at the CRF.
- The following week, Research Team member will collect all the samples. Check that all samples are appropriately labelled (study number; date; type of sample i.e. whole BAL, Spun BAL [supernatant]) and take it to the Portex freezer (–80°C) for long-term storage.

**Blood**

- Three mLs of blood in a large brown or white top bottle will be obtained when the child is anaesthesized for the CT/ bronchoscopy for inflammatory marker assessment.
- The sample will need to be mixed by inversion 8–10 times in theatre.
- The sample will be labelled with the child's study number, date and time of the sample, type of sample.
- It will be then be taken to the CRF by Research Team member and allowed to stand for at least 30 minutes to clot.
- Within 1 hour of sampling, the sample will be centrifuged for 10 minutes at 2,000g at 4°C.
- The serum will then by aspirated into two bottles and labelled with the child's study number and date the sample was taken.
- The sample will be stored in a freezer at –80°C at the CRF.
- The following week, Research Team member will place the sample in a specimen bag with an additional label, stating child's study number, date and type of sample, and take it to the Portex freezer (–80°C) for long-term storage.

* * * * * * * * * * * * * * *

### ii) SAMPLES TO MICROBIOLOGY

- The microbiology lab (Garth Dixon and Peter Watson) must be informed about the bronchoscopy and BAL beforehand so that they are expecting the sample. Will need to reach the lab before 3pm.

**BAL**

- This sample will also need to be assessed for respiratory viruses and a portion will need to be sent to the virology department for respiratory viral immunoflurescence (adenovirus, influenza, parainfluenza and RSV).

- The BAL sample sent to the microbiology department will be assessed for microscopy and culture. On the form it is important to clearly state: **Infant CF BAL Sample – For Colony Counts**.

- The BAL sample for microbiology will be taken to the microbiology reception in the Camelia Botnar Lab by Research Team member.

## Useful contacts at GOSH Somers Clinical Research Facility

| | |
|---|---|
| Beth Towlson (CNS) | Tel ext: 6893 bleep 0161<br>email: towlse@gosh.nhs.uk |
| Jignasha Gajera (Lab tech) | tel ext: 6934<br>email: GajerJ@gosh.nhs.uk |
| Catherine Irvine (Lab tech) | tel. ext: 6934<br>email: IrvinC@gosh.nhs.uk |
| Reception desk | tel ext: 6872 |

# LCFC Infant CF Bronchoscopy Samples

**CONTROL sample**

**5ml saline sucked through bronch prior to insertion** into patient

Send to **microbiology for MC&S** [PIMS form test code = MCS]

State on form:

"CONTROL SAMPLE, Infant CF BAL sample for colony counts"

**1st BAL sample** from patient pooled with **4th BAL sample** for patient

SPLIT into 2 aliquots

1. Smallest aliquot, send to **virology for Routine Respiratory screen** [PIMS form test code = V047]
2. Biggest aliquot, send to **microbiology** as 1 sample for
   **MC&S** [PIMS form test code = BAL]
   **TB culture** [PIMS form test code = B027]
   **Fungal culture** [PIMS form test code = FUNG]

State on form:

"Infant CF BAL sample for colony counts"

**2nd BAL sample** from patient

SPLIT into 2 aliquots

1. Smallest aliquot, send to **haematology** for
   **Fluid cell count** [PIMS form test code = H059]
2. Biggest aliquot, send to **histology for cytology** [PIMS form test code = T100]

State on form:

"Any inflammatory cells, if so which and are they raised mild/mod/severe and % count. Any fat laden macrophages, are they raised mild/mod/severe"

**3rd BAL sample** from patient

SPLIT into **2 aliquots** (in 2 universal bottles)

Give both samples to Research team member for freezing & storage.

**Research samples**

**4th BAL sample** from patient pooled with 1st BAL sample from patient – **see above**

## Brody II Scoring analysis

| | | | | | | |
|---|---|---|---|---|---|---|
| Bronchiectasis score<br>(range 0 to 12) | = ( ( | Extent of bronchiectasis in central lung<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | + | Extent of bronchiectasis in peripheral lung<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | ) x ) | Average bronchiectasis size multiplier<br>0.5 = 0<br>1 = 1<br>1.5 = 1.25<br>2.0 = 1.5<br>2.5 =1.75<br>3 = 2 |
| where | | | | | | |
| Average bronchiectasis size | = ( ( | Size of largest dilated bronchus<br>1 = <2x<br>2 = 2x-3x<br>3 = >3x | + | Average size of dilated bronchi<br>1 = <2x<br>2 = 2x-3x<br>3 = >3x | ) / ) | 2 |
| Mucous plugging score<br>(range 0 to 6) | = | Extent of mucous plugging in central lung<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | + | Extent of mucous plugging in peripheral lung<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | | |
| Peribronchial thickening score<br>(range 0 to 9) | = ( ( | Extent of peribronchial thickening in central lung<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | + | Extent of peribronchial thickening in peripheral lung<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | ) x ) | Severity of peribronchial thickening<br>1 = mild<br>1.25 = moderate<br>1.5 = severe |
| Parenchyma score<br>(range 0 to 9) | = | Extent of dense parechymal opacity<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | + | Extent of ground glass opacity<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | + | Extent of cysts or bullae<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe |
| Air Trapping score<br>(range 0 to 4.5) | | Extent of air trapping<br>0 = none<br>1 = 1/3 of lobe<br>2 = 1/3 to 2/3 of lobe<br>3 = >2/3 of lobe | X | Appearance of air trapping<br>1 = subsegmental<br>1.5 = segmental or larger | | |

**Figure E1: CFCT Scoring Sheet**

**ID no:_____Lobe: RUL/RML/RLL/LUL/Ling/LLL**

**Bronchiectasis**
**Size***
Largest — None — SP(spurious) — 2x — 3x
Average — 2x — 3x

**Appearance** — cylindrical — varicose — saccular

**Extent**
Central — 0 — 1/3 — 2/3
Peripheral — 0 — 1/3 — 2/3

**Mucous Plugging**
**Extent**
None — SP
Central — 0 — 1/3 — 2/3
Peripheral — 0 — 1/3 — 2/3

**Peribronchial thickening** — None — SP

**Severity** — mild — moderate — severe

**Extent**
Central — 0 — 1/3 — 2/3
Peripheral — 0 — 1/3 — 2/3

**Opacity**† — SP — 0 — 1/3 — 2/3

**Ground Glass** — SP — 0 — 1/3 — 2/3

**Cysts/Bullae** — SP — 0 — 1/3 — 2/3

**Hyperinflation**
**Extent** — SP — 0 — 1/3 — 2/3

**Appearance** — subsegmental — segmental or larger