Lung clearance index in children with non-Cystic Fibrosis, non-Primary Ciliary Dyskinesia bronchiectasis and in a healthy population

by

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Abstract

Introduction:

Non-CF, non-PCD bronchiectasis in children is a chronic, suppurative lung disease diagnosed by high resolution computed tomography (HRCT) imaging of the lungs. Spirometry can be abnormal in bronchiectasis but has been shown to be insensitive to early disease in other related conditions such as cystic fibrosis. Lung clearance index (LCI) could have a role in assessment of the disease.

Lung clearance index is calculated from multiple breath washout tests. There are limited data on the normative values expected using some devices, in a range of ages, and between ethnic groups.

The aim was to establish normative values in children aged between 6 years and 12 years, to investigate differences between ethnic groups, and to establish the relationship between lung clearance index and other measures of disease in children with bronchiectasis.

Methods:

Healthy children were recruited from a range of settings and reviewed to ensure no previous lung disease. Children with bronchiectasis diagnosed on HRCT were recruited from the outpatient service of Birmingham Children's Hospital.

All participants performed lung function tests including LCI and spirometry. Basic demographic data was collected.

Results:

72 healthy children and 13 children with bronchiectasis were recruited and performed lung function. All children with disease were able to complete testing, 70.8% of healthy children were able to perform lung clearance index.

The mean LCI of healthy children was 7.19 (0.6 SD), children with bronchiectasis had significantly higher LCI (9.51, 2.2 SD, $p<0.0001$). There was no difference between healthy children from White-British and Indian Subcontinent ethnicity (7.04 vs. 7.20, $p=0.33$). LCI in children with bronchiectasis correlated with HRCT scores, FEV_1, FVC and MMEF. LCI was also more sensitive to disease than these spirometry metrics.

Conclusion:

LCI is feasible in children (both inexperienced and experienced in previous lung function testing). We have demonstrated a normative value for our cohort and have shown no difference between ethnic groups. LCI was more sensitive to disease and correlated well with other markers of disease highlighting a potential role for LCI in the monitoring of non-CF, non-PCD bronchiectasis and as a potential therapeutic outcome measure.

Dedication

This work is dedicated to Sophie, Lucy and Maddie

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Abbreviations

BTS	British Thoracic Society
CF	Cystic Fibrosis
ERS	European Respiratory Society
HRCT	High resolution computed tomography
MBW	Multiple breath washout
PCD	Primary ciliary dyskinesia

Chapter 1: Introduction:

The structure of the airways and lung parenchyma influences the efficiency of ventilation. The main bronchus divides approximately 22 times, resembling an inverted tree, and as they divide, the diameters of the 'branches' gets smaller, eventually to less than 1mm. Branches less than 2mm, in adults, are known as the small airways; these lead to the air sacs, the alveoli, where gas exchange takes place. The airways are therefore key in the process of ventilation.

The respiratory drive (the neurological stimulation to breathe), and the respiratory pump (the musculoskeletal system that helps mechanically pull air into the lungs) are essential for normal ventilation (1). Following ventilation, the vital step of diffusion of the gases takes place across the blood:gas barrier. The final process needed in breathing is perfusion, the supply of blood to the relevant, ventilated parts of the lung.

The architecture of the airway tree promotes even distribution and optimal mixing of inhaled gas with the resident gas already in-situ. Humans have presumably evolved this way to achieve optimal gas exchange, and therefore, maximise the ability of the body to remove the harmful waste gas and obtain the necessary fresh gas needed for respiration.

It is the distribution of air and its mixing within the lungs that is of interest in this work; in particular, one non-invasive technique for measuring and quantifying ventilation distribution, and how it may help in the assessment of lung disease in children.

1.1 Bronchiectasis :

1.1.1 Definition and classification:

Bronchiectasis is a chronic, suppurative lung disease defined radiologically as an abnormal dilatation of the airways diagnosed by high resolution computed tomography (HRCT) of the lung (2). The symptoms are generally those of chronic productive or wet cough and the presence of these symptoms should alert clinicians to the possibility of the diagnosis of bronchiectasis. Bronchiectasis can be caused by a range of underlying conditions such as cystic fibrosis (CF), primary ciliary dyskinesia (PCD) or other conditions such as post infection, immunodeficiency, congenital malformation, foreign body inhalation or aspiration. These other causes (which could be described as non-CF, non-PCD bronchiectasis), will be henceforth described as bronchiectasis.

The condition is heterogenous and has been classified previously as localised (one lobe) or more generalised (multi-lobe. Or alternatively classified as exclusively lower airway disease or involving both lower and upper airway disease. These descriptions are, to some extent, arbitrary and do not define the underlying aetiology of development of bronchiectasis.

Multiple risk factors are known to be associated with bronchiectasis in children, but it is clear that chronic cough and repeated exacerbations (that include airway infection and inflammation which persist if left untreated), are the key features (2). It is therefore important to stop the repeated cycle of infection and inflammation as early as possible to stop disease progression and the progression of permanent, structural lung damage (3).

1.1.2 Prevalence:

It is a rare disease in children, although has become more frequently recognised in recent times (4). In the United Kingdom, bronchiectasis has been reported at rates of up to 566 per

100,000 population in adults with an incidence that has increased by 40% in the previous 10 years (5). Although considered to be reducing, a number of studies have shown increasing incidence such as in the United States of America (213 cases per 100,000 with an annual growth rate of 8%) (6) and in China, where it is estimated that in those over the age of 40, there is a prevalence of 1200 per 100,000 people (7). It is hypothesised that this apparent increase over recent years could be due to recent advances in, and the increased use of, diagnostic radiology techniques and the use of national bronchiectasis registries which allow easier identification of patients. Diagnosis is often delayed. The diagnosis varies amongst different populations and with physician awareness and the availability of HRCT scans (8–10).

The geographical variation described here has been noted and there are clear ethnic and cultural influences on the prevalence of bronchiectasis (3) such as the indigenous population in Australia having a higher incidence of bronchiectasis and studies in China demonstrating higher frequencies of Pseudomonas aeruginosa infections (which could be due to differences in exposure, genetic susceptibilities or patterns of antibiotics use) (11,12). Some of this variation can in part be explained by the access to and use of the previously mentioned diagnostic techniques but also environmental exposures (such as to a potential causative pathogen such as tuberculosis), host factors and differences in data capture and health records.

These data apply to adults, with the frequency being clearly described as increasing with increasing age (for age 18 to 34 years, 7 per 100,000, and for those age over 75 years, 812 per 100,000) demonstrating the acquisition of bronchiectasis over time as a population ages (6). Bronchiectasis in pre-school (13) and school aged children (14) has been described but its frequency is not as widely reported.

The incidence of bronchiectasis in children aged 0-14 years has been reported as 0.5 per 100,00 child-years to 3.7 per 100,000 child years (in Finland and New Zealand respectively), but illustrating the differences within various ethnic groups, it has been shown that among Aboriginal infants in Australia, incidence is up to 200 per 100,000 child years (10).

1.1.3 Pathogenesis of bronchiectasis:

There are several differences between children and adults with bronchiectasis including the underlying aetiology, microbiology and treatment outcomes (15–17). There has been guidance from the European Respiratory Society (ERS) and British Thoracic Society (BTS) for the care of adults with bronchiectasis, and more recently the ERS have produced guidance for children (18–20).

The most often cited model of the development of bronchiectasis is the 'vicious cycle hypothesis' described by Cole (21). The model suggests an initial event, such as a viral illness, that impacts on mucociliary clearance and gives respiratory tract infection. This causes inflammation with further impairment of the natural defences of the airway which results in further infection and inflammation. This is a cycle that when established, leads to lung damage (22). This proposed cycle has been added to with the development of a more comprehensive frameworks such as that proposed by Chang et al (see Figure 1.1). Here they highlight the importance of other factors that influence the development of bronchiectasis including predisposing factors from birth (such as genetics), modifiable lifestyle and environmental/modifiable factors and the presence of additional co-morbidities (asthma, gastro-oesophageal reflux disease, nutritional deficiencies). This useful model also reinforces the role prevention strategies should play in the avoidance of significant disease and lung damage (17). The model illustrates the hypothesised process by which an acute respiratory infection becomes a protracted bacterial bronchitis, then chronic suppurative lung disease,

before radiographic bronchiectasis is apparent. This lung damage is manifest as dilatation of the airways which become inflamed, tortuous and filled with secretion. There can be lung fibrosis and lung parenchymal disease.

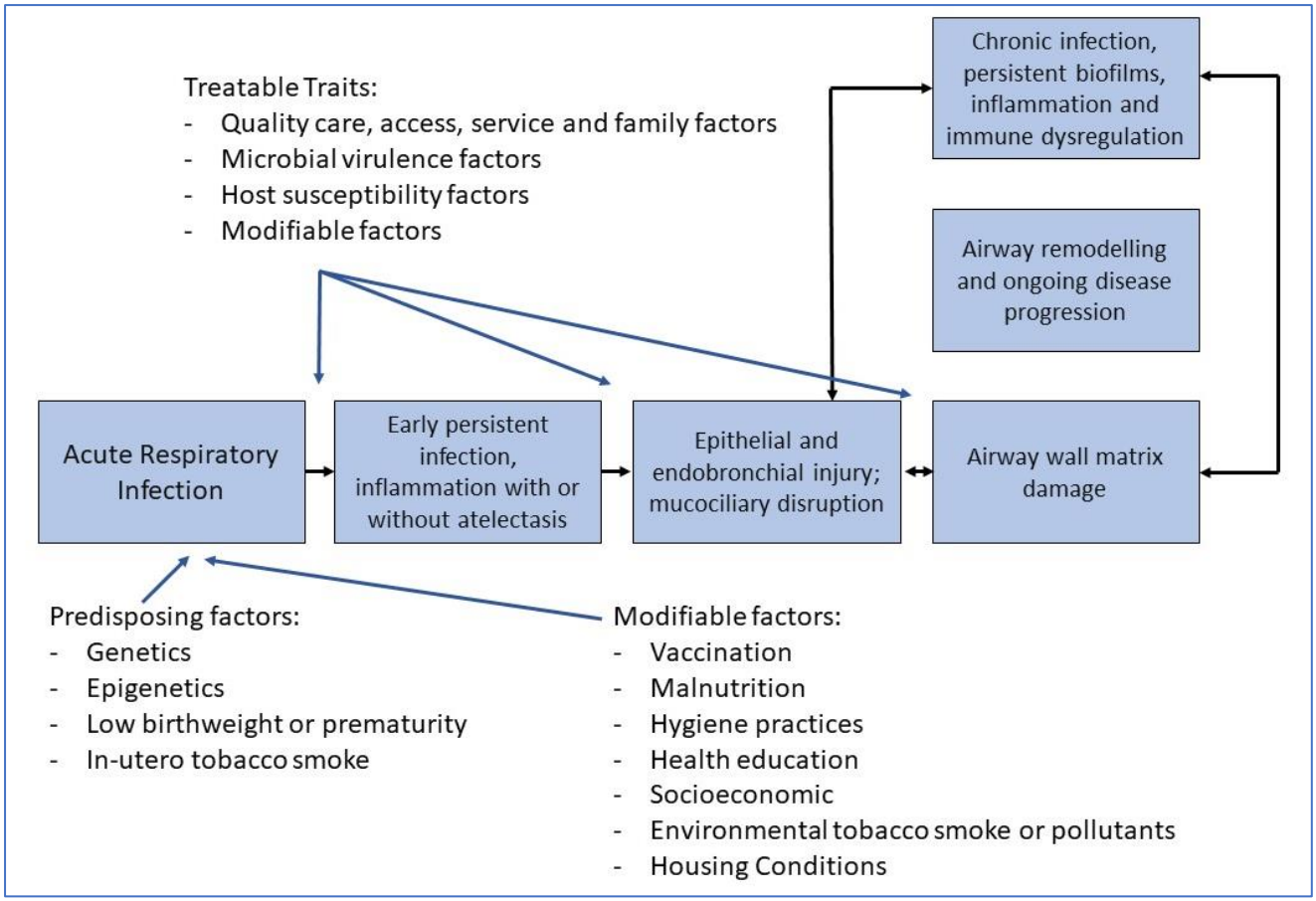

Figure 1.1: Framework for the development of paediatric bronchiectasis.

Adapted from "Bronchiectasis in children: diagnosis and treatment", Chang et al (17)

1.1.4 Investigations and diagnosis:

As previously discussed, bronchiectasis has a number of possible underlying causes or co-morbidities that may contribute and the diagnosis can often be delayed (8). There are several investigations undertaken to identify the underlying cause or co-morbidity, such as specific tests for Cystic Fibrosis (sweat test, nasal potential difference, genetics (23)), Primary Ciliary Dyskinesia (nasal nitric oxide, cilia ultrastructure and function analysis, genetics (24)), inhaled foreign body (bronchoscopy) or immune dysfunction (blood tests analysis for blood count,

immunoglobulin levels, functional antibodies levels, or the presence of human immunodeficiency virus; HIV).

One key feature in the care of children with bronchiectasis is early diagnosis and appropriate intervention. Awareness of the symptoms and signs of bronchiectasis is important, with the presence of these symptoms triggering further investigation (17). The most appropriate test for diagnosis of bronchiectasis and to assess its distribution and severity is HRCT. Other investigations can be helpful in either supporting the diagnosis or assessing disease progression / functional impairment. The most used assessment of lung function in an outpatient setting is spirometry. Chronic bacterial infection is common in patients with bronchiectasis and respiratory microbiology culture of the sputum or cough swab is an important part of routine clinical assessment. These important investigations are discussed in more detail below.

1.1.4.1 High Resolution Computed Tomography (HRCT):

Currently, bronchiectasis can be assessed accurately with high resolution computed tomography scans which gives a precise measure of disease and has high sensitivity for diagnosis when compared with chest x-ray and bronchography. HRCT is considered the gold standard test for diagnosis of bronchiectasis (25). Serial chest HRCT scans, due to risks of radiation exposure, are not recommended except where the decision to repeat a HRCT scan, based on the clinical condition of the patient, answers a question that will change management (2).

The main HRCT features of bronchiectasis are:

- an increase in the broncho-arterial ratio (the inner diameter of the airway as a ratio to the outer diameter of the accompanying blood vessel),
- bronchial wall thickening,
- a lack of bronchial tapering (from central to the peripheries),

- the presence of bronchial structures in the lung periphery,
- mucus plugging,
- mosaic perfusion (representing air-trapping) (17).

There are several controversies when considering the radiological signs for the diagnosis of bronchiectasis in children. The ratio at which the definition of broncho-arterial size becomes pathological was traditionally based on adult data (suggesting >1 to 1.5). It has become more clear that this may under diagnose children with bronchiectasis as it has been demonstrated that this ratio correlates with age (26). Guidance instead suggests a ratio of >0.8 combined with the key clinical features in making the diagnosis. Imaging can show false positive findings (such as the airways influenced by surrounding fibrosis or atelectasis), or indeed HRCT imaging can appear normal if suboptimal images are obtained or there is motion artefact. Airway size (and therefore the diagnosis of bronchiectasis) has also been shown to be dependent on lung volume and so consideration has been given to the standardisation of protocols of lung volume during HRCT acquisition (27). It is therefore important the bronchiectasis diagnosis is not purely be based on radiological findings, rather should be supported by clinical findings (28). Additional, future practice may include automated computer programmes that analyse images providing further standardisation.

Findings on HRCT could be quantified with standardised scoring systems such as Bhalla (29) or Brody score (30). These scores were developed and validated with imaging from patients with cystic fibrosis and provide a composite score of key radiological features. The Bhalla score identifies features on a segmental basis, the Brody score providing a more detail lobar scoring system. HRCT has been used as an outcome in trials of patients with bronchiectasis recently and was demonstrated to be sensitive in detecting a treatment response (31). The Brody score has been shown to be a valid outcome measure in both adults and in children (30).

HRCT imaging is an established tool in the diagnosis and assessment of bronchiectasis and is included in international guidance for children with the condition (2).

1.1.4.2 Spirometry:

Non-invasive lung function testing has been established as part of the care of children with bronchiectasis and includes spirometry due to its ease of use, repeatability and its role in monitoring (32) Spirometry can be helpful in determining disease severity and as an indication of functional impairment. The spirometry is often normal in mild disease, but obstructive or restrictive spirometry changes can be seen in severe bronchiectasis (33).

Clinical guidelines recommend that spirometry should be performed as a routine test as a means of monitoring and detecting complications however it is relatively insensitive when compared to HRCT scans (2,10). Spirometry can be attempted in children from the age of about three years but this can be challenging. If children regularly perform the technique, with good training, interpretable data can be collected from the age of 4 to 5 years (34).

Reference ranges are clearly established with age, size and ethnicity specific equations to assess the spirometry values of children and young people (35). Spirometry is a well established tool and are included in international guidance on the assessment and monitoring of children with bronchiectasis (2).

1.1.4.3 Microbiology:

Identifying organisms from specimens of the respiratory tract is important as certain organisms have prognostic information in adults (such as the presence of *Pseudomonas aeruginosa* being associated with increased mortality) (36). This can help guide treatment of exacerbations and can identify patients for whom certain long term therapy (such as antibiotic) may be indicated (5).

Specimen are obtained as sputum expectorated by the patient. This is far more challenging in children and not possible in pre-school children without specific manoeuvres to extract it. Cough swab specimen or cough plate specimen are used but these have been shown to be a poor surrogate for lower airway microbiology in patients with cystic fibrosis (37). Induced sputum (the induction typically performed with nebulised saline and physiotherapist support) is an alternative method of obtaining lower airway specimen or performing a broncho-alveolar lavage during bronchoscopy for a gold standard assessment.

The frequency for airway microbiology testing is not clear but is recommended on expert guidance to be performed every 6-12 months but would depend upon the patient's clinical condition, ability to expectorate, and the patient's ease of accessing the appointment. This guidance is included in international guidelines on the care of children with bronchiectasis (2).

1.1.5 Treatment of bronchiectasis:

The goal of treatment of bronchiectasis in children is to maintain lung function by preventing further airway damage, optimising lung growth, and avoiding respiratory exacerbations and the development of complications with an aim to reduce future morbidity and mortality. It has been demonstrated in some cases (using HRCT), that with appropriate management, that bronchiectasis can even be reversed (14). There are different elements to the management of bronchiectasis which have been adopted largely on expert consensus that are discussed below; it is noted that there are relatively few randomised controlled trials upon which practice decisions can be based.

1.1.5.1 Antibiotic Therapy:

Pulmonary exacerbations in children with bronchiectasis is usually caused by (in no particular order); *Haemophilus influenzae, Morazella catarrhalis* and *Streptococcus*

pneumoniae. Other pathogens such as *Aspergillus, Pseudomonas aeruginosa,* and non-tuberculous mycobacterial species are less commonly found (17).

The goal of antibiotic therapy is to reduce bacterial load in the airway, so interrupting the cycle of infection and inflammation (see Figure 1.1). Antibiotics might be used to treat an exacerbation, aim to eradicate a specific pathogen, or be used as a prophylactic therapy to prevent future complications.

Exacerbations are also triggered by viruses, but guidelines suggest treatment antibiotic for a minimum of 10-14 days (2). The diagnosis of an exacerbation in children can be challenging and relies upon reported clinical symptoms (such as increased cough, change in cough characteristics, chest pain, dyspnoea, haemoptysis and chest signs) (20). Antibiotic selection is based on known microbiology of the patient (if information available), or empirical choice based on local practice.

As discussed above, *Pseudomonas aeruginosa* is associated with an increase in mortality, but also an increase in exacerbation frequency. There have been several studies of patients with cystic fibrosis with *Pseudomonas aeruginosa* infection and on the strategies used to detect, eradicate, and control infection. Highlighting an issue of a lack of specific data for children with bronchiectasis, it is common practice to use the strategies found in the care of patients with Cystic Fibrosis to treat those with bronchiectasis. This usually include oral or intravenous therapy, with or without a longer course of inhaled antibiotic.

Long term prophylactic antibiotic is recommended for patients who have had a hospital admission for a respiratory exacerbation of who have had three or more non hospitalised exacerbations in the previous one year (2). Caution is advised with the prospect of inducing antibiotic resistance therefore requiring monitoring of microbiology specimen, and also of the awareness of encountering side effects of long term antibiotic therapy; such as drug induced QTc interval prolongation with Azithromycin.

In summary, antibiotic therapy remains a mainstay of treatment of patient with bronchiectasis but the clinician should be aware of the important issues highlighted above.

1.1.5.2 Airway clearance techniques:

Managing airway secretions is another standard recommendation supported in guidance, again based on expert opinion rather than high quality evidence (2). Therapy should be individualised with guidance provided by a physiotherapist with expertise in respiratory disease. These therapies will likely change over the years, suited to patients based on age, comprehension of the exercise and some therapies specific for certain underlying conditions (such as manual insufflation – exsufflation devices for those with neuromuscular disease).

Sometimes these physiotherapy techniques are combined with mucolytics such as dornase alfa or hypertonic saline. There is little evidence base to support their use, and as such, there is no recommendation for them to be used in routine practice. Instead, some patients, based on a careful review of that individual, particularly if their clinical progress is poor, may well find benefit from the use of such therapies.

1.2 Challenges in management of bronchiectasis:

Regarding the care of patients with bronchiectasis, there is a relative paucity of data to help make decisions on treatment success or failure, guide prognosis for patients, or assess the value of new treatments in randomised control trials. Large, international collaborations such as the European Multicentre Bronchiectasis Audit and Research Collaboration (EMBARC) have been established to identify research priorities that include understanding the natural history of bronchiectasis, conduct therapeutic trials and understand the mechanisms that are involved with various genetic, microbiological and inflammatory pathways.

The paucity of evidence that already exists may be due to a lack of clinical endpoints in trials that are able to detect a significant change (38). This may be due to the heterogeneous nature of the disease; several previous clinical trials have found it difficult to demonstrate a treatment response in bronchiectasis to therapy (39,40). This important question, whether certain investigations could be used as an outcome measure for interventional studies, is being examined carefully to support future interventional studies (41).

1.2.1 Potential non-invasive assessment of bronchiectasis:

While serial HRCT images would provide sensitive information on airway structure, repeated assessment using HRCT carry risks such as increased exposure to radiation. Non-invasive assessments of pulmonary function could be a surrogate but the test needs to be sensitive enough to detect subtle changes that are apparent on the highly sensitive imaging that HRCT provides. The BronchUK Clinimetrics study is a longitudinal observational study in adults exploring the clinimetric properties of a range of outcome measures to support recommendations for bronchiectasis clinical studies (ClinicalTrials.gov Identifier: NCT02468271. This work is ongoing and will provide valuable insight into the use of a range of outcome measures.

One option explored in this thesis is the measure of Lung Clearance Index, provided by performing multiple breath washout.

1.3 Multiple Breath Washout:

The branching structure of the lung is made up of 23 airway generations that deliver gas to the over 100m^2 of gas transfer area (42). The normal healthy lung is not homogenous with respect to ventilation in all of the 300 million alveoli (43). Inhomogeneity could be caused by differences in ventilation between two lung regions, (i.e. where differential filling and

emptying of each lung 'unit' can occur (44)) or architectural asymmetry that exists in the lung (45). A degree of inhomogeneity has also been described as because of the effects of gravity within the lung (46). Various techniques have been used to evaluate the sites and mechanisms of ventilation inhomogeneity to explore disease processes that may affect this feature of the ventilation. Previous commonly used lung function tests such as spirometry, are not affected by changes in ventilation homogeneity. Spirometry largely gives information about the larger airways and mostly ignore the small airways (those under 2mm as previously described).

One test to evaluate the small airways includes inert gas washout (both multiple breath and single breath). Multiple breath washout tests were introduced in the 1950s with the single breath washout being described sightly earlier in 1949 (47,48). Multiple breath washout tests have therefore existed for decades and were explored further in the 1970's and 1980's as a non-invasive method of exploring the distribution of gases within the lungs (49,50). A multiple breath washout assesses the efficiency of an inert marker gas being cleared from the lungs by computing an index derived from the washout curve (with the curve representing the expired concentration of the inert gas for each breath of the washout) (51). With a similar basic principle, a single breath washout test explores gas mixing within the time frame of a single breath (52).

To perform a multiple breath washout, the gas concentrations in the expired breath must be measured very quickly and accurately. These gas concentrations are measured during tidal breathing (at the end of expiration), and so this is a test of ventilation inhomogeneity assessed at the functional residual capacity of the lungs (44). The gases measured during the washout must be inert and most commonly include nitrogen (N_2), sulphur hexafluoride (SF_6) and helium (He). The inert tracer gas that is resident in the lungs (either naturally as is the case with N_2, or following a 'wash-in' phase with an exogenous gas such as SF_6 or He) is then 'washed out' using 100% oxygen for N_2, or room air for SF_6 or He. The principle of the washout is to assess

the concentration of the inert gas throughout, along with other information on the volume and flow of gas. Different parameters can be generated from the analysis of the data.

1.3.1 Washout parameters (multiple breath and single breath):

Excellent theoretical descriptions of some of the various parameters of analysis of the washout are in work from Verbanck and colleagues and are summarised below with other important references as needed (51,53). The parameters of washout analysis described below, illustrate important clinical features and allow meaningful, easy to understand metrics, to be extracted from the vast amount of data gather during a washout.

The multiple breath washout gives the continuous concentration of the tracer gas which can be plotted on a graph. This is the concentration of the gas; y axis, against time; x axis and will display the gas concentration progressively decreasing with each subsequent breath in a healthy person (51) (Figure 1.2). The curve can also be translated to a 'washout curve' on a semi-logarithmic scale; this is the mean expired tracer gas against the number of 'lung turnovers'. The lung turnover is literally the number of times the volume of the lung has been exchanged and is calculated from the cumulative expired volume at that point, divided by the functional residual capacity of that test subject. This allows for comparison between participants with different lung volumes (54). The start of analysis for the washout begins with the calculation of the functional residual capacity (by examining the cumulatively expired tracer gas washout) (53).

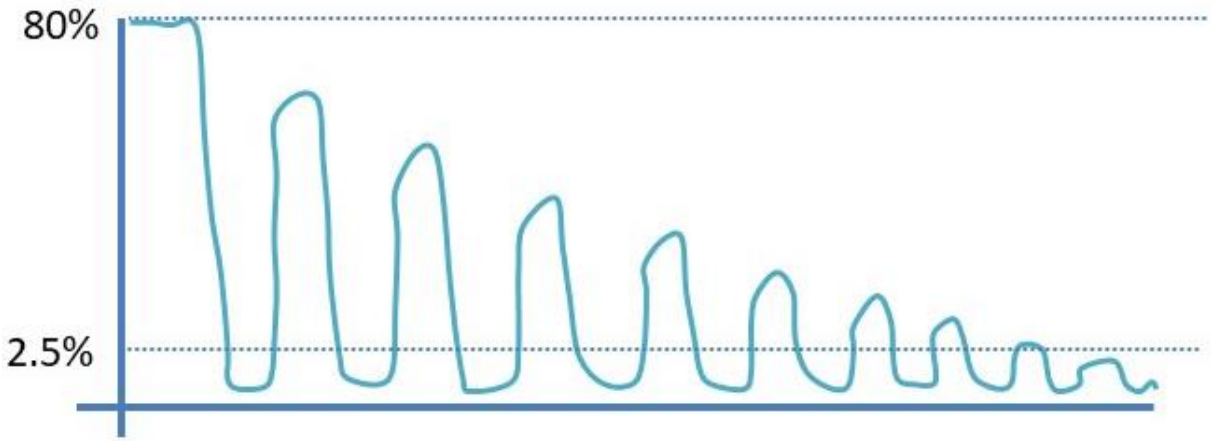

Figure 1.2 Nitrogen concentration change over time

Y axis: concentration of nitrogen, X axis: time. Adapted from "Ventilation distribution during histamine provocation", Verbanck et al, 1997 (51)

With a single breath washout of a tracer gas (or treating each breath of a multiple breath washout as such), you can view data as a plot of the tracer gas concentration against volume (Figure 1.3). This single breath washout plot can be divided into different phases, i.e. as the air from the lungs leaves the body, different portions of the plot represent the anatomical location of the gas while it was resident in the lungs and airways. The first phase (Phase I) is the beginning of exhalation and is made up primarily of exhaled gases from the test system and airway deadspace. Phase II (the bronchial phase) is where the nitrogen concentration rises rapidly and is a mixture of airway and alveolar gas. Phase III (the alveolar phase) is where the nitrogen concentration plateaus; the angle of slope depends upon the gas distribution within the lungs and homogeneity of mixing. Phase IV is a fast rising phase at the end of expiration (52).

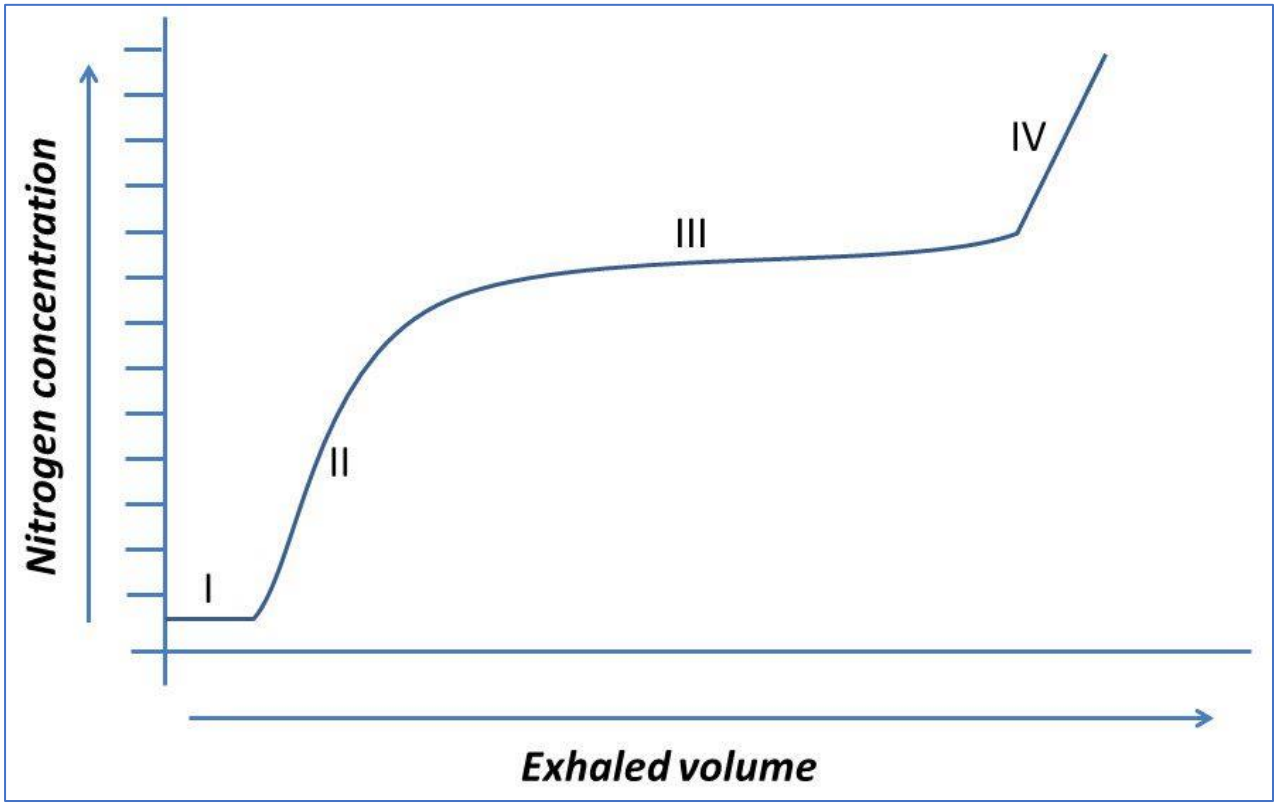

Figure 1.3 Single breath nitrogen washout test

I: First phase, II: Second phase, III: Third phase, IV: Fourth phase

The alveolar slope (Phase III slope) can be 'normalised' by dividing it by the mean nitrogen concentration of that particular breath (50). The larger the ventilation inhomogeneity between lung units, the larger the normalised alveolar slope. Different mechanisms are responsible for this ventilation inhomogeneity and by examining the alveolar slope during a washout, it illustrates the different physiological and pathophysiological features that can affect ventilation inhomogeneity.

1.3.2 What affects ventilation inhomogeneity?:

There are several theoretical and laboratory demonstrated features of the physiology of ventilation that have been described as influential on ventilation inhomogeneity within the lungs:

- Convection dependent ventilation inhomogeneity: This is due to flow difference between different 'lung units'. If one lung unit was relatively more poorly ventilated (and so had a larger N_2 concentration), this would empty relatively late in the breath and gives a more positive alveolar slope. Convection dependent ventilation inhomogeneity becomes more apparent as the washout progresses.
- Diffusion convection dependent inhomogeneity. This happens where there is interaction of diffusion and convection within an asymmetric acinus and results in inhomogeneity of gas concentrations (45). In abnormal lungs, asymmetry can be increased giving an increased N_2 alveolar slope.

1.3.3 S_{cond} and S_{acin}:

The contributions of the conductive and acinar airways to ventilation inhomogeneity (described in the above theory) can be calculated by examining the normalised alveolar slope (S) of the whole multiple breath washout (Figure 1.4). Parameters describing the contributions are termed S_{cond} and S_{acin} respectively.

S_{cond} is the normalised slope difference per lung turn-over unit (this is determined by calculating the linear regression in the part of the washout where only conductive airways are known to contribute to the rate of rise of S, e.g. between lung turn-over 1.5 to 6). S_{acin} is found by subtracting the contribution attributed to the conductive airways from the slope of the first

breath. (In multiple breath washout, the alveolar slope of the first breath is predominately generated by the diffusion convection dependent ventilation inhomogeneity in the acinar lung unit area.). These complex metrics can be visualised graphically on a plot of the normalised alveolar slope against the number of lung turnovers (see Figure 1.4).

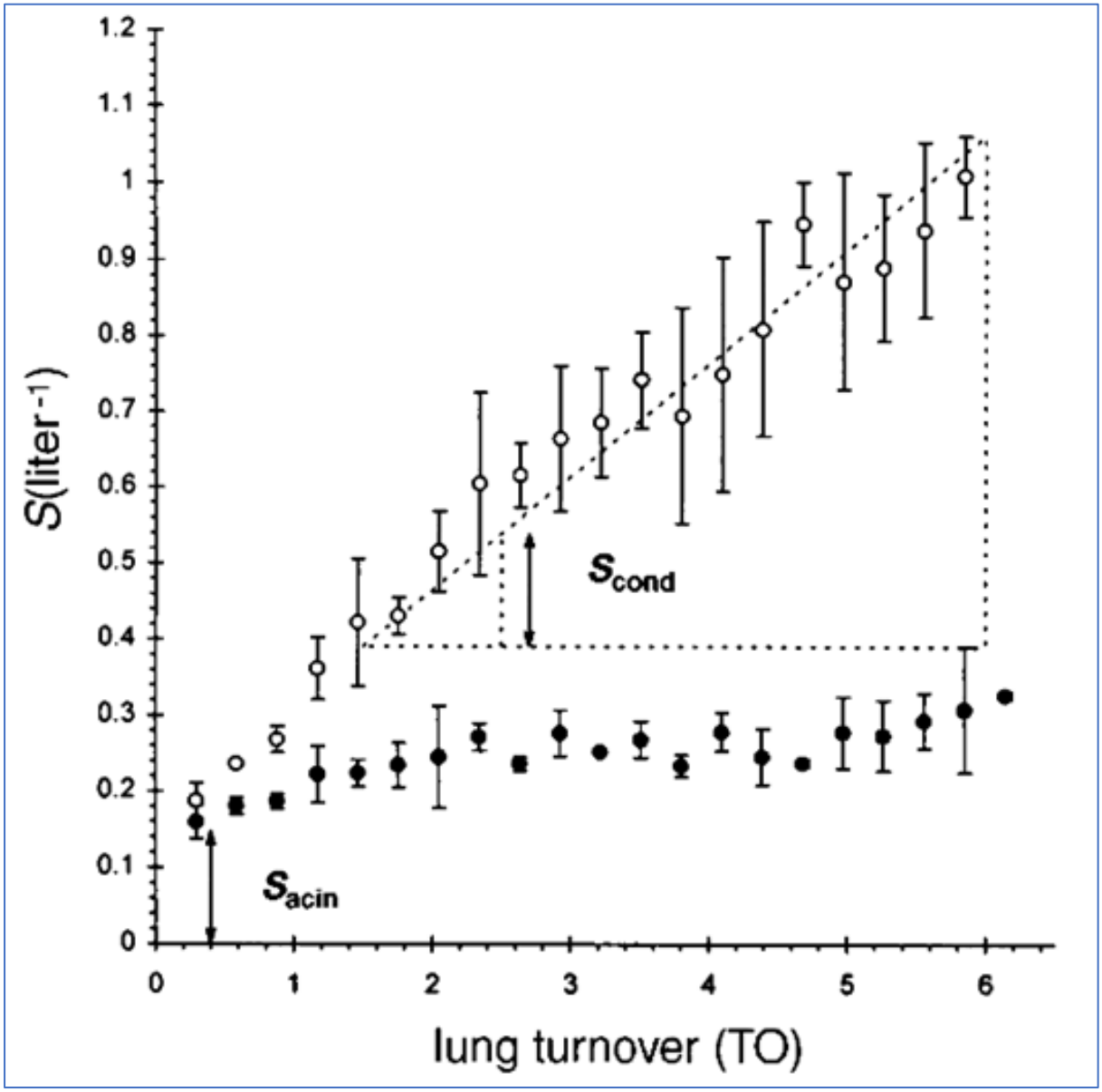

Figure 1.4 Visualisation of S_{cond} and S_{acin} calculation

Reproduced with permission from "Ventilation distribution during histamine provocation", Verbanck et al, 1997 (51)

It is worth noting, that phase III slope analysis is affected by age, body size and tidal volumes of the breath. The modelling and experimental studies are mostly from adults performing controlled breathing and it has been clear that these metrics are of less use in young people and children due to the challenges of data acquisition (51,55,56).

1.3.4 Indices of ventilation inhomogeneity:

Other indexes of ventilation inhomogeneity exist that are derived from the data measured during multiple breath washout; Mixing ratio and Moment ratio have been described recently. Other measures have also been explored historically (multiple-breath alveolar mixing inefficiency, pulmonary clearance delay, and the Becklake Index).

Mixing ratio has been well described and is calculated as the ratio between actual and ideal number of breaths needed to lower end tidal tracer gas concentration to one 40^{th} of the starting value (55,57). An increase in the mixing ratio represents worsening inhomogeneity. Moment ratio has also been studied. This incorporates the entire washout to assess the shape of skewness of the washout curve which reflects ventilation homogeneity of the lungs (58,59). The potential attraction of Moment ratio analysis is that it is reported to be less affected by irregular breathing patterns, making it perhaps more suitable in children. None have gained the popularity of the most reported index from multiple breath washout, lung clearance index (LCI) and none have robust, age specific reference ranges for normality. The challenge of appropriate reference ranges for LCI will be explored in section 1.5.

1.4 Lung Clearance Index:

Lung clearance index (LCI) has been the most often reported index of ventilation inhomogeneity and is the simplest index to calculate from a multiple breath washout. It is

calculated by dividing the cumulative exhaled volume of gas needed to washout the tracer gas to one 40^{th} of its starting concentration, by the functional residual capacity (57).

As described previously, ventilation maldistribution can be from asymmetric narrowing of the airway lumen at branch points throughout the airway tree (this narrowing or obstruction could come from inflammation, scarring, mucus, or changes in airway tone). The increase in ventilation maldistribution raises the lung clearance index and indicates worsening disease) Several studies during the 1980's examined ventilation inhomogeneity in cystic fibrosis and although not introduced in to clinical practice at this stage, lung clearance index was demonstrated to provide valuable information in a research setting (60–62).

Over time, work has continued with a resurgence of interest in lung clearance index over the last 20 years. The attraction is the potential ability of lung clearance index;

- to identify lung disease at an earlier stage than other non-invasive lung function tests (such as spirometry),
- to assess the lungs in a harmless, non-invasive manner that could be repeated on multiple occasions as (opposed to CT scans)

This early work on lung clearance index was limited by the significant resource requirements. The equipment needed to perform reliable multiple breath washout tests was essentially restricted to specialist research laboratories with mass spectrometry equipment. Commercial devices have subsequently been developed with growing evidence for their assessment of lung clearance index; these issues will be explored through this thesis.

The following sections will discuss the clinical use of Lung Clearance Index and its influence on practice today.

Lung Clearance Index in Cystic Fibrosis:

1.4.1.1 Background:

Cystic fibrosis (CF) is the most common inherited genetic disease in the Caucasian population, affecting more than 10,000 people in the UK (63). Most morbidity and mortality arises from lung disease that is progressive and irreversible. Prognosis has improved considerably over recent decades with improvements in treatment and care with a recent study on UK survival predictions noting that at least half of babies born today can expect to survive into at least their fifth decade of life (64).

The disease arises after a mutation within the cystic fibrosis transmembrane conductance regulator gene that affects the subsequently produced protein's function. The protein is a chloride channel and so changes in its function affects chloride secretion, sodium reabsorption and water movement across the membrane. What follows is mucus that is more thick and viscous (65).

Dehydrated secretions within the chest leads to airway infection, an exaggerated inflammatory response, and the progressive development of structural airway disease (bronchiectasis) and eventually, respiratory failure. Respiratory disease is the leading cause of morbidity and mortality arising due to cystic fibrosis.

1.4.1.2 Treatment:

The priority for care of patients with cystic fibrosis has been prompt treatment of respiratory tract infections, identification of respiratory pathogens and secretion clearance therapies to maintain good lung health. Alongside this, excellent nutrition and general care of other systems

(such as the liver and assessment for diabetes) is important. Treatment and understanding of the care of CF has improved over the years, driving up the quality of care.

The most promising treatment development of recent years has been that of personalised, small molecule medicines. These molecules target the cystic fibrosis transmembrane conductance regulator (CFTR) protein and are specific for certain protein errors resulting from certain genetic mutations and are known as CFTR modulators. These add on treatments have revolutionised care, with current CFTR modulator options for about 90% of patients, demonstrating improved lung function, reduction in pulmonary exacerbation, and what will hopefully translate to increased survival (63).

1.4.1.3 Monitoring:

Periodic but regular assessment of lung function is an important part of the respiratory care of patients with CF as it provides an objective record of some aspects (depending on the lung function test used) of the workings of the lung Spirometry has been a well-established investigation for patients with CF. Its ease, reliability, and cost make it an attractive prospect to use. As the health of patients with CF improves, their lung function assessed by spirometry also improves.

Additional monitoring of the chest can be performed with intermittent radiological assessment, either chest xray, or more detailed HRCT imaging. Chest xray is relatively insensitive to the important pathological changes within the lung (bronchiectasis), and despite HRCT imaging being able to detect early signs of lung disease, it does carry with it a risk of radiation exposure (66).

1.4.1.4 Lung Clearance Index in cystic fibrosis:

As previously mentioned, lung disease in cystic fibrosis occurs following repeated infective and inflammatory processes that cause small airways disease. These changes can be identified on CT imaging, even at a very young age (such as from 8 months (67,68). When attempting to identify these changes using lung function tests, it was found that lung clearance index was far more sensitive than spirometry at detecting disease (55,69). As described previously, lung clearance index is primarily influenced by the small airways where ***early*** disease occurs. The flow and volume of air measured by spirometry is primarily influenced by the larger airways, an anatomical location that would usually be affected at a stage of ***later*** disease progression (65). When comparing the use of LCI and FEV_1 in the assessment of CF lung disease, it is important to be aware that FEV_1 is a test performed to forced vital capacity whereas LCI is performed in normal, 'quiet' breathing. It may be that while performing the tidal breaths needed in multiple breath washout, some diseased airways remain closed (and therefore not forming part of the assessment of LCI). It has been described that LCI and FEV_1 provide different information about a participants airways (55).

Lung clearance index reflects disease in the small, distal airways better than spirometry measures such as FEV_1 (70). This does mean that LCI lends itself to the monitoring of early lung disease in CF. LCI was found to be abnormal in early life in CF, with changes even demonstrated shortly after birth (71–73).

Structural HRCT changes are also more sensitively detected by LCI (71,72). It was demonstrated in a cohort of children with CF, that LCI detected abnormality in 85% (greater than other measures such as spirometry and plethysmography) and that a normal LCI essentially precluded abnormalities in these other measures. LCI was also far more sensitive than any spirometric or plethysmographic measure to the presence of abnormality on HRCT imaging. An important finding in this study was noting that should LCI be abnormal, the

HRCT image would also be abnormal, opening up the prospect of LCI being used as a non-invasive indicator of the need to perform an invasive tests.

LCI is also elevated in the presence of infection. Upon testing a group of children and adults with CF, it was noted that those with a current positive growth of pathogen had higher LCI than those with no significant growth. There was a further increase in LCI between those patients with a positive growth of *Pseudomonas aeruginosa* when compared to those with non-*Pseudomonas aeruginosa* groups (74).

LCI has been used extensively as an outcome marker within clinical trials. The European Cystic Fibrosis Society Clinical Trials Network Standardisation Committee has reviewed LCI as part of a review of clinical endpoints in multicentre clinical trials for patients with CF (75). The review describes the reliability and validity of the measure, which is good, along with the reasonable feasibility in patients with CF. Importantly, the review highlights the potential use of LCI in patients with mild CF lung disease with FEV_1 in the normal range due to the increased sensitivity previously highlighted. This is an attractive test in the current population of young people with CF who have better lung health, and therefore normal FEV_1.

Numerous, high impact studies, particularly examining the effects of small molecule drugs on cystic fibrosis, have been published making use of LCI as an outcome measure. In one example, a phase 3, randomised, double blind, placebo-controlled study examining the effects of lumacaftor/ivacaftor, demonstrated a mean improvement of just over 1 unit (see **Error! Reference source not found.**) (76). This improvement is similar in magnitude to other studies describing the effects of treatments such as hypertonic saline (77) and dornase alfa (78).

The decision as to what magnitude of change in LCI constitutes a clinically meaningful change in CF (either improvement or worsening) has been challenging and there is still not universal agreement on this and is explored in a further section (70). Appropriate references ranges of

normality suitable for the particular device, washout gas and the patient are required to assist with this challenge.

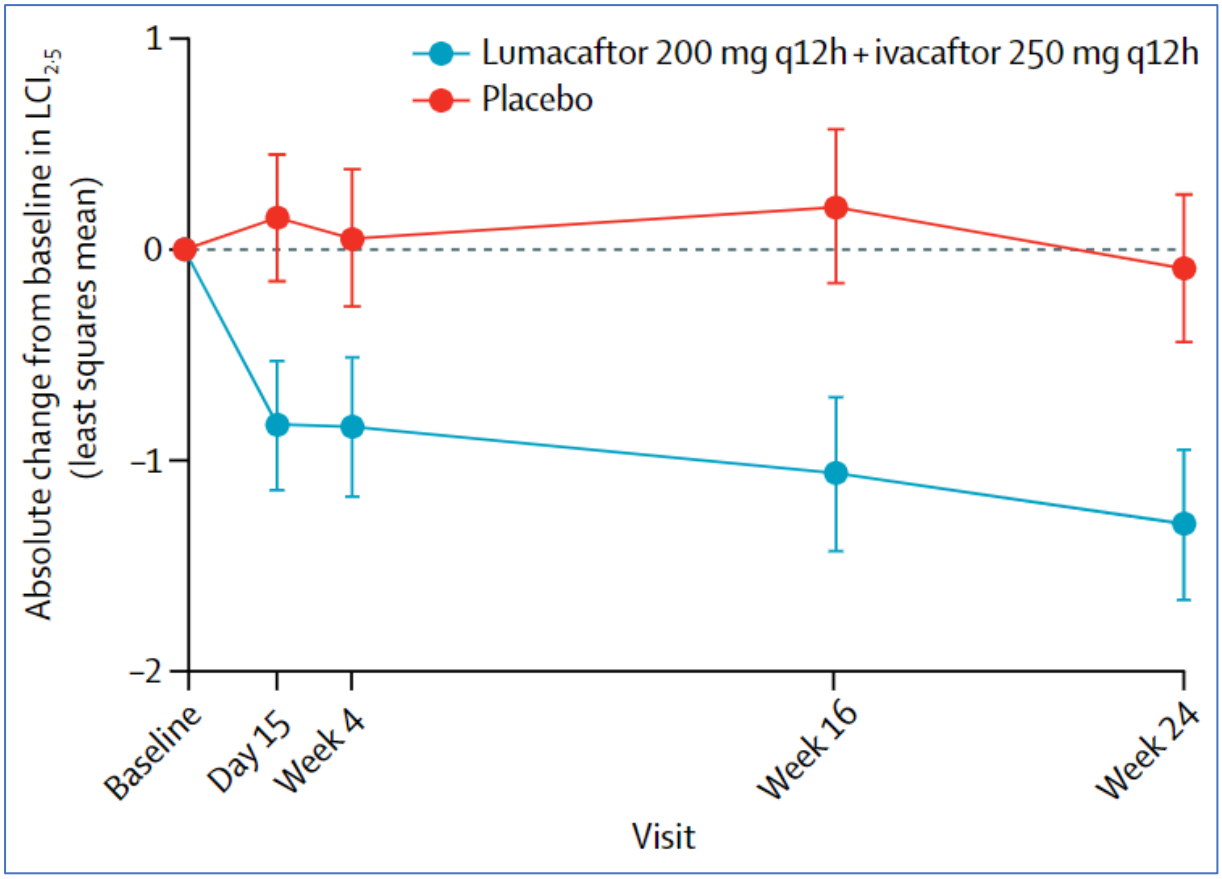

Figure 1.5 Change in LCI with treatment with lumacaftor/ivacaftor

Reproduced with permission from Elsevier, (Ratjen et al, Efficacy and safety of lumacaftor and ivacaftor in patients aged 6-11 years with cystic fibrosis homozygous for F508del-CFTR: a randomised, placebo-controlled phase 3 trial, Lancet Respi 2017 (76)***)***

Absolute change in LCI from baseline. Error bars are 95% CI. Values shown were adjusted for mixed effects model for repeat measures covariates. Q12h: every 12 hours.

1.4.2 Lung Clearance Index in Primary Ciliary Dyskinesia:

1.4.2.1 Background:

Primary ciliary dyskinesia (PCD) is a genetic disease mostly inherited as an autosomal recessive condition. The genetic changes affect the function of the cilia who's main role is to clear airway mucus, bacteria and debris by coordinated beating (79). PCD is caused by abnormalities in this process and are responsible for the characteristic symptoms of recurrent and chronic lower and upper respiratory tract infections. Further to this, there are important extra pulmonary symptoms due to the involvement of cilia found in the middle ear, sinuses, reproductive tract and brain. The ciliary dysfunction mainly cause them to be immobile, but stiff, uncoordinated and ineffective ciliary beats have also been reported (80).

The diagnosis of PCD is often delayed with one study demonstrating that 70% of patients have been seen more than 50 times by a doctor before the diagnosis is made. Contributing to this is the important point of the clinical phenotype of PCD being very wide (24). In preschool and school age children, daily wet cough due to repeated episodes of bronchitis is a universal finding that may result in the development of chronic suppurative lung disease and is an important cause of bronchiectasis (81).

1.4.2.2 Treatment:

The prognosis of PCD is less clear than other conditions due to the severity of lung disease being highly variable. It has been demonstrated that some patients have a steady progression of their lung disease with some people requiring lung transplantation (82).

There are several therapeutic strategies for the treatment of PCD, but these are typically not based on robust, disease specific evidence. It is common for treatment options from other

chronic respiratory diseases such as cystic fibrosis or bronchiectasis to be extrapolated for use in PCD.

Similar to CF, airway clearance is vital and a variety of techniques are employed including physiotherapy and exercise, with or without airway adjuncts (83). There are however important differences between CF and PCD. The impairment of muco-ciliary clearance in PCD is primary and so present from birth, whereas in CF it is secondary and may be initially preserved although the data on the course of early PCD lung disease remains limited (83,84).

1.4.2.3 Monitoring:

High resolution computed tomography (HRCT) is a sensitive imaging modality for investigating PCD lung disease and to detect bronchiectasis but requires a more significant radiation exposure than a conventional chest xray (24). Chest magnetic resonance imaging has been explored as an alternative with good agreement with HRCT findings (85). As for cystic fibrosis, spirometry is routinely performed for the ease of use and repeatability. An obstructive pattern is typically seen.

1.4.2.4 Lung clearance index in primary ciliary dyskinesia:

The potential for lung clearance index to be a valuable tool in the assessment of lung disease caused by PCD is based on the premise of the need for a non-invasive test, that could be performed readily in clinic, and that it be a sensitive test that could monitor a treatment response or be used as a trial outcome. LCI in PCD has been studied less frequently than in CF but has been shown to demonstrate abnormality, even in young children with the disease (86,87). LCI is also more abnormal in those with certain ciliary structural pathologies (microtubular defects demonstrated to show greater LCI than dynein arm defects) (88). LCI has also been compared

to other lung function tests such as spirometry. Interestingly, there is discordance between studies. Green et al (86) and Irving et al (89) found that LCI and FEV_1 showed no correlation in a group of patients with PCD. Other studies have shown a statistically significant negative correlation similar to that seen in CF (90).

When comparing LCI to imaging Boon et al (87) demonstrated LCI correlated with HRCT scores and with some sub-scores of airway thickening, mucus plugging and bronchiectasis. LCI and HRCT agreed in the demonstration of disease in 83% of cases, FEV_1 and CT agreed in only 53% of cases implying the greater sensitivity of LCI compared to FEV_1. This has been repeated in other studies examining LCI and lung disease seen on lung MRI imaging where in a group of children and adults with PCD related lung disease on MRI, 83% had abnormal LCI, and only 27% abnormal FEV1 (91).

LCI has been examined longitudinally with patients assessed over a one year period (90). Reporting on the variability and evolution of LCI over this time period, it was found that LCI significantly increased (albeit by a small amount), with LCI shown to be abnormal in a group of patients who had normal FEV_1, therefore providing important, additional information.

When compared to other disease groups, LCI in PCD has been shown to be more abnormal than cystic fibrosis (84). The feasibility of completing LCI in PCD is similar to CF and also can demonstrate disease in young children (84,92).

Lung clearance index does have potential as a non-invasive measure of disease in PCD, but caution must be employed in extrapolating findings from other lung diseases into the clinical interpretation of LCI in PCD. CF is a patchy disease of secondary ciliary dyskinesia as opposed to PCD which is a diffuse disease and of primary ciliary pathology. The relationship between lung structure and function in PCD also requires further exploration prior to the test being universally adopted (93,94).

1.5 Clinically relevant factors in the assessment of lung clearance index:

1.5.1 Medical gas and gas analyser choices:

As previously discussed, early studies of lung clearance index were performed with the wash in of an inert gas, usually sulfur hexafluoride (SF_6), with the analyser being a mass spectrometer. This restricted access to LCI testing significantly with the mass spectrometer being a large, expensive, laboratory based piece of equipment. The gas, SF_6, is a green house gas and its use is being restricted. Commercial companies have therefore provided alternative options in both device and inert gases which have improved access but also raised challenges that will be discussed below.

1.5.1.1 Tracer gas choice:

LCI results obtained using different tracer gases are not interchangeable. This has been demonstrated in different settings and on different devices. The tracer gas gives information about the location of gas mixing within the lungs and this is determined by the gas density. The heavier the gas, the more distal the mixing point. This will change the anatomical location at which gas mixing in the lungs is mostly from diffusion to that of convection and diffusion. SF_6 is a more heavy gas than other inert gases, such as helium and so theoretically would give a higher LCI value in the same subject.

SF_6 is a potent greenhouse gas and has been described as the *'world's worst greenhouse gas'* with it being many times more potent than CO_2 (95,96). There is also the hypothesis that with a heavy gas, (with a more distal diffusion front), that the LCI would be higher as previously mentioned, plus the washout longer (increasing the test time), something important when considering feasibility (97,98). There are some potential benefits to consider. When washing

out SF_6, you only require room air (which does not contain SF_6). This is readily available, and would not need to necessarily be supplied via medical gases (although this is the case in some device set ups). An alternative washout gas would be 100% oxygen. While this would achieve the same washout properties of inert gas removal, there are some concerns of the effect of 100% oxygen on the breathing pattern of the subject, particularly young infants. 100% oxygen may affect ventilation and pulmonary blood flow in the lungs (99). The use of SF_6 with room air washout would remove the impact of the potential change in tidal breathing pattern due to 100% oxygen from the analysis (98).

Nitrogen (N_2) is the most used alternative gas to SF_6. It is already resident in the lungs of test participants and so an obvious benefit is the availability. The only medical gas required is 100% oxygen to achieve the washout, something almost universally available in most modern healthcare settings and is affordable. Nitrogen though, is present in the tissue of the body, not just the lungs. Nitrogen from the body can back-diffuse in to the lungs, contributing to the gas being washed out during the multiple breath washout test. This is difficult to measure and to account for and could potentially affect results. This would particularly be a problem during longer washouts (100,101).

LCI will assess the ventilation of the lungs, but only the parts in which the washout gas is present. Particularly diseased lungs may have areas which are difficult for an exogenous gas to permeate (particularly during the time limited wash in phase required e.g. for SF_6). Proceeding with the washout with the more diseased areas of the lungs not having adequate wash in would produce an under read of the LCI for that subject. This different distribution of the washout gas is another factor potentially influencing the different results between exogenous and endogenous inert washout gases (101,102).

	EasyOne Pro LAB	**Exhalyzer D**	**Innocor**
Manufacturer	ndd	Eco medics AG	Innovision
Gas measure	Ultrasonic transit in time	Ultrasonic transit in time	Photoacoustic spectroscopy
Device structure	Open circuit washout design using bias flow Self-contained, portable. Can do Phase III in research software	Open circuit wash in and washout using bias flow Semiportable, need a linked computer Can do phase III slope analysis	Closed re-breath circuit was in with CO2 scrubber Open circuit washout design Self-contained, portable Cannot do Phase III
Gas options	N_2 with 100% O2 washout	N_2 and SF_6 Medical air and 100% O2 for washout	SF_6
Application	Over 4 years and >18kg	Those over 3kg	Anyone able to comply

Table 1.1: Commercial medical devices measuring lung clearance index

The impact of the tracer gas has been examined in several studies demonstrating a significant difference. In participants with CF and those without lung disease, LCI was performed with simultaneous washout of SF_6 and N_2 (measured by Innocor and Exhlyazer devices respectively). Clearance of SF_6 occurred with fewer breaths and produced a lower LCI in both participants with CF and in healthy controls when compared to N_2 (97). Interestingly, in this study, when the washout was continued beyond the usual end of the test, the N_2 signal remained persistently elevated (compared to SF_6 which washed out to reach zero). This may have represented nitrogen contribution from the tissues.

In a further study in infants and preschool children, SF_6 and N_2 multiple breath washout was performed on the Exhalyzer D commercial device, demonstrating again, greater results using nitrogen compared to SF_6 (absolute difference in healthy controls 1.1 (0.9 to 1.3) LCI units and 2.1 (1.4 to 2.8) for participants with CF). Importantly, the absolute difference between LCI when measured using different tracer gases is greater with higher LCI. Again, the rationale for the higher LCI using nitrogen was thought to be due to tissue release of nitrogen which has been reported to be related to cardiac output (103). This is relevant due to the unpredictability of this factor which would be difficult to adjust for.

When choosing a washout gas, these factors must be considered carefully and further work is required to optimise the results of LCI obtained with nitrogen.

1.5.1.2 Medical device choice:

Several commercial options have been developed over the previous 10 years to give a range of choices to clinicians looking to being using LCI in their workplace. There are important differences between these devices, and like the tracer gas choice, the results are not interchangeable between devices. Different acquisition techniques are required, different

training is required, regular calibration is needed and time must be invested by the operator in understanding their chosen device. A summary of three devices is shown in Table 1.1.

1.5.1.2.1 ndd EasyOne Pro LAB:

This self-contained device (see Figure 1.6 and Figure 1.7) is produced by ndd Medical Technologies, Zurich, Switzerland. It uses nitrogen as the tracer gas and requires a 100% medical oxygen supply to act as the washout gas. It is an open circuit design; the 100% medical oxyegn is delivered during inspiration to washout resident nitrogen and expired gas is exhaled to the room. A valve system is used to deliver the gas as required.

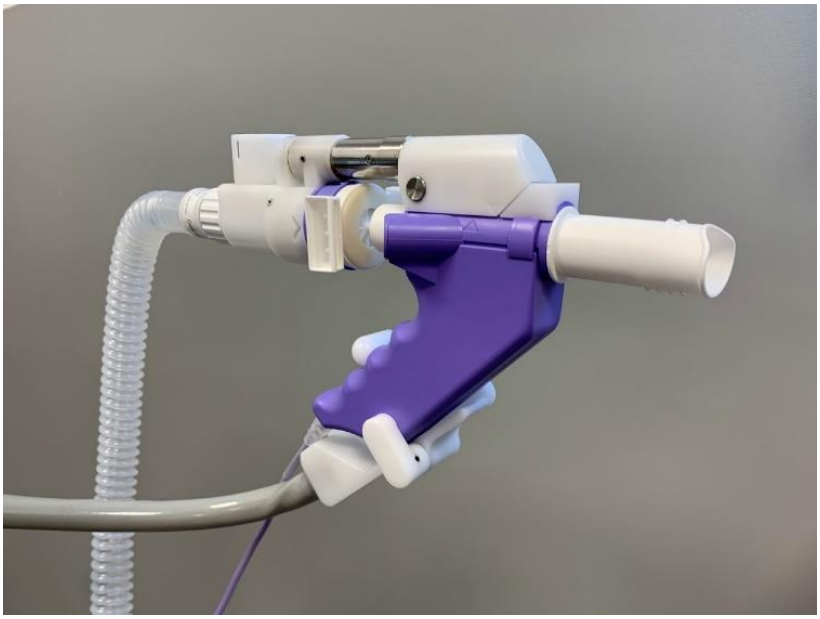

Figure 1.6: ndd EasyOne Pro LAB subject interface including mouthpiece (photograph)

Figure 1.7: ndd EasyOne Pro LAB (photograph)

The gas sensors include a main stream and side stream flow and molar mass sensor to calculate the concentration of gases exhaled. Included in this calculation is the concentration of CO_2 determined from a side stream CO_2 sensor that used infrared absorption technology to generate the result that is used to indirectly calculate the nitrogen concentration. The company report that this sensor provides accurate measurements without the need for regular calibration. The device assumes a fixed respiratory quotient (that being the ratio of volume of CO_2 produced by the lungs to the volume of oxygen consumed during respiration). The device adjusts the measured CO_2 signal for any potential change in sensor output using a correction factor based on the respiratory quotient. There is concern that this method overestimates the nitrogen concentration (and therefore multiple breath washout outcomes such as LCI) and highlights the important influence of software algorithms in the generation of LCI. The oxygen sensor measuring molar mass is directly calibrated with a pulse of 100% oxygen before and after each measure and calibrated using the known molar mass of oxygen and air.

As described, the EasyOne Pro LAB calculates the concentration of nitrogen indirectly, calculating using Dalton's law of partial pressure. The calculation assumes that only four gases vary in concentration (nitrogen, argon, CO_2 and O_2) and that nitrogen and argon are always present in a fixed ratio. Two equations are performed:

1) The sum of all gas concentrations equals 100% (Dalton's Law)

$$f_{N2} + f_{O2} + f_{CO2} + f_{H2O} + f_{Ar} = 1$$

2) The molar mass of the gas equals the gas concentrations of all involved gases multiplied by the molar mass of the gas:

$$f_{N2}.MM_{N2} + f_{O2}.MM_{O2} + f_{CO2}.MM_{CO2} + f_{H2O}.MM_{H2O} + f_{Ar}.MM_{Ar} = MM$$

(f_X references the fraction of the gas. MM_{XX} references the molar mass value of the involved gas (104).

The EasyOne Pro LAB has been validated both in vitro and in research participants. A novel lung model was used to show that the functional residual capacity measured by the device was very close to a precisely delivered volume of air from a ventilator using SF_6, it has also been validated using nitrogen as the tracer gas with similar results (105,106). Reproducibility was assessed in both short (one hour) and long term (six to fifteen months) tests showing low variability and also generating normal values for the device using SF_6 as the tracer gas (107)

The device is feasible to use and has been assessed in multi-centre settings. The success rate for achieving LCI in a group of children and young adults was 75.5% (slightly better in participants with cystic fibrosis compared to healthy control participants) (108).

1.5.2 Other devices measuring LCI:

The other devices described in Table 1.1 are the Exhalyzer D from Eco Medics AG, Duernten, Switzerland, and the Innocor LCI from Cosmed, Glamsbjerg, Denmark.

The Exhalyzer D uses a similar set up to the EasyOne Pro LAB with mainstream and sidestream sensors to measure oxygen and CO_2 and an ultrasonic transit in time measure for flow. The nitrogen is calculated indirectly with the known concentration of O_2 and CO_2 and an estimated argon fraction. It can be used to measure LCI by nitrogen washout or SF_6 washout. It requires daily calibration of CO_2 and O_2 sensors and is used in research and clinical settings (109,110).

The Innocor uses photoacoustic spectroscopy for multi-gas analysis and a differential pressure pneumotachnometer for a flowmeter. It is only available to use with SF_6 as the washout gas but uses a closed circuit rebreathe technique for the wash-in to reduce time required. It too has been used in research and in the clinical setting (111,112).

1.5.3 Differences in devices measuring LCI

As previously mentioned, the results generated from different devices cannot be considered interchangeable and has been demonstrated in multiple studies. In two groups of participants, one with CF and one of healthy participants, both the Exhalyzer and EasyOne Pro LAB were used to measure LCI using nitrogen washout. LCI was higher when measured on the Exhalyzer compared to the EasyOne Pro LAB (8.98 vs 7.68) as well as differences noted with higher FRC (113). The differences were associated with the magnitude of the LCI (with a greater LCI demonstrating a greater difference between devices). Multifactorial reasons were described for this difference with the nitrogen measurement algorithm described as one, and possibly the impact of the different breathing patterns between devices being the other.

These findings have been repeated with in vitro and in vivo FRC measurements compared between the Exhalyzer and EasyOne Pro LAB (114). When compared to plethysmography, the Exhalyzer was found to report FRC close (within 5%) to the true measured volume, with the EasyOne Pro LAB underestimating the FRC by up to 20%. They again demonstrated the importance of the analysis software with re-analysis of the EasyOne Pro LAB data using a newer software version reducing the FRC difference to a mean of 11%. In vivo findings were similar and have been repeated elsewhere (115). Other studies have looked further at the inherent differences of the EasyOne Pro LAB and other devices with several causes being reported and summarised below:

- The algorithms used to compute outcomes (100)
- Dead space vol (115) (100)
- Impact of on demand O2 on breathing pattern (115)
- Potential sensor errors (115)

These studies have shown that the available equipment does not produce interchangeable results and highlights the importance of consistency between measurements using the same device set up for longitudinal follow up and the importance of taking in to account software updates. It is likely that with the current systems available, that reference ranges will be specific for each device and for each gas (116).

1.5.4 Influence of height and age

In the development of normative data, the question of variation by subject demographics is considered. In the reporting of spirometry data, subject sex, age, ethnic group, and height are used to report the test results as a percentage predicted of expected values (35).

Lung clearance index is higher during infancy and decreases over the first five years of life (117). The reasons for this have been postulated, with the rapid period of alveolarization that occurs with growth during this time thought to contribute. This has been measured with a drop in the 'upper limit of normal' of approximately 0.8 LCI units over the first 5 years which has been described as therefore necessary to have different ranges of normal for these age groups. Over the age of 6 years, LCI is more consistent until adulthood (with a change in the upper limit of normal of less than 0.3 LCI units) (118).

The evidence of more heterogenous gas mixing in younger children has been explored, with work demonstrating that the phase III slope in multiple breath washout in sedated infants decreases with increasing age, showing that ventilation becomes more homogenous with lung growth and maturation (119).

Using the EasyOne Pro LAB, in participants age 6 years to 20 years, LCI was shown to be stable and independent of age, weight or height (107,120). In a further large study using the Exhalyzer, LCI was negatively associated with height, (coefficient -0.005, -0.0087 to -0.0019, p=0.002), weight (coefficient -0.0049, -0.009 to -0.0008, p=-.02) and age (coefficient -0.0225, -0.0421 to -0.003, p=0.024). The was no relationship found with sex (101). This expected change would equate to 0.04 units per year, and would therefore demonstrate a 0.5 unit change over the 6 to 18 year age period (describe as being negligible, see Figure 1.8). Based on this, the fixed upper limit of normal for this childhood to adult range would be supported.

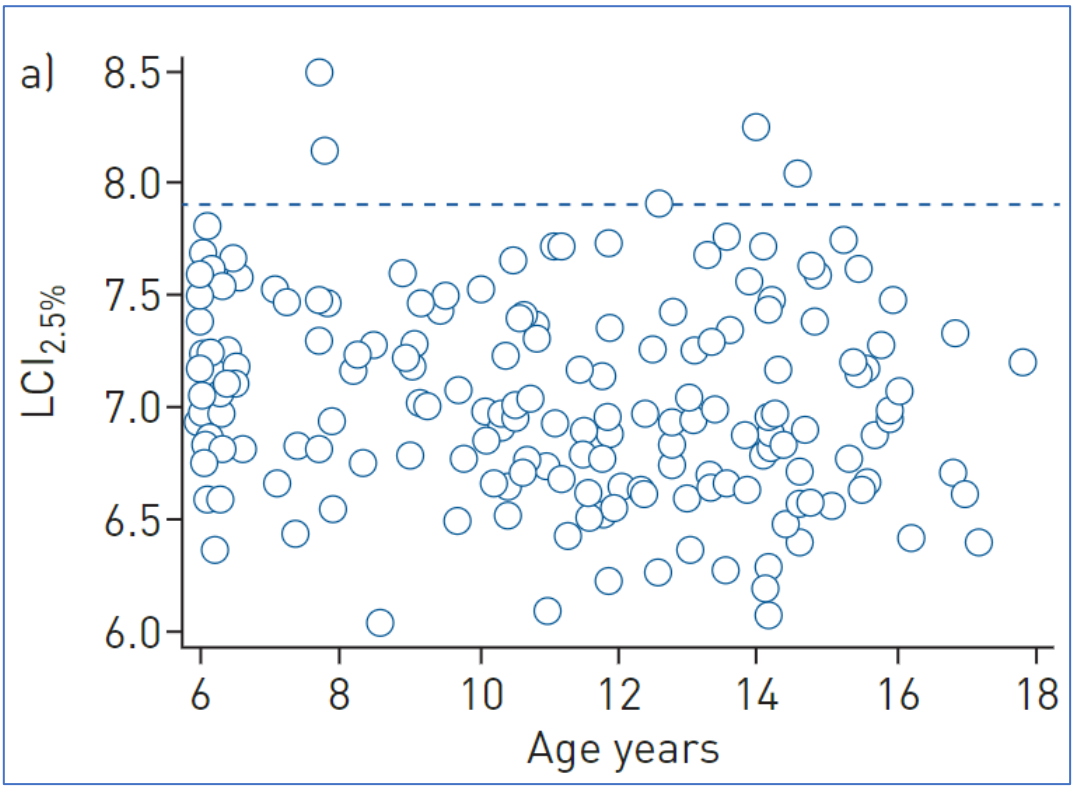

Figure 1.8: Lung clearance index plotted against age using the Exhalyzer

Reproduced with permission from the European Respiratory Society; Anagnostopoulou et al, 2020 (101)

Paediatric reference equations have been developed for LCI to reflect these small changes seen through childhood including for younger, shorter children using a mass spectrometer (118). The most significant changes in LCI exist in the first 5 years of life with reduction in LCI being of a quantity to be clinically significant. Beyond the age of 6, LCI stabilises and analysis of children older than 6 demonstrated no significant relationship with height or age (see Figure 1.9). The Innocor device has also demonstrated no significant relationship between age and LCI in a dataset of children and adults (121). They suggest a single upper limit of normal for all participants between the age of 5 years and 39 years.

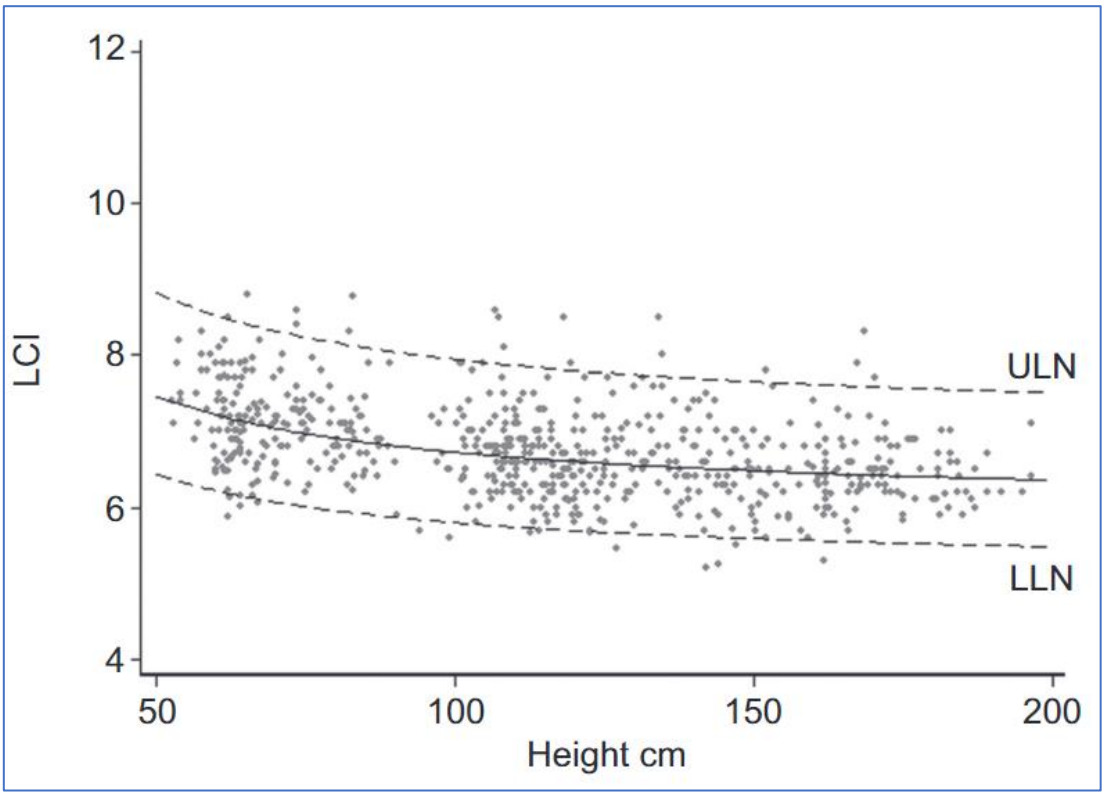

Figure 1.9: Lung clearance index from infancy to 19 years of age.

(Solid line representing the 50th centile predicted, the dashed lines represent the upper and lower limit of normal, 97.5th centile and 2.5th centile). Reproduced with permission from the European Respiratory Society Lum et al, 2013 (118)

The findings of these studies support the prospect of LCI in its use of monitoring children above the age of 5 or 6 years. This is concordant with the age at which the test becomes generally feasible. Different approaches may well be required for younger, preschool children and further work is required before commercial use is possible.

1.5.5 Other influences on Lung Clearance Index:

The relatively novel status of lung clearance index means that we can be less clear about which environmental factors may influence the result. This is important in the assessment of

patients particularly when comparing groups or attempting to generate reliable normative data sets.

Pollution has been linked to increased respiratory symptoms, respiratory tract infection and prolonged symptoms infants across several studies (122,123). Spirometry has been shown to be reduced with exposure to increased concentration of particular matter 2.5 ($PM_{2.5}$), NO_2, and some metals (zinc and iron) (124). This is particularly important in childhood (125).

The location of the childhood home is also important to understand. When lung function is compared between groups from urban and rural areas, there are important differences (126). It is worth noting that the relationship between residence and pulmonary health is complex and may include a number of contributing factors such as socioeconomic status, environmental exposure and nutrition (126).

Additional environment insults could also be important. Living in a household with a smoking adult has an important association with pulmonary function tests (127). In this particular study, they demonstrated lower spirometry indices in those exposed to household smoke and has been replicated elsewhere (128).

These known potential confounding factors of lung function should be assessed and recorded in the gathering of data, and if possible, the association of these factors should be assessed against measures of lung function (129). In this study, lung clearance index was not found to be associated with maternal smoking during pregnancy, nor maternal asthma.

1.5.6 Clinically relevant change in Lung Clearance Index:

Lung clearance index is not a perfect measure with perfectly repeatable figures. Having an understanding of the variation that can occur in successive measures is an important feature of any test. Other lung function tests such as spirometry have clear guidelines that aim to minimise this variability by defining what the acceptable test standards are (130). There is extensive normative data already published for spirometry (35).

There are different ways of measuring the variability between a 'set' of lung function tests performed on one test occasion. The coefficient of variation is a relative measure of variability that indicates the size of the standard deviation in relation to its mean (sometimes called a relative standard deviation). It can be used to compare between different groups and so is valuable in comparing groups of lung function tests.

The coefficient of variation (CV%) for spirometry has been assessed and documented at hourly, daily and weekly time intervals, and between different spirometry technicians and devices (131,132). In a large study of multiple teams of pulmonary function technicians the CV% has been shown to be between 1.8 to 4.9% for both FVC and FEV_1 across multiple tests (133). A further study of multiple biological quality control data points, in more than 100 pulmonary function test labs, over several years, noted that the CV% for FVC and FEV_1 were almost universally below 5% (134).

1.5.6.1.1 LCI short term variability:

Lung clearance index has been shown to have a similar CV% to spirometry in multiple settings across multiple devices. Within a single test setting, Horsley et al, examined both healthy children (mean age 11) and adults (mean age 33) and those with cystic fibrosis (mean age 30) using a modified Innocor device. Analysis showed a CV% of 3.2% for healthy adult FRC,

3.9% for healthy children FRC, 3.6% for healthy adult LCI and 5.4% for healthy children LCI. Participants with cystic fibrosis had a CV% of 3.5% for FRC, and 4.4% for LCI (the children and adult data were combined for those with disease) (111). This is in keeping with other variability data using later versions of the Innocor device (135).

Again, in a single test setting, the EasyOne Pro device was used in several groups of healthy participants and those with cystic fibrosis (mean age 12.5 and 11.8 respectively) across different sites. They demonstrated subject intra-test CV% from 3.47 to 7.14% for healthy participants and 4.3 to 6.31% for those with cystic fibrosis (108). This comparable mark of 'quality' of the test is important in supporting the use of the same device across different locations by different clinical teams. LCI using mass spectrometry has shown similar results, with both healthy children age 6 to 16 years and age matched participants with cystic fibrosis demonstrating a CV% of 5.2% and 6.2% respectively (69). Similar results have been shown using the Exhalyzer in both health and in participants with cystic fibrosis (CV% of 3.9% and 3.8% respectively) (136). In younger children, the variability in a single test setting was similar. Measured using mass spectrometry, healthy children age 2 to 5 years showed a CV% of 5.2%, and those of similar age with cystic fibrosis 7.8% (56).

It is helpful to review the within test variability of LCI to support its use in clinical practice. The guidance that exists to help perform LCI does aim to reduce the variance that may occur. Upon completion of the washout trials it is suggested to critically examine the FRC and LCI results for each trial to determine inclusion or not in the aggregate final result. Excessive variation of either of these figures from the mean mandates exclusion of that particular trial as an indication of a potentially discrepant result (52). It has been suggested that perhaps these quality control criteria limit the use of lung clearance index in those with worse lung disease; worse lung disease would give a higher LCI and depending on the nature of the lung disease may give greater variation during a test occasion (e.g. shifting mucus secretions during a test).

The balance between excluding certain trials to maintain the quality of the lung function test against the inclusion of washout trials that demonstrate greater variation from the mean must be carefully considered and international guidance helps to determine the levels of acceptability for these matters (135).

LCI has been examined with repeat measures after a range of short time periods. Repeating LCI after just 5 – 10 minutes in children under three years of age showed a mean difference in the result of -0.07 LCI units (95% confidence interval -0.69; 0.54) in healthy children and 0.11 LCI units (95% confidence interval -0.48; 0.70) in those with cystic fibrosis (136). It is these results showing the 'between test' reproducibility that are important in helping to start to understand the physiological variance of LCI (in health and disease) to form an idea of what the minimal clinically important difference might be. Over longer time periods and in older populations, the results are similar. After a mean of 36 days, the between visit reproducibility of LCI was shown to be 0.6 LCI units (95% confidence interval -0.78; 0.46) in a group of adults in good health (111).

Other work has examined the variation in LCI using different statistical approaches. The coefficient of repeatability, CR, calculated as 1.96 multiplied by the standard deviation of the difference between measures, implies that between two measurements, the difference with be less than the CR in 95% of cases (137). This metric is useful as the result is in the same units as the measurement being assessed, but clearly one disadvantage is that it cannot be used to compare repeatability of measurements of differing units. Another method, the intra-class correlation coefficient (ICC), examines the correlation within repeated measures in a class of data and gives a result between 0 and 1 (138). Other publications use Bland Altman to describe the variation, a method of studying the mean difference between measures and constructing the limits of agreement (139,140).

Using these measures has helped assess the variability of LCI over longer time periods. LCI has been examined on two, stable visits, a mean of 213 days apart (range 67 to 614). The CR in adults with cystic fibrosis was 1.2 LCI units, and 1.3 in children with cystic fibrosis. In this study, repeat measurements across a period of 8 months demonstrated good intra-class correlation coefficient for LCI (0.96, 95% confidence interval 0.94 to 0.98), this was similar to FEV_1 and FEF_{25-75} (135). This was the first study examining longer term variability of LCI (over a time period greater than three months) in those with 'stable' disease and adds support to its use as a monitoring tool. The CR in this study of 1.2 in adults and 1.3 units in children represents 12% and 17% of the mean LCI value respectively and is similar to other earlier suggested work on the change in LCI units.

1.5.6.1.2 Lung Clearance Index clinically relevant change:

An important factor in the use of Lung Clearance index is the understanding of what constitutes a clinically relevant change. There will be natural variability in respiratory function tests and research on the clinically relevant change for LCI has developed over the last few years. The decision as to what magnitude of change should be (either improvement or worsening) has been challenging and there is still not universal agreement on this (70).

There have been several important studies examining this key issue. LCI SEARCH was a multicentre prospective study examining the utility of lung clearance index in patients of all ages with cystic fibrosis. The patient's examined were described as having mild disease, they were free from chronic infection with pseudomonas and had preserved FEV_1 above 50%. A mean of 8 study visits (that included LCI) were performed over the course of the study. When comparing lung clearance index variation (in clinically stable patients across a mean time span

of 105 days), the mean absolute difference was 0.01 LCI units. The range of variation around this was -18.8% to 20.7% on Bland Altman analysis with an ICC of 0.93 (112).

Other studies report variability using different statistical approaches. Green et al describe the variability of LCI with monthly nitrogen multiple breath washout over a one-year period in children with cystic fibrosis. They used the Exhalyzer device with participants aged 5 to 18 years of age. Coefficient of variation was used to describe the within subject variability which was found to be 8.2%, described as being good (141). The difference between two sessions however, from one to up to 12 months apart was up to 25% and used this figure as a proposed clinically relevant change. This 25% difference was greater than previously described variability in a similar cohort of patients with between session variability of LCI being 19% (142). This decreased to 17% if visits during period of pulmonary exacerbation were excluded.

A different approach to assessing the clinical variation has been recently published in a further attempt to understand a clinically meaningful change (143). Analysing previous data, LCI was performed three monthly in a group of children with cystic fibrosis. Multiple breath washout was performed on the Exhalyzer. All tests performed with a 10% change were categorised as signal (i.e. a relevant change), or noise (variability of the test), or uncertain (with all decisions achieved by consensus decision). A 15% change in LCI was suggested as a threshold due to sensitivity in detecting clinically relevant events while limiting inappropriately highlighted noise. 10% and 20% changes in LCI were explored with better sensitivity to signal but greater noise, or less sensitivity to signal but reduced noise respectively. They summarised by suggesting there should be no single threshold, but that changes in LCI could help stratify patients who need further consideration for escalation in care.

There is not yet consensus on this important issue, but as summarised above, the current work provides an outline of the issues that remain to be explored and propose an approximate strategy to be further researched.

1.5.7 Race and Lung Clearance Index:

Appropriate normative data should also be specific for the population in which it will be used. Ethnicity has not traditionally been defined well within research with one study noting that a minority of studies comparing ethnic differences in lung function actually defined race and/or ethnicity (144). Differences have been described in the lung function test results in children from different ethnic backgrounds. In spirometry assessment, clinical interpretation of spirometry results commonly use ethnic-specific values, developed by the Global Lung Function Initiative (GLI) (145) but important questions have been raised about the use of 'race correction' in the interpretation of lung function results (146).

LCI is a measure that is internally adjusted for the size of an individual's resting lung capacity (the total volume of gas required for washout is divided by the functional residual capacity) which theoretically will remove the influence of an individual's anatomical differences that may affect lung volumes (118,147,148). Sonnappa et al examined LCI in two groups of children identified from White and South Asian ethnicity (148). These data showed no significant difference between the groups but it has not been clearly demonstrated elsewhere whether ethnicity influences lung clearance index (101). LCI has a potential to be used both in research and routine clinical settings in many childhood respiratory diseases and therefore, there is a need to evaluate normative data in children from a range of ethnic backgrounds.

1.5.8 Feasibility of lung clearance index:

For any test to be of value to a patient it must be feasible to perform, ideally in an outpatient clinic setting, and should be something that could be repeated on multiple occasions as

required. As described above, three acceptable quality multiple breath washout readings are used to generate the LCI. Many more recent studies and consensus documents have accepted two washout readings should three not be possible (52,84).

Mass spectrometry, the Exhalyzer and the ndd EasyOne Pro LAB have been compared both in children with CF and in healthy school aged children. A maximum of 8 attempts was performed on each with three acceptable multiple breath washout readings achieved in 100% using mass spectrometry, 75% using ndd EasyOne Pro LAB, and 47% using the Exhalyzer (149). A further, more recent study demonstrated much improved success with the Exhalyzer device with 97% of readings accepted (this was a smaller group of children performing serial readings over a number of months and also used a research software package that could assess the washout data in a more detailed way that perhaps a commercial user would, potentially rescuing some washouts that would have been rejected otherwise) (150). The Innocor device has also been examined, again in a group of patients performing serial readings, with an overall success rate of 92.7% (67 'failed' visits from 112 patients with 913 visits total) (112).

Performing serial tests has been shown to generate a clear progression of success with one study reporting an initial success rate of 66% in preschool children, improving with each interval (and therefore repeated tests) to 75%, 86% and 90% at 6 months. This was demonstrated in both young children with CF and those without (151).

This range of feasibility is also reflected when feasibility of the test is examined by age group. Using these commercial device, success under the age of three years is rare (152), with improved success for the remaining pre-school ages (50 to 80%) (56,153). Older, school age, children have been reported to have success rates as reported above from 63-100% (55,69,108).

The time taken to complete the test is also important if it is to be practicable in a 'real world' lab setting. This range has been reported from 20 (112) to 40 (111) minutes per patient per

LCI test. The time has been shown to be shorted in children compared to adults (17.8 minutes vs 23.5 minutes) with correlation of the test time to the age using the Innocor device (121). Examining time taken using the Exhalyzer device, if the total test time was limited to 20 minutes, 90% of participants were shown to obtain at least one LCI measure, but with only 41% able to perform more than one (154).

The most common reasons for failure are often an inability to perform controlled tidal breathing, technical issues with the equipment, leak into the analysis circuit, or participants unable to perform reproducible readings (112,152,154).

Understanding the logistical demands of the test allows an institution to properly plan for its use and allows reliable results to be generated without the concern of resource and time pressure.

1.5.9 Summary:

Bronchiectasis is a rare but increasingly recognised chronic suppurative disease in children resulting in significant morbidity in children. Careful assessment and monitoring of patients is important in identifying those who need escalation in treatment; the optimal method for achieving this is not yet agreed. Lung clearance index is a sensitive measure of lung disease as shown in Cystic Fibrosis and Primary Ciliary Dyskinesia. However, there is paucity of data on the utility of LCI in children with bronchiectasis.

There is data reporting the feasibility, repeatability, and demonstration of the variation between the available devices of lung clearance index. There is a paucity of data demonstrating lung clearance index from a range of children from differing ethnic backgrounds that requires further research.

1.5.10 Hypotheses, Aims and Objectives:

The thesis evaluates two main hypotheses:

- Hypothesis 1: There are no differences in Lung Clearance Index in healthy children from differing ethnic backgrounds
- Hypothesis 2: In younger children with non-cystic fibrosis, non-primary ciliary dyskinesia bronchiectasis, with evidence of disease on CT imaging, that Lung Clearance Index is a more sensitive disease marker than FEV_1.

Chapter 2: Systematic review of lung clearance index in non-cystic fibrosis, non-primary ciliary dyskinesia bronchiectasis

This systematic review was performed to review the existing evidence for the use of lung clearance index in patients with non-cystic fibrosis, non-primary ciliary dyskinesia bronchiectasis. An abstract of this review was presented at the European Respiratory Society International Congress 2021 (155).

This chapter has been published in the journal Respiratory Medicine, titled **"A systematic review of lung clearance index in non-cystic fibrosis, non-primary ciliary dyskinesia bronchiectasis"** (156). This chapter details the manuscript as published.

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Christopher Hine: Formal analysis, Writing – original draft, designed the study, analysed the data and wrote the first draft. **Maya Desai:** Formal analysis, Writing – original draft, designed the study, analysed the data and wrote the first draft. **Jane Davies:** Writing – original draft, designed the study, **Elizabeth Sapey:** Writing – original draft, designed the study, **Prasad Nagakumar:** Formal analysis, Writing – original draft, designed the study, analysed the data and wrote the first draft. All authors have approved the final manuscript.

2.1 Introduction:

Bronchiectasis is a chronic, suppurative lung disease defined radiologically as an abnormal dilatation of the airways diagnosed by high resolution computed tomography (HRCT) of the lung (2). In children, chronic productive or wet cough is the main symptom, while in adults, the clinical manifestations include chronic cough with sputum production, dyspnoea, fatigue and haemoptysis.

Bronchiectasis unrelated to CF or PCD (henceforth referred to as bronchiectasis) has been increasingly recognised as a disease contributing to respiratory morbidity worldwide (up to 566.1 per 100,000 population (4)).

The diagnosis, along with the assessment of disease severity, is important for early initiation of intervention. The severity of bronchiectasis can be assessed with HRCT which gives an accurate measure of disease and has high sensitivity for diagnosis when compared with chest x-ray and bronchography (157). Serial chest CT scans, due to risks of radiation exposure, are not recommended (2,157). The bronchiectasis severity index (BSI) is a composite severity score that is useful in adults (158). More generic symptom scores exist, such as the St George's Respiratory Questionnaire (SGRQ), which is a widely used tool for assessing health related quality of life (159). The limitations of the serial CT scans and clinical scoring systems highlights the need to explore the role of non-invasive tests that can be used in children and adults to monitor disease severity.

Lung clearance index (LCI) is an index of uneven ventilation and is measured by multiple breath washout (MBW) of an inert tracer gas from the lungs, most commonly nitrogen (N_2) or

sulphur hexafluoride (SF_6) (55). LCI is the most commonly reported outcome of MBW, the standard definition being the number of lung turnovers required to reduce the tracer gas concentration to $1/40^{th}$ (2.5%) of the starting concentration. LCI has been widely explored in CF (where it is a sensitive marker of early lung disease) (151) and in primary ciliary dyskinesia (PCD), (87,88) (where due to the heterogeneity of the disease, studies have not shown a consistent relationship between LCI, structural lung changes and spirometry).

This systematic review aimed to assess the evidence for the role of LCI in non-CF, non-PCD bronchiectasis as a non invasive disease biomarker .

2.2 Methods:

This review is reported according to the PRISMA statement (Preferred Reporting Items for Systematic reviews and Meta-analyses) and is registered on the PROSPERO (International Prospective Register of Systematic Reviews) database (#CRD42020203012) (160,161).

2.3 Search Strategy:

We searched MEDLINE, CINAHL, HMIC, Emcare, AMED, Embase, the Health Technology Assessment Database, the Cochrane Central Register of Controlled Trials, and the Clinical Trials Register. No geographical or language restrictions were applied. Studies were reviewed if published within the last 20 years. Search results were downloaded to Rayyan and duplicates were removed (162). (Search criteria are shown in Table 2.1).

Systematic Review Search Strategy

Main Search

1. lung clearance index.ti,ab
2. LCI.ti,ab
3. multiple breath washout.ti,ab
4. MBW.ti,ab
5. ventilation inhomogeneity.ti,ab
6. sulphur hexafluoride.ti,ab
7. SF6.ti,ab
8. nitrogen washout.ti,ab
9. helium washout.ti,ab
10. inert gas washout.ti,ab
11. **OR/1-10**
12. exp BRONCHIECTASIS/
13. Bronchiectasis.ti,ab
14. **OR/12-13**
15. **AND/11,14**

Secondary Searches

To what extent is lung clearance index abnormal compared to forced expiratory volume in one second (FEV_1)?

16. FEV1.ti,ab
17. lung* function.ti,ab
18. lung* volumes.ti,ab
19. forced expiratory volume.ti,ab
20. exp FORCED EXPIRATORY VOLUME/
21. exp RESPIRATORY FUNCTION TESTS/
22. **OR/16-21**
23. **AND/15,22**

Does lung clearance index change in relation to the severity of disease assessed by clinical indicators?

16. resp* exacerbation.ti,ab
17. pulm* exacerbation.ti,ab
18. resp* deterioration.ti,ab
19. pulm* deterioration.ti,ab
20. exacerbation.ti,ab
21. acute exacerbation.ti,ab
22. quality of life.ti,ab
23. QOL.ti,ab
24. health-related quality of life.ti,ab
25. symptom score.ti,ab
26. resp* symptom score.ti,ab
27. exercise test.ti,ab
28. exercise tolerance test.ti,ab
29. exercise.ti,ab
30. physical activity.ti,ab
31. exp DISEASE PROGRESSION/
32. exp SYMPTOM FLARE UP/

33. exp RESPIRATORY TRACT INFECTIONS/ 34. exp QUALITY OF LIFE/ 35. exp HEALTH STATUS INDICATORS/ 36. exp OUTCOME ASSESSMENT, HEALTH CARE/ 37. esp HEALTH STATUS/ 38. exp SIGNS AND SYMPTOMS, RESPIRATORY/ 39. exp EXERCISE TOLERANCE/ **40. OR/16-39** **41. AND/15,40**
Does lung clearance index change following an intervention? 16. resp* exacerbation.ti,ab 17. pulm* exacerbation.ti,ab 18. resp* deterioration.ti,ab 19. pulm* deterioration.ti,ab 20. exacerbation.ti,ab 21. acute exacerbation.ti,ab 22. antibiotic.ti,ab 23. antibiotics.ti,ab 24. physio*.ti,ab 25. secretion clearance.ti,ab 26. airway clearance.ti,ab 27. physical therapy.ti,ab 28. exp DISEASE EXACERBATION/ 29. exp SYMPTOM EXACERBATION/ 30. exp RESPIRATORY TRACT INFECTIONS/ 31. exp PHYSICAL THERAPY/ 32. exp EXERCISE THERAPY/ **33. OR/16-32** **34. AND/15,33**

Table 2.1: Example search strategy

Review Question	Exclusion	Inclusion
Can LCI be used to support clinical decision making in the care of children with non-CF, non-PCD bronchiectasis and what evidence exists to support its use	Diagnosis of cystic fibrosis Diagnosis of primary ciliary dyskinesia	Lung clearance index reported (ATS/ERS standards for reporting) Diagnosis of bronchiectasis confirmed on CT imaging
Additional review questions and additional exclusion/inclusion criteria		
To what extent is LCI abnormal, compared to FEV_1, in children with non-CF, non-PCD bronchiectasis confirmed on Chest CT imaging?	No formally reported CT imaging score or specifically diagnosis of bronchiectasis	Forced expiratory volume in one second (FEV_1) reported (ATS/ERS standards for reporting)
Does LCI change in relation to the severity of disease (assessed by clinical indicators) in children with non-CF, non-PCD bronchiectasis?		Reported clinical measure: o Treatment for pulmonary exacerbation o Quality of life/Severity score o Respiratory symptom score o Exercise Tolerance test o Airway microbiology assessment
In children with non-CF, non-PCD bronchiectasis, does LCI change following an intervention	No pre- and post-intervention assessment of LCI	Therapeutic intervention performed including: o Antibiotic therapy o Airway clearance manoeuvre o Novel therapy

Table 2.2: Inclusion and Exclusion criteria use for study selection for review questions
CF: cystic fibrosis; PCD: primary ciliary dyskinesia; ATS: American Thoracic Society; ERS: European Respiratory Society; CT: computed tomography; FEV_1: forced expiratory volume in one second; LCI: lung clearance index

2.4 Study screening and eligibility criteria:

We included studies of any design. Case studies were included if they included groups of greater than 10 subjects. One author (CH) screened all titles and abstracts according to the pre-specified inclusion and exclusion criteria (see Table 2.2) and screened full reports of potentially relevant studies. Two independent reviewers (PN and MD) examined a 20% selection of the titles, abstracts and full reports to ensure consistency (163). Discrepancies were resolved by discussion.

2.5 Data extraction and analysis:

One reviewer (CH) extracted all outcome data and information necessary to assess study quality. Two independent reviewers (PN and MD) examined a 20% selection to ensure consistency as previously described. All extracted data was entered into Excel (Microsoft Corporation, Redmond, WA, USA).

The outcomes of the included studies were compared, and a narrative analysis was performed. The studies had a high degree of heterogeneity and therefore meta-analysis of included studies was not possible.

2.6 Results:

276 citations were found after completing the main search (see Figure 2.1). Of these studies, 28 full text articles, applying to at least one review question, were assessed for inclusion.

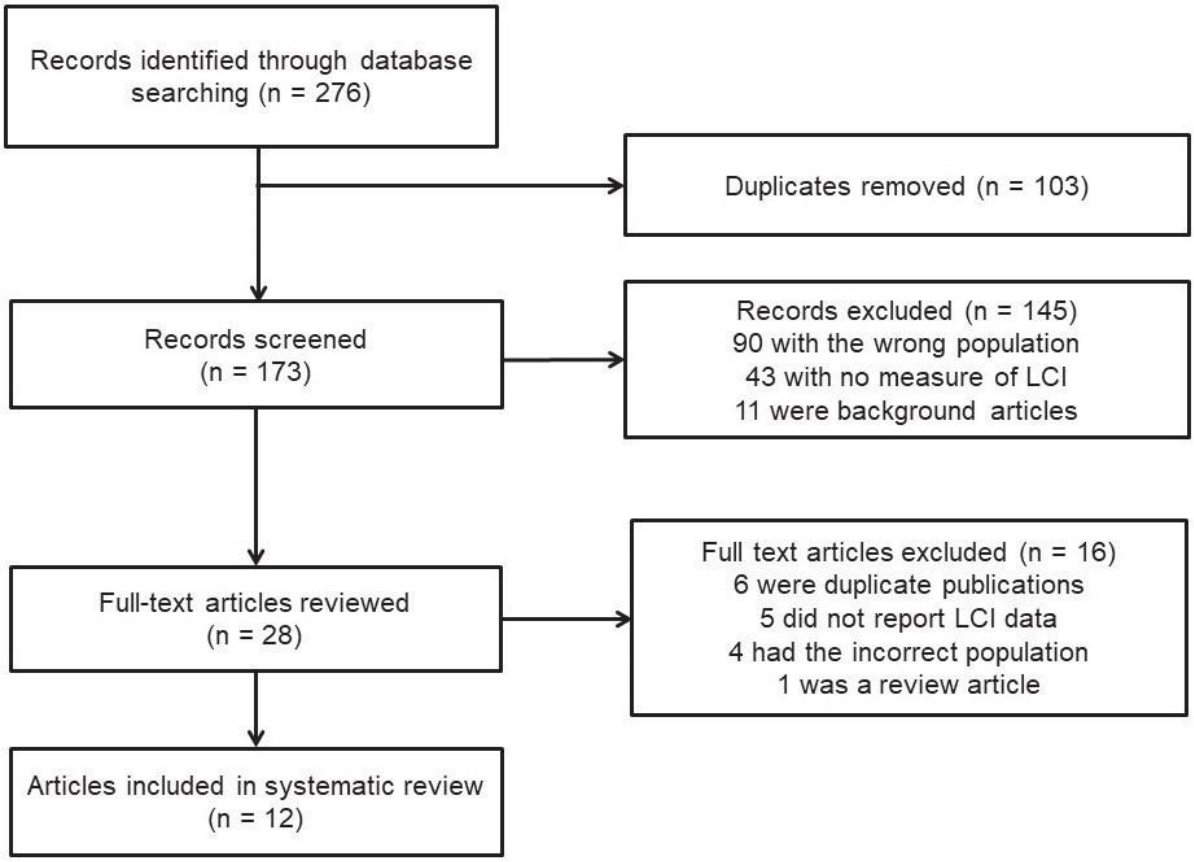

Figure 2.1: Flow diagram of literature search and study selection
LCI: lung clearance index

12 articles were eligible to be included in the review. Of these, five included paediatric (defined as age up to 18 years) data totalling 68 patients (164–168). The paediatric data was exclusively presented in conference abstracts with limited information available. The remaining seven articles included 451 adult patients (169–175). (See Table 2.3 for an overview of the articles).

All articles were observational studies with a majority (10 articles, 83%) being exclusively, or largely of cross-sectional study design

Article		Patients	Study Design	Device	Tracer Gas	LCI Protocol
Paediatric Data						
Lung clearance index (LCI) is a sensitive predictor of high resolution computed tomography (HRCT) scores in children with non-CF bronchiectasis, Irving et al, 2014 (164)	London, United Kingdom	12 children (mean age not specified)	Cross sectional	Not specified	Not specified	Not specified
Lung clearance index and exercise capacity among children with bronchiectasis, Hatziagorou et al, 2012 (166)	Thessaloniki, Greece	14 children (mean age not specified)	Cross sectional	Not specified	Not specified	Not specified
Lung clearance index and exercise capacity among children with CF and non-CF bronchiectasis over a two year period, Hatziagorou et al, 2015 (168)	Thessaloniki, Greece	13 children (mean age 13.0 years)	Cohort	Not specified	Not specified	Not specified
Lung clearance index and exercise capacity among children with mild CF- and non-CF bronchiectasis, Hatziagorou et al, 2013 (167)	Thessaloniki, Greece	15 children (mean age not specified)	Cross sectional	Not specified	Not specified	Not specified
Sensitivity of lung clearance index and chest computed tomography in early lung disease among children with non-CF bronchiectasis, Hatziagorou et al, 2012 (165)	Thessaloniki, Greece	14 children (mean age not specified)	Cross sectional	Not specified	Not specified	Not specified
Adult Data						
Composition of airway bacterial community correlates with chest HRCT in adults with bronchiectasis, O'Neill et al, 2020 (169)	Belfast, United Kingdom	21 adults (mean age 64.0 years)	Cross sectional	Innocor® LCI, Cosmed, Italy	Sulfur hexafluoride	Specified
Lung clearance index in adults with non-cystic fibrosis bronchiectasis. Gonem et al, 2014 (170)	Leicester, United Kingdom	43 adults (mean age 67.4 years)	Cross sectional	Innocor® LCI, Cosmed, Italy	Sulfur hexafluoride	Specified
Lung clearance index is a repeatable and sensitive indicator of radiological changes in bronchiectasis, Rowan et al, 2014 (171)	Belfast, United Kingdom	Group 1: 30 Adults, (mean age 56.7 years) Group 2: 60 Adults, (mean age 62.4 years)	Cross sectional	Innocor® LCI, Cosmed, Italy	Sulfur hexafluoride	Specified
The reproducibility and responsiveness of the lung clearance index in bronchiectasis. Grillo et al, 2015 (172)	London, United Kingdom	*(Group 1 excluded)* Group 2: 32 adults (mean age 63.1 years)	Case series	Innocor® LCI, Cosmed, Italy	Sulfur hexafluoride	Specified
Maximal mid-expiratory flow is a surrogate marker of lung clearance index for assessment of adults with bronchiectasis, Guan et al, 2016 (173)	Guangzhou, China	115 Adults, (mean age 44.6 years)	Cross sectional and case series	Quark PFT, Cosmed, Italy	Nitrogen	Specified
Residual volume/total lung capacity ratio confers limited additive significance to lung clearance index for assessment of adults with bronchiectasis, Guan et al, 2017 (174)	Guangzhou, China	135 Adults (mean age 44.4 years)	Cross sectional	Quark PFT, Cosmed, Italy	Nitrogen	Specified
The quantitative link of lung clearance index to bronchial segments affected by bronchiectasis, Verbanck et al, 2018 (175)	Sydney, Australia	15 Adults, (mean age not specified)	Cross sectional	Bag-in-box setup	Nitrogen	Specified

Table 2.3: Summary of included articles, lung clearance index device, gas and assessment protocol
LCI: lung clearance index; PFT: pulmonary function tests; HRCT: high resolution computed tomography

2.7 Risk of bias in included studies:

Risk of bias was assessed using the National Institute of Health Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies (Figure 2.2) (176). Of the five paediatric articles reviewed, four were rated ‘high’ and one ‘moderate’ for risk of bias.

The adult data was presented in full manuscripts and therefore was more comprehensive. All articles were rated ‘low’ for risk of bias. Part of the Risk of Bias Tool assesses demonstration of a causal relationship, however, all but one of the articles were of cross-sectional design and would score poorly in these areas. As the research aims are of exploration of the relationship between a disease and possible marker of disease, rather than a causal relationship, we concluded that this would not increase the overall risk of bias and those articles have been graded accordingly.

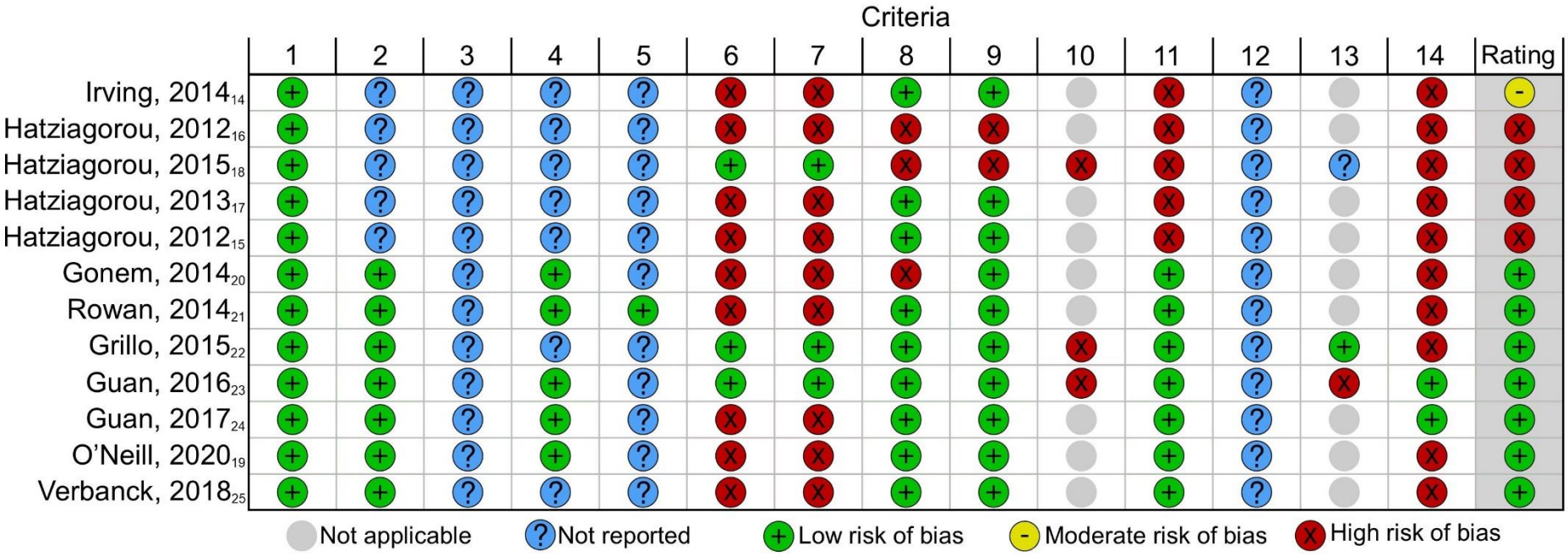

Figure 2.2: Quality Assessment – National Institute of Health Quality Assessment Tool for Observational Cohort and Cross-sectional studies

Criteria 1: Was the research question or objective in this paper clearly stated?

Criteria 2: Was the study population clearly specified and defined?

Criteria 3: Was the participation rate of eligible persons at least 50%?

Criteria 4: Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?

Criteria 5: Was a sample size justification, power description, or variance and effect estimates provided?

Criteria 6: For the analyses in this paper, were the exposure(s) (*bronchiectasis*) of interest measured prior to the outcome(s) (*Lung Clearance Index*) being measured?

Criteria 7: Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?

Criteria 8: For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g. categories of exposure, or exposure measured as continuous variable)?

Criteria 9: Were the exposure measures (assessment of bronchiectasis) clearly defined, valid, reliable, and implemented consistently across all study participants?

Criteria 10: Was the exposure(s) assessed more than once over time?

Criteria 11: Were the outcome measures (the measure of LCI) clearly defined, valid, reliable, and implemented consistently across all study participants?

Criteria 12: Were the outcome assessors blinded to the exposure status of participants?

Criteria 13: Was loss to follow-up after baseline 20% or less?

Criteria 14: Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?

2.8 Paediatric Data:

Of the five paediatric studies included, one assessed LCI and disease severity on CT imaging (164), one assessed the diagnostic value of LCI and spirometry (165), and three examined LCI and clinical condition assessed by exercise testing (166–168).

Irving et al (164) described 12 children with bronchiectasis. Theirs was the only paediatric study to compare LCI with spirometry, demonstrating a correlation between LCI and forced expiratory volume in one second (FEV_1) that was similar to a cohort of CF patients ($r=-0.6$, $p=0.009$). They also described a range of features of bronchiectasis on CT imaging and their correlation with LCI (see Table 2.4).

Hatziagorou et al (165) assessed 15 patients (aged 6 to 21 years) with bronchiectasis and normal FEV_1 (greater than 80% predicted), LCI was abnormal in 12 patients (80%). Hatziagorou et al also described groups of patients with bronchiectasis across three abstracts, it is not clear if some patients overlap between these groups (166–168). One describes 14 children with reduced FEV_1 (75.1%), reduced peak oxygen uptake (V'O peak, 77.3%) and high LCI (11.8) (166). A second describes 15 patients with bronchiectasis with similar Bhalla scores, V'O peak and LCI compared to patients with CF (167). Finally, 13 patients with bronchiectasis were monitored over two years with similarly affected lung function tests and stable LCI over this time period.

<table>
<tr><th>Article</th><th>CT Scoring system</th><th>LCI Correlation with features on imaging</th></tr>
<tr><td>Lung clearance index (LCI) is a sensitive predictor of high resolution computed tomography (HRCT) scores in children with non-CF bronchiectasis, Irving et al, 2014 (164)</td><td>Modified Bhalla (177)</td><td>Feature on CT (correlation: Pearson’s r)
<table>
<tr><td></td><td>Correlation</td><td>P value</td></tr>
<tr><td>Extent of bronchiectasis</td><td>0.8</td><td>0.002</td></tr>
<tr><td>Severity of bronchiectasis</td><td>0.7</td><td>0.01</td></tr>
<tr><td>Airway wall thickening</td><td>0.7</td><td>0.01</td></tr>
<tr><td>Air trapping</td><td>0.8</td><td>0.0006</td></tr>
</table></td></tr>
<tr><td>Lung clearance index is a repeatable and sensitive indicator of radiological changes in bronchiectasis, Rowan et al, 2014 (171)</td><td>Brody 2004 (178)</td><td>Computed Tomography Scan Abnormalities (correlation: Pearson’s r)
<table>
<tr><td>Percent Bronchiectasis</td><td>R = 0.41, p<0.01</td></tr>
<tr><td>Percent Airway Thickening</td><td>R = 0.21, not significant</td></tr>
<tr><td>Percent Mucus Plugging</td><td>R = 0.49, p<0.001</td></tr>
<tr><td>Percent Parenchymal</td><td>R = 0.56, p<0.001</td></tr>
<tr><td>Percent Air Trapping</td><td>R = 0.36, p<0.01</td></tr>
<tr><td>Percent Total</td><td>R = 0.55, p<0.001</td></tr>
</table></td></tr>
<tr><td>Maximal mid-expiratory flow is a surrogate marker of lung clearance index for assessment of adults with bronchiectasis, Guan et al, 2016 (173)</td><td>Modified Reiff (179)</td><td>Computed Tomography Scan Scoring (correlation: Pearson’s r)
<table>
<tr><th>CT Score</th><th>LCI (median (95% CI))</th><th>Correlation</th><th>P Value</th></tr>
<tr><td>1≤ HRCT score <7</td><td>12.40 (11.84, 13.18)</td><td>-0.48</td><td><0.01</td></tr>
<tr><td>7≤ HRCT score ≤12</td><td>16.20 (14.60, 16.69)</td><td>-0.71</td><td><0.01</td></tr>
<tr><td>HRCT score ≥13</td><td>19.15 (18.73, 22.69)</td><td>-0.12</td><td>0.66</td></tr>
</table>
LCI Agreement with CT features (Concordance: kappa statistic)
<table>
<tr><th>CT Feature</th><th>Concordance</th><th>95% CI</th></tr>
<tr><td>≤3, >3 bronchiectatic lobes</td><td>0.527</td><td>(0.376, 0.678)</td></tr>
<tr><td>≤9, >9 HRCT Total score</td><td>0.527</td><td>(0.376, 0.678)</td></tr>
<tr><td>Uni vs. Bi lateral Bx</td><td>0.291</td><td>(0.126, 0.456)</td></tr>
<tr><td>Tubular vs. Cystic Bx</td><td>0.291</td><td>(0.126, 0.456)</td></tr>
</table></td></tr>
<tr><td>Residual volume/total lung capacity ratio confers limited additive significance to lung clearance index for assessment of adults with bronchiectasis, Guan et al, 2017 (174)</td><td>Modified Reiff (120</td><td>‘High’ LCI (greater than median) correlation (Chi squared test) with HRCT features
<table>
<tr><th>Clinical Feature</th><th>X^2 value</th><th>P value</th></tr>
<tr><td>>3 bronchiectatic lobes</td><td>31.69</td><td>P<0.001</td></tr>
<tr><td>HRCT total score >9</td><td>30.27</td><td>P<0.001</td></tr>
<tr><td>Cystic bronchiectasis</td><td>8.98</td><td>P=0.003</td></tr>
<tr><td>Mosaicism</td><td>15.01</td><td>P<0.001</td></tr>
</table></td></tr>
<tr><td>The quantitative link of lung clearance index to bronchial segments affected by bronchiectasis, Verbanck et al, 2018 (175)</td><td>Bhalla (180)</td><td>(Correlation: Pearson’s r)
<table>
<tr><td>Number of segments affected by bronchiectasis</td><td>r = 0.76 , p<0.001, 95% CI (0.41 – 0.92)</td></tr>
</table></td></tr>
</table>

Table 2.4: Summary of findings on imaging and lung clearance index in included patient cohorts.
CT: computed tomography; LCI: lung clearance index; Bx: bronchiectasis; HRCT: high resolution computed tomography

2.9 Adult Data:

2.9.1 Assessment of Lung Clearance Index:

Lung clearance index was assessed using different devices (see Table 2.3). Four studies used the Innocor® LCI system (Cosmed, Italy), a commercial device that uses a photoacoustic infrared gas analyser to record washout of a tracer gas (sulfur hexafluoride, SF_6) (169–172). Two studies used the Quark PFT system (Cosmed, Italy), a commercial device with CO_2 and O_2 gas analysers to indirectly measure washout of nitrogen (N_2) (173,174). Finally, one study used a non-commercial, nitrogen washout, laboratory set up with direct N_2 measurement (175). All studies reported details of the assessment protocol used and quality control measures in place. The repeatability of LCI in patients with bronchiectasis was reported in two adult studies with intraclass correlation coefficients of 0.86 to 0.94 (170,171).

2.9.2 Lung Clearance Index and Forced Expiratory Volume in one second:

The correlation of LCI and FEV_1 was reported in three adult articles all demonstrating an inverse relationship: decrease in FEV_1 with rising LCI: ($r = -0.37$ to -0.61) (170–172) (see Table 2.5).

Article	Mean FEV_1%		Mean LCI	LCI correlation with FEV_1%
Composition of airway bacterial community correlates with chest HRCT in adults with bronchiectasis, O'Neill et al, 2020 (169)	76.5% (17.2%)		9.5 (1.7)	NR
Lung clearance index in adults with non-cystic fibrosis bronchiectasis. Gonem et al, 2014 (170)	82.0% (3.8%)		9.99 (0.31)	R = -0.61, p<0.0001
Lung clearance index is a repeatable and sensitive indicator of radiological changes in bronchiectasis, Rowan et al, 2014 (171)	Group 1	84.8% (20.7%)	9.2 (1.8)	R = -0.37, p<0.05
	Group 2	76.5% (18.9)	9.1 (2.0)	R = -0.51, p<0.0001
The reproducibility and responsiveness of the lung clearance index in bronchiectasis. Grillo et al, 2015 (172)	*(Z score)* -2.97 (1.30)		12.76 (3.47)	R = -0.548, p<0.05
Maximal mid-expiratory flow is a surrogate marker of lung clearance index for assessment of adults with bronchiectasis, Guan et al, 2016 (173)	Group 1	NR	15.3 (NR)	NR
	Group 2	NR	16.65 (NR)	NR
Residual volume/total lung capacity ratio confers limited additive significance to lung clearance index for assessment of adults with bronchiectasis, Guan et al, 2017 (174)	NR		14.6 (range 12.0-17.6)	NR
The quantitative link of lung clearance index to bronchial segments affected by bronchiectasis, Verbanck et al, 2018 (175)	NR		7.8, (95% CI 7.2 to 8.4)	NR
Lung clearance index (LCI) is a sensitive predictor of high resolution computed tomography (HRCT) scores in children with non-CF bronchiectasis, Irving et al, 2014 (164)	NR		NR	R = -0.6, p=0.009
Lung clearance index and exercise capacity among children with bronchiectasis, Hatziagorou et al, 2012 (166)	75.1%		11.8	NR
Lung clearance index and exercise capacity among children with CF and non-CF bronchiectasis over a two year period, Hatziagorou et al, 2015 (168)	76.6%		9.71	NR
Lung clearance index and exercise capacity among children with mild CF- and non-CF bronchiectasis, Hatziagorou et al, 2013 (167)	NR		NR	NR
Sensitivity of lung clearance index and chest computed tomography in early lung disease among children with non-CF bronchiectasis, Hatziagorou et al, 2012 (165)	NR		NR	NR

Table 2.5: Summary of correlation of lung function tests and lung clearance index.
HRCT: high resolution computed tomography; NR: not reported; NA: not applicable. Data expressed as mean (standard error) unless otherwise noted.

2.9.3 Predictive value of Lung Clearance Index in disease detection:

LCI was examined for its ability to identify patients with disease vs. healthy controls in two articles (170,171). Both used Receiver Operating Characteristics (ROC) to display the discriminatory ability of LCI with the Area Under the Curve (AUC) used as a measure. Gonem et al showed that the AUC for LCI was 0.90 (compared to 0.83 for FEV_1) (170). This was similar in the data from Rowan et al; AUC for LCI 0.96 and 0.82 for FEV_1 (171). Neither study identify a value of LCI above which the risk of bronchiectasis is defined as being at particular risk.

Guan et al demonstrated the value of LCI in detecting disease at differing levels of severity (173). Severity was judged using both BSI and HRCT. When grouped into mild, moderate and severe (using BSI tertials of mild; 0-4, moderate; 5-8, and severe; 9 or more [4]), LCI was able to accurately discriminate between mild and moderate/severe bronchiectasis (AUC 0.67, p=0.003) and between mild/moderate and severe bronchiectasis (AUC 0.70, p=0.001). HRCT imaging was scored using the modified Reiff system (179), with scores grouped into mild, moderate and severe cohorts (181). The predictive value of LCI to discriminate between mild and moderate/severe was high (AUC 0.83, $p<0.001$) but again was better when distinguishing between mild/moderate and severe (AUC 0.920, $p<0.001$).

Another publication by Guan et al of a slightly larger group of patients from the same cohort examined the discrimination of mild from moderate/severe and mild/moderate from severe bronchiectasis (severity judged by BSI) (174). The results were similar to the previous publication (AUC 0.73 and 0.70 respectively).

2.9.4 Lung Clearance Index Correlation with other Clinical measures:

2.9.4.1 Relationship to severity score:

Two articles examined the relationship between lung function and BSI and demonstrated a clear association. Guan et al showed increasing lung clearance index (12.90, 14.60, and 16.90) with increasing severity of BSI tertials (mild, moderate and severe) with significant differences between these groups (173). The second study by Guan et al (174) demonstrated that BSI significantly correlated with lung clearance index ($r = 0.45$, $p<0.001$) (see Table 2.6).

<table>
<tr><th>Article</th><th>LCI correlation with clinical indicator</th><th>LCI change pre/post intervention</th></tr>
<tr><td>Composition of airway bacterial community correlates with chest HRCT in adults with bronchiectasis, O'Neill et al, 2020 (169)</td><td>Bacterial community composition<table><tr><td>Richness (S)</td><td>R = -0.19, p = 0.4</td></tr><tr><td>Evenness ($e^{H/S}$)</td><td>R = -0.22, p = 0.3</td></tr><tr><td>Shannon Wiener Index (H)</td><td>R = -0.29, p = 0.2</td></tr><tr><td>Dominance (D)</td><td>R = 0.29, p = 0.2</td></tr></table></td><td>NA</td></tr>
<tr><td>Lung clearance index in adults with non-cystic fibrosis bronchiectasis. Gonem et al, 2014 (170)</td><td><table><tr><td></td><td>LCI</td></tr><tr><td>No chronic bacterial growth, n=26</td><td>10.02 (0.36)</td></tr><tr><td>Chronic colonisation with bacteria, n=17</td><td>9.95 (0.57)</td></tr></table>No significant difference (Student's T Test)</td><td>NA</td></tr>
<tr><td>Lung clearance index is a repeatable and sensitive indicator of radiological changes in bronchiectasis, Rowan et al, 2014 (171)</td><td>Respiratory symptoms<table><tr><td>SGRQ</td><td>R = 0.24, p = 0.03</td></tr></table></td><td>NA</td></tr>
<tr><td>The reproducibility and responsiveness of the lung clearance index in bronchiectasis. Grillo et al, 2015 (172)</td><td>NA</td><td><table><tr><td></td><td>LCI change</td><td>P value</td></tr><tr><td>Start exacerbation physio</td><td>0.11 (0.85)</td><td>0.5</td></tr><tr><td>End exacerbation physio</td><td>0.14 (0.93)</td><td>0.4</td></tr><tr><td>Admission for IV antibiotic</td><td>0.38 (1.59)</td><td>0.2</td></tr></table></td></tr>
<tr><td>Maximal mid-expiratory flow is a surrogate marker of lung clearance index for assessment of adults with bronchiectasis, Guan et al, 2016 (173)</td><td>BSI Score (LCI adjusted for age, sex and BMI)<table><tr><th>BSI Score</th><th>LCI (95% CI)</th><th>P Value</th></tr><tr><td>0≤ BSI <5</td><td>12.90 (12.57, 15.05)</td><td>N/A</td></tr><tr><td>5≤ BSI ≤8</td><td>14.60 (13.68, 15.96)</td><td>P < 0.01</td></tr><tr><td>BSI ≥ 9</td><td>16.90 (15.89, 19.37)</td><td>P < 0.01</td></tr></table>LCI Agreement with clinical indicator<table><tr><th>Clinical Indicator</th><th>Concordance</th><th>95% CI</th></tr><tr><td>BSI≤5 vs. BSI>5</td><td>0.309</td><td>(0.133, 0.485)</td></tr><tr><td>No pseud. vs. pseud colonised</td><td>0.200</td><td>(0.035, 0.365)</td></tr><tr><td>FEV≤80% vs. FEV1>80%</td><td>-0.364</td><td>(-0.52, -0.21)</td></tr></table></td><td><table><tr><td></td><td>LCI (unit change)</td><td>P value</td></tr><tr><td>Admission for IV antibiotic</td><td>-0.06</td><td>0.3</td></tr></table></td></tr>
<tr><td>Residual volume/total lung capacity ratio confers limited additive significance to lung clearance index for assessment of adults with bronchiectasis, Guan et al, 2017 (174)</td><td>'High' LCI (greater than median) correlation<table><tr><th>Clinical Feature</th><th>X^2 value</th><th>P value</th></tr><tr><td>BSI >5</td><td>15.00</td><td>P=0.001</td></tr><tr><td>FEV_1 predicted ≤80%</td><td>21.17</td><td>P<0.001</td></tr></table><table><tr><td>BSI</td><td>R = 0.45, p < 0.001</td></tr></table></td><td>NA</td></tr>
</table>

Table 2.6: Summary of treatment effect and clinical condition on lung clearance index in included patient cohorts. HRCT: high resolution computed tomography; SGRQ: St George's Respiratory Questionnaire; NR: not reported; NA: not applicable; BSI: bronchiectasis severity index; 95% CI: 95% confidence interval; BMI: body mass index. Data expressed as mean (standard error) unless otherwise noted.

2.9.4.2 Relationship to symptoms:

Only one article reported the correlation of lung clearance index with a health-related quality of life questionnaire. Rowan et al (171) showed evidence of a relationship between LCI and SGRQ with a significant positive correlation between increasing symptoms and LCI ($r=0.24$, $p=0.03$).

2.9.4.3 Relationship to disease detected by imaging:

Four adult studies comprising 325 patients were included that described LCI and its relationship to CT imaging (Table 2.4) (171,173–175). Two studies had CT imaging and LCI performed at the same visit (171,175) and two studies reported a group of patients across two publications with CT images taken within one year of LCI assessment (173,174).

Rowan et al (171) assessed CT images with a formal scoring system (Brody (178)). They report the degree of correlation of LCI with CT features with a positive relationship between worsening CT changes and increasing LCI.

Verbanck et al (175) demonstrated the relationship between the number of lung segments with bronchiectasis on imaging and LCI for populations of patients with both cystic fibrosis ($r=0.72$, $p<0.001$) and bronchiectasis ($r=0.76$, $p<0.001$).

Guan et al reviewed CT scores calculated using a modified Reiff score (173,174). In their earlier cohort (173) they describe the range of severity of bronchiectasis using HRCT (44 mild patients, 1≤ Reiff score <7; 46 moderate patients, 7≤ Reiff score ≤12; 20 severe patients, Reiff score ≥13). They found those with higher LCI (above their median of 14.7) more consistently reflected those with worse changes on CT (using kappa statistic to assess agreement).

2.9.5 Lung Clearance Index change with intervention:

Two articles examined the effect of a clinical intervention on lung clearance index (173,174). Guan et al monitored a group of adults during a period of clinical stability (exacerbation free for four weeks) (173). Those who subsequently experienced an exacerbation were invited to repeat further LCI prior to treatment, and again one week after completion of 14 days of antibiotic therapy. The changes in LCI were small and were not statistically significant (see Table 2.6).

Grillo et al assessed a group of patients experiencing an exacerbation of bronchiectasis and again found that there was no significant difference in LCI nor FEV_1 z-scores from the start to the end of exacerbation (see Table 2.6). Patients were recruited at the onset of exacerbation, with LCI performed within 48 hours of commencing intravenous antibiotics and on reaching clinical recovery at discharge which was determined by clinician decision (172). This cohort of patients undergoing treatment for exacerbation also performed LCI pre and post

physiotherapy sessions (performed both at the start, and at the end of the exacerbation) with no significant difference found in LCI.

2.9.6 Other measures of potential clinical impact:

2.9.6.1 Relationship to microbiome:

Several studies also reported the relationship of LCI to metrics of bacterial infection. O'Neill et al, presented microbiological data (169) from a subgroup of a larger study (171). LCI had no significant correlation with any of the microbiological indices (measured as bacterial species richness (S), the Shannon-Wiener diversity index (H), species evenness ($e^{H/S}$) and species dominance (D) (see Table 2.6). There was however a link between the microbiological indices and some features on CT expressed as Spearman's rank correlation coefficient (r_s):

- Percent bronchiectasis vs. (D) r_s=0.654, p=0.001; (H) r_s=-0.655, p=0.001; ($e^{H/S}$) r_s=-0.474, p=0.030; (S) r_s=-0.567, p=0.007
- Percent airway thickening vs. ($e^{H/S}$) r_s=-0.443, p=0.044
- Percent parenchymal change vs. (D) r_s=0.453, p=0.039; (H) r_s=-0.489, p=0.024; (S) r_s=-0.453, p=0.039

This implies that worsening bronchiectasis on imaging correlates with decreasing diversity and increasing dominance of a bacterial species. Despite not finding a correlation between LCI

and the microbiological indices, they did demonstrate that LCI was able to detect a significant difference when patients were grouped into 'never', 'intermittent' and 'chronic' colonisation groups for *Pseudomonas aeruginosa* infection (defined using the Leeds criteria (182) (p=0.04)). Gonem et al, however, did not find a difference in LCI between their cohorts of patients with 'chronic bacterial colonisation' and 'no chronic bacterial colonisation' of any pathogen, defined as isolation on sputum culture on at least two occasions during the previous year (see Table 2.6) (170).

Pseudomonas aeruginosa colonisation (defined as a growth on two occasions, at least three months apart) was also examined by Guan et al (173). There was poor level of agreement of 'high' LCI (above their median) with the presence of *Pseudomonas aeruginosa* colonisation (kappa statistic, 0.200).

2.10 Discussion:

This systematic review confirms that in adults with bronchiectasis, LCI is abnormal and that it correlates with FEV_1 and markers of disease on CT. These findings suggest that LCI may be a marker of disease severity but the volume of data and heterogeneity of the studies limits conclusion. Unlike the adult data, there were no high-quality studies identified including paediatric patients. Further work is required to explore the value of LCI in the paediatric population and currently no clear conclusions can be drawn. To describe the most clinically applicable data, this review did not explore the role of other multiple breath washout metrics

(S_{acin}, S_{cond} etc…) and is a weakness of the study; further work is needed to investigate the value of these other measures.

LCI has been shown to be a more sensitive marker of disease than FEV_1 in cystic fibrosis (72). This seems to be true also for adults with bronchiectasis. This is clearly important clinically with the potential use of LCI in identification of patients who require escalation of management. No longitudinal LCI data was found in this review describing its role in the long-term disease monitoring. Furthermore, no data exists describing the minimal clinically important difference for LCI. In children, the data was very limited but results showed the potential for LCI to be used as a measure of disease severity due to the correlation between disease on CT imaging and LCI. LCI appears to be repeatable in adults with intraclass correlation coefficients similar to the ranges described in cystic fibrosis (75).

Treatment of chronic infection and acute infective exacerbations are key in the management of bronchiectasis. O'Neill et al (169), show a link between some CT features and the metrics of bacterial composition; LCI, however, did not show a relationship with any. A relationship between raised LCI and groups of differing frequency of *Pseudomonas aeruginosa* infection was noted. These findings are similar to patients with cystic fibrosis with a raised LCI in those with active pseudomonas infection (56). *Pseudomonas aeruginosa* infection is more common in those with more severe lung disease and is associated with poorer clinical outcomes

including worse lung function (183,184). Whether *Pseudomonas aeruginosa* is associated with worsening lung function or is a marker of severity only, is not clear.

LCI has been shown in this review to have a clear relationship with CT findings, which emphasises its role in disease monitoring. This relationship was explored by Verbanck et al who examined a group of patients with bronchiectasis and described LCI in relation to the number of lung segments affected by bronchiectasis (175). The lobar distribution of bronchiectasis varies with aetiology and it is not known if patients with disease of different severity and different distribution can be compared, limiting the utility of LCI in this group (e.g. more mild but widespread disease compared to severe, focal changes). Further work is required to identify the variation of LCI in patients with bronchiectasis of different aetiologies. This will be important to support the use of LCI as an outcome measure for clinical trials by ensuring patient selection is appropriate.

There is a need for further research on the role of LCI in bronchiectasis, particularly in children. The widespread use of LCI in cystic fibrosis research, due to its demonstrated value in identifying early disease and correlation with CT changes, has yet to be replicated in bronchiectasis. Specifically, longitudinal data is required to demonstrate variability in LCI over time and relate this to the severity of disease. Availability of portable devices is encouraging for point of care testing. A minimal clinically important difference will need to be clarified to make the index a practically useful tool.

2.11 Conclusions:

In adults, LCI was a sensitive measure of disease severity and correlated with clinical assessment tools. Contrary to cystic fibrosis, the review did not identify good quality studies defining the role of LCI in children with non-CF, non-PCD bronchiectasis. With the lack of measures of disease in this condition, LCI can be explored as a potential metric of disease severity and research in this area should be a priority.

Chapter 3: Methods

3.1 Ethical approval:

The study was conducted from October 2020 to September 2021 at Birmingham Children's Hospital, UK and was approved by the Health Research Authority, Yorkshire and the Humber – Leeds West Research Ethics Committee (Reference 20/YH/0028).

3.2 Participants:

3.2.1 Non cystic fibrosis, non primary ciliary dyskinesia bronchiectasis:

Children with bronchiectasis were recruited from specialist bronchiectasis and general respiratory clinics at Birmingham Children's Hospital, Birmingham Children's and Women's NHS Trust. Subjects were identified after clinical coding and patient database review. The hospital coding database was interrogated for patients aged between 6 years and 12 years who were coded with the International Classification of Disease (ICD) 10 coded clinical entry descriptions:

- Cough (R05)
- Bronchiectasis (J47)
- Asthma (J45)
- Unspecified chronic bronchitis (J42)
- Respiratory Infections (J98.7)

- Unspecified acute lower respiratory tract infection (J22)

All patients were then cross referenced against a list of all Computed Tomography (CT) images of the chest performed within the hospital. Patients appearing on both lists were reviewed for appropriate inclusion into the trial. In addition to a coding search, clinic lists were reviewed for other patients who would be suitable using the inclusion and exclusion criteria described below (see Table 3.1).

Inclusion criteria	**Exclusion criteria**
Diagnosis of bronchiectasis on HRCT image (reported by Consultant Radiologist)	Diagnosis of: - neuromuscular disease - neurological disability - congenital structural airway / gut / thoracic malformation - cystic fibrosis - primary ciliary dyskinesia Current exacerbation of bronchiectasis

Table 3.1: Inclusion and exclusion criteria

The exclusion of patients with neuromuscular disease removes patients who likely have pathology that would affect the typical respiratory pattern of the patient; the exclusion of those with significant neurological disability is due to the participants need to be able to follow the

instructions necessary to complete the LCI measurement, the exclusion of participants with structural airway/gut/thoracic malformations remove the potential influence of anatomical malformations affecting the ventilation homogeneity in addition to any underlying bronchiectasis. Finally, patients were excluded if they had an exacerbation of bronchiectasis (diagnosed as an increase in symptoms requiring antibiotics or an increase in their physiotherapy) within 4 weeks. The intention of not assessing participants at this time point is to remove variability introduced by excessive airway secretions or inflammation that might affect the LCI.

CT imaging was accepted if within two years of the date of study assessment. While this may bring a delay between CT imaging and lung function assessment, a significant change in the structural lung disease noted on image is unlikely. Furthermore, the decision not to perform a repeat HRCT for children due to study participation was a decision taken on safety to avoid exposure to radiation. Much of the data examining HRCT progression over time is from participants with cystic fibrosis. In a group of children and adults with cystic fibrosis, repeat CT imaging was compared to earlier images showing a statistically significant, but small, increase in CT scores generated by the Brody scoring system (178) (1.55% per year noted on the composite score and 1.52% per year on the score for peripheral bronchiectasis only) (185). This change is similar to that reported elsewhere when looking exclusively at CT imaging for children with CF (186,187). Here, the mean change in participants was 1.09% per year to 2.01% per year, again using the Brody scoring system. The mean interval between the images was 30 months. The underlying pathology in these children is different from those being examined in this study, but there is a paucity of data examining the progression of CT changes

in non-CF, non-PCD bronchiectasis. In these children with CF, the underlying pathological process is continual, although these children are typical managed carefully with close monitoring and therapies to minimise the development of lung disease. Although care must be taken in extrapolating from one lung disease to another, it would be reasonable to judge that the pathological processes responsible for the lung disease in non-CF, non-PCD bronchiectasis participants is not significantly worse than in CF. Previous comparisons of lung clearance index between groups of adults with bronchiectasis of non-CF and CF causes have shown the reverse (higher LCI for those with CF) (170).

It is possible that some of the CT changes may have improved. The most common previous general consensus was that bronchiectasis is irreversible (27) but current data suggests this may not be the case (17). While improvement might be possible for some patients with adequate treatment and care, this has been demonstrated in children over longer time intervals than the two year period between imaging and LCI planned for this study (13).

3.2.2 Children without lung disease:

The cohort of healthy children were recruited from a range of sources (although these varied through the study due to the introduction of COVID-19 infection and control policies):

- Outpatient:
    - Orthopaedic general clinic
    - Orthopaedic fracture clinic
    - Ear, Nose and Throat clinic

    - General Medicine clinic
    - Ophthalmology clinic
- Inpatient:
    - Waiting area for day case surgery
    - Emergency Department observation area

Participants were identified aged between 6 years and 12 years of age with an absence of conditions or symptoms that would suggest underlying pathophysiological processes that might affect Lung Clearance Index.

Inclusion Criteria:

- No previous hospital admission for a respiratory condition
- No physician diagnosis of asthma at any time
- No history of chronic cough (defined as greater than 6 weeks in length), during the last one year
- No history of recurrent wheeze (defined as greater than two episodes of wheeze during the previous one year)

Exclusion Criteria:

- Born at a gestation of less than 37 weeks
- Born following intrauterine growth restriction
- A previous medical history that includes congenital heart disease

The process for recruitment was a time consuming procedure that included the contact of patients and families to highlight the research that was being conducted and asking their interest in hearing more about it, delivering both written and verbal information about the study and the necessary involvement of participants, allowing adequate time for those potential participants to consider the information, and then ultimately, arranging the logistics for a research visit to the hospital.

In the later stages of the study, free from logistical restrictions of the COVID-19 pandemic, potential participants were identified via social contacts, or family/relations of staff and patients. We were able to conduct many of the necessary steps, including distribution of study literature, prior to the arranging of the study visit. However, a majority of the study took place during a period of time where the hospital was under a level of restriction for staff, patients and visitors.

During this time, attendance at the hospital for a member of the public was restricted to those who needed to attend for a clinical contact (i.e. an assessment by staff, a scan, or an appointment). Additional family members or repeat visits that were not clinically necessary were not permitted. Therefore, my approach of potential participants was restricted to hospital patients.

To overcome this, I identified scheduled outpatient clinics where there were likely to be patients who would not meet the exclusion criteria detailed above (such as orthopaedic, ophthalmology, ENT and emergency department visits). People attending these clinics would have phone calls from the hospital in the days preceding the appointment to ensure they were attending, and to remind them of the various infection control restrictions in place at the time.

I worked with the outpatient logistics team to arrange an additional message passed to certain patients (often parents/guardians); that being, 'that there were research studies being conducted at the time of their upcoming visit' and asking 'if they would be interested in hearing more about potential studies'. I restricted this message being passed only to the families of patients of the correct ages. This allowed me to contact participants in advance and arrange a study visit either before or after their clinical visit to hospital.

On occasions where I was unable to arrange a participant in advance, I would use advertisement within the hospital waiting rooms as a method of highlighting the research study. Outpatient nurses would inform families of potential studies, and then I would approach to offer involvement as above. I would always consider the impact of the patient's clinical journey in hospital prior to recruitment, and indeed, for a number of potential participants, the logistics of their hospital visit would prevent their involvement in the study (i.e. needing to attend a scan at a particular time). Meeting potential participants prior to their clinical appointments would give them sufficient time through the rest of their hospital journey to consider their involvement in detail prior to consenting. The non-invasive nature of the study meant that prolonged consideration or copious study literature was not required.

3.3 Subject Assessments:

All participants had height and weight measured using hospital equipment. The parents/guardians accompanying the subject completed a brief questionnaire providing further

details on the ethnicity, the household location and the presence of household smoking (see Appendix 1)

3.3.1 Ethnicity data collection

The parent/guardian of the subject was presented with the United Kingdom Office for National Statistics ethnicity groupings used for the UK census (188). They were asked to select the option that best applied to the subject, with the further option to enter a free text entry if they felt it was necessary (189).

3.3.2 Multiple Breath Washout

What follows is a description of the procedure developed in the acquisition of multiple breath washout data. This was based on existing guidance on multiple breath washout in general and using the ndd EasyOne Pro LAB, with then adjustment following personal experience in the use of the device.

3.3.2.1 Device set up

ndd's promotional material for the EasyOne Pro LAB describes how *"all EasyOne products are designed to require no calibration and minimal maintenance. The device is designed to remain in calibration throughout its lifetime. No calibration procedures are required prior to testing"* (104). This feature of the device meant there was minimal test specific preparation

required and manufacturer instructions were followed in the equipment set up, cleaning and use of consumables. In addition to the manufacturer recommended steps, I added a tablet device via a mobile arm to the EasyOne Pro LAB that could be manipulated to be seen by the subject during use. This would play a preferred video during the time of the washout. Audio of the video was delivered through over ear, noise cancelling headphones. These steps became necessary to support the participants tidal breathing pattern. During multiple breath washout, using the EasyOne Pro LAB, the valve delivery system supplies 100% oxygen on demand with inspiration. This gives an easily audible noise of moving gas which I found provided positive feedback to the subject, encouraging a higher than usual respiratory rate and smaller than expected tidal volume. The thorough use of distraction techniques reduced this and I developed this process over the period of the study.

3.3.2.2 Multiple Breath Washout Acquisition:

Manufacturer advice was followed in the use of the device to acquire multiple breath washout data. Participants were instructed prior to use of the preferred technique and coached between washouts to improve technique. Washouts were abandoned immediately should it be apparent that the quality was not sufficiently acceptable. Washout attempts were continued until the required number of acceptable washouts were performed, or the subject was unable to perform further tests. Following a washout attempt, the subject was required to rest, breathing room air, in order to wash back in the previously washed out nitrogen.

Each nitrogen washout, was performed using the EasyOne Pro, (ndd Medical Technologies, Zurich, Switzerland). 100% wall oxygen was supplied as the washout gas. Each washout was performed in the seated position, with participants distracted via a video with headphones. LCI was performed before spirometry in all cases. LCI was calculated from a mean of three acceptable washouts, two washouts were accepted if the patient was unable to perform three. Repeated coaching, encouragement, distraction, and visual cues were employed to create a better, more stable, tidal breathing pattern.

3.3.2.3 Quality Control:

Quality was assessed in a standard manner following previous guidance (52,190) and broken down in to three stages:

- Individual washout pre-phase breathing pattern
- Individual washout breathing pattern
- Overall multiple breath washout test quality assessment

3.3.2.3.1 Pre-phase breathing pattern assessment:

1. Tidal volume (Vt): Assess the Vt and judge if appropriate for the subject size (8-15ml/kg of ideal body weight). Ensure the Vt stable for 5 breaths prior to the start of the washout. Ensure the last breath of the pre-phase is not irregular.
2. End expiratory lung volume (EELV): Ensure the EELV is stable by examining the volume/time trace stable.

3. Time between trials: Ensure the starting end-tidal concentration of nitrogen of the first trial is more than 77%, and all subsequent washout end-tidal starting concentrations of nitrogen are within 1.5% of the baseline.

3.3.2.3.2 Washout breathing pattern assessment:

1. Tidal volume (Vt): Ensure the Vt is appropriate for the subject size (8-15ml/kg of ideal body weight), ensure the first breath of the washout is regular, ensure the Vt is stable over the washout. Identify episodes of panting (short rapid breaths approximately half mean Vt) or sighing (large breath approximately 1.5 time mean Vt) judging for a premature test end with panting, and trapped gas released with a sigh.
2. End expiratory lung volume: Ensure the EELV is stable, identify evidence of breath stacking or incomplete exhalation.
3. Hyper/hypoventilation: Ensure end tidal CO_2 ($etCO_2$) between 4% and 6% through the washout, ensure the $etCO_2$ is not continuously increasing or decreasing across the washout.
4. Leak: identify evidence of a leak

3.3.2.3.3 Overall multiple breath washout test quality assessment:

All washout trials judged to be acceptable were then assessed for quality aiming for key features to indicate a high-quality test occasion:

1. Tidal breathing throughout all trials

2. No testing events, irregular breaths, or evidence of any leaks
3. 3 acceptable trials

Using the guidance above, test occasions were either accepted or rejected. A valid LCI reading was achieved with at least two acceptable trials, with no test events (such as leaks of other previously described issues), and a reasonably stable tidal breathing pattern.

3.3.3 Spirometry

Spirometry was performed following Lung Clearance Index using the ndd EasyOne Pro LAB with patient incentive screen. The measurement was performed as per manufacturer instruction with three good quality measures taken. The best of the three measurements was used in accordance with ERS/ATS guidelines.

3.3.4 High Resolution Computed Tomography (HRCT)

HRCT was reviewed for each patient with bronchiectasis with the imaging being obtained from our hospital, or the hospital who originally performed the scan. This was reviewed by two senior radiologists with experience in thoracic imaging in children. Diagnosis of bronchiectasis was confirmed and images were formally scored as guided by the Brody score for HRCT (178). To summarise this approach, an overall score is obtained within which are specific scores for bronchiectasis, mucous plugging, peribronchial thickening, and parenchymal disease. This score includes Hyperinflation and air trapping but was not assessed in this study as many of the participants did not have expiratory images as part of their HRCT as was local practice.

score for HRCT.

The two radiologists had significant experience in thoracic radiology and routinely examined chest HRCT as part of their clinical work. A specific scoring system for non-CF bronchiectasis does not exist (although there is work ongoing (181), and so an additional scoring method needed to be used. The radiologists were familiar with the Brody scoring system which was chosen predominately due to its previous use within research in bronchiectasis (171). The Brody system has been validated in the assessment of changes on HRCT in Cystic Fibrosis, and while this is a different disease pathology to that being examined in this study, there are very similar pathological airway changes that exist in Cystic Fibrosis and non-CF bronchiectasis seen on imaging. Finally, the Brody scoring system provides detail on the main pathologies seen on imaging (bronchiectasis, mucus plugging, peri-bronchial thickening and parenchymal changes), within all lobes which is more granular 0 to 243 (with alternative scores such as Bhalla and Reiff scores scoring from 3-25 and 0-18 respectively).

I developed an Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheet that included all scoring components for all lobes. The radiologists would simply enter their clinical interpretation (for example, the extent of bronchiectasis in the central lung in the right upper lobe, with options of none, 1/3 of the lobe, 1/3 to 2/3 of the lobe, or more than 2/3 of the lobe). The spreadsheet would allocate the appropriate score and ask the next question. For each HRCT, the spreadsheet would then give a subtotal and total for each anatomical area and grand total for the patient. Scoring was conducted on several non-study scans as a ‘practice’ with subsequent comparison of scores to ensure clinical interpretation was consistent. Scoring of the study patients was analysed with a statistical test as described in the results section to ensure

further agreement. HRCT scores used in the analysis of correlation with lung function measures were generated from an average of the two radiologists scores. The spreadsheet used to help generate the scores can be seen in the appendix (see section 8.2).

3.4 Statistical Approach

All statistical analyses were performed using GraphPad Prism® version 5.00 for Windows (GraphPad Software, San Diego California, USA). Parametric data was assessed using unpaired t-test to assess group difference, one way ANOVA was used to assess multiple group differences. Non-parametric data was assessed using Mann-Whitney test to assess group differences, Spearman's rank correlation coefficient. Fishers exact test was used to compare categorical data. Univariant linear regression was performed to assess lung clearance index and association with height, weight and age. Repeatability of measurements of lung clearance index was assessed using coefficient of variation. Receiver operating characteristic (ROC) curve was used to evaluating the performance of lung clearance index as a diagnostic test. A p value of less than 0.05 was considered significant.

Recruitment of participants was opportunistic from presentation to outpatient clinics and through database and coding reviews as above. Limited data is available for children with bronchiectasis and in healthy children performing lung clearance index limiting formal power calculation. Previous studies have used samples of 30 subjects per group in order to detect a

10% variation in lung function due to ethnic origin with at least 80% power at the 5% significance level (148).

Chapter 4: Lung clearance index in healthy children from a range of ethnic backgrounds

4.1 Introduction:

This chapter includes an assessment of the feasibility of lung clearance index, normative data and the challenge of defining ethnically specific normative data.

Lung clearance index has been shown to be of value in the assessment of lung disease in children with cystic fibrosis and other diseases (52). LCI has been widely used in cystic fibrosis (CF, where it is a sensitive marker of early lung disease) (55,69), primary ciliary dyskinesia (PCD) (86,87), and there is developing evidence in non-CF, non-PCD bronchiectasis. It has been shown to be feasible in a range of different settings and guidance has been produced on the necessary operator training, experience and support required.

To enable LCI to be used as a robust tool in research and clinical practice, specific protocols, operator training and certifications have been developed. There is a potential for MBW to be used for non-invasive monitoring of chronic diseases like bronchiectasis, asthma and interstitial lung disease. (70,75,190,191).

Several commercial devices for measuring LCI have been developed over the previous 10 years. There are important differences between these devices and the results have been shown to not be interchangeable between the devices, as explained in section 1.5.1 (“Medical gas and gas analyser choices”).

4.1.1 Device specific normative data:

Lung clearance index (LCI) is a measure of uneven ventilation measured by multiple breath washout (MBW) of an inert tracer gas from the lungs. The use of LCI as a clinical tool has been aided by the availability of several commercial systems that are designed to be easy to use and relatively cheap to operate. However, there are important differences between available devices including the method of data acquisition and the tracer gas used. The results from these devices are not interchangeable. Normative data is not available for all devices and all inert gas setups.

To interpret the results for a particular commercial device with a particular tracer gas, there must be a good understanding of the range of normal that exists for the population. This work examines the EasyOne Pro LAB with a nitrogen gas washout setup. Research has previously examined the range in groups of children up to early adulthood (Figure 4.1). This has demonstrated a suggested range of normal but the groups of children have been small (less than 45 in total), and there is no detail as to the characteristics of these children. The range of mean LCI for these cohorts of healthy children is from 6.78 to 7.40 lung clearance index units with age range from 5 years to 17 years. This compares to a range of mean LCI for healthy children using the Exhalyzer with nitrogen washout of 6.1 to 7.05 lung clearance index units with children of a similar age (113,154)

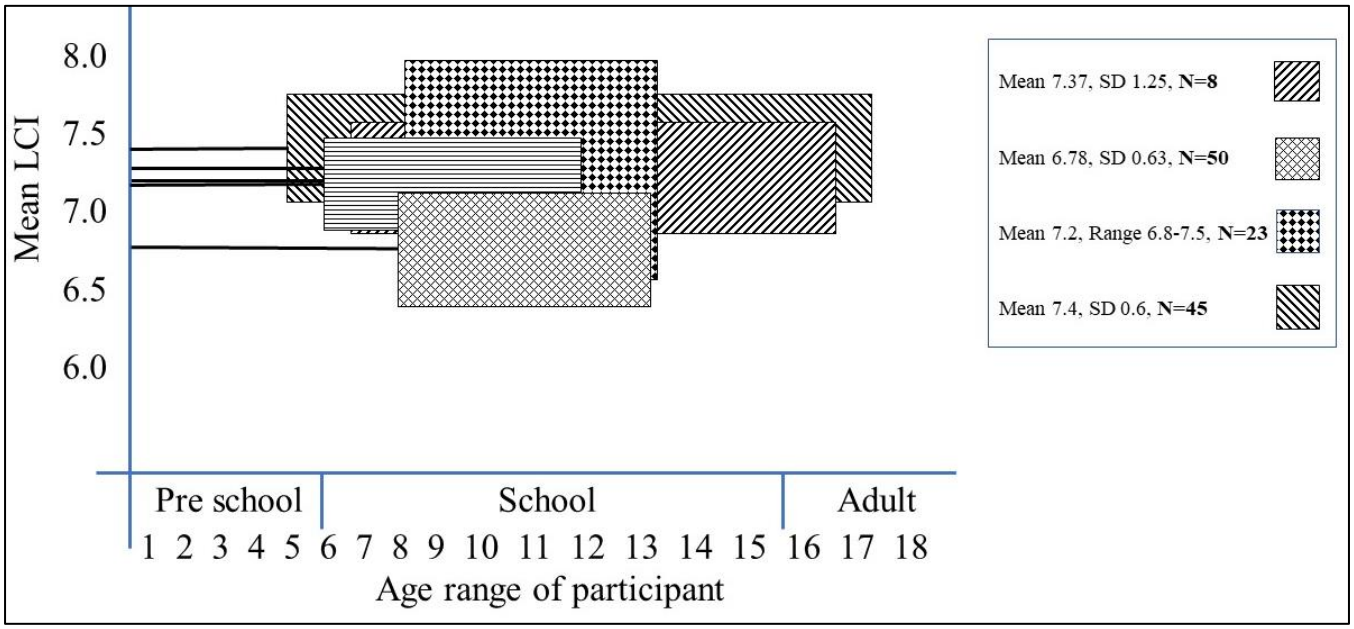

Figure 4.1: ndd EasyOne Pro LAB nitrogen washout assessment of lung clearance index y axis: cohort mean LCI; x axis: cohort age range. Height of box represents 1+/- standard deviation (note- SD for Zwiterloot et al not available, range shown), width of box represents age range of cohort.

Oestreich et al, A non-systematic signal-correction error in a commercial multiple-breath washout device significantly impacts outcomes in children and adults, medRxiv, 2022 (192)

Poncin et al, Agreement between multiple-breath nitrogen washout systems in children and adults, Journal of Cystic Fibrosis, 2017 (113)

Zwitserloot et al, Differences in lung clearance index and functional residual capacity between two commercial multiple-breath nitrogen washout devices in healthy children and adults, ERJ Open Research, 2020 (115)

Isaac et al, Validation of the NDD easyone pro lab multiple breath nitrogen washout system in children, Pediatric Pulmonology, 2018 (193)

4.1.2 Ethnicity specific normative data:

Population specific normality data is crucial for wider application of LCI measurement in routine clinical practice. As previously discussed, ethnicity has not been well defined in research and there have been differences described in measures of lung function between populations of varying ethnicities (144,145).

Sonnappa et al have reported normative LCI values from 'White' and 'South Asian' backgrounds (148). These data (of 37 and 31 children respectively) showed no significant difference between the groups but it has not been repeated in other data sets or in other groups of ethnicities (118). The description of each group (White and South Asian) does not provide more detail as to the ethnicity of the participants which has been highlighted as an important factor in the assessment of normative data in lung function (144).

Differences have been described in the lung function test results in children from different ethnic backgrounds. In spirometry assessment, clinical interpretation of spirometry results commonly use ethnic-specific values, developed by the Global Lung Function Initiative (GLI) but important questions have been raised about the use of 'race correction' software.

LCI is a measure that is internally adjusted for the size of an individual's resting lung capacity (the total volume of gas required for washout is divided by the functional residual capacity) which theoretically will remove the influence of an individual's anatomical differences that may affect lung volumes (118,147,148).

Lung clearance index has previously been demonstrated to be influenced by changes in age (infancy and old age) whilst reportedly remaining stable through childhood and early

adulthood. This is discussed in detail with the section on clinically relevant factors affecting lung clearance index, starting on page 47.

4.1.3 Feasibility and practically measuring Lung Clearance Index:

Any test that is consider for use in the clinical setting should be easy to use, practical, repeatable, and realistically achievable by the population intended for investigation.

The technological advances in commercial MBW devices have resulted in the availability of portable machines using standard medical oxygen and air supply which can be used in an office setting. Two devices using nitrogen as the tracer gas are commonly used (Exhalyzer D (Eco Medics, Duernten, Switzerland), the EasyOne Pro LAB (ndd Medical Technologies, Zurich, Switzerland).

The successful measurement of LCI using the Exhalyzer D is dependent on the age and the prior experience of the participants and the professionals performing the test (194). Various studies have reported success rates ranging from 24 - 100% (75,195). The time and the number of attempts required to obtain a valid LCI measurement in children has not been described in detail for the Exhalyzer nor the EasyOne Pro LAB.

4.1.4 Summary:

This chapter will describe the normative data for lung clearance index in healthy children of school age from a range of ethnic backgrounds using nitrogen washout and the ndd EasyOne

Pro LAB device. We assessed the feasibility, success rate, number of attempts and time taken to obtain acceptable LCI results in these healthy primary school children.

4.2 Hypothesis:

- There are no differences in Lung Clearance Index in healthy children from differing ethnic backgrounds

4.2.1 Aims:

1. To establish LCI normative data for children age 6 to 12 years of age using nitrogen washout on the ndd EasyOne Pro LAB
2. To determine differences in LCI between healthy children from different ethnicities
3. To establish the feasibility and practicality of the use of the ndd EasyOne Pro LAB in obtaining LCI in children naïve to its use

4.3 Methods:

Children were recruited into the study from a range of sources as previously described on page 99. They completed written assent where able with parents/guardians providing written consent. Ethical approval had been obtained from the Health Research Authority (Yorkshire and the Humber-Leeds West Research Ethics Committee, reference 20/YH/0028). Demographic data was measured including height, weight, and sex; participants (or their parent/guardian) self-reported their ethnicity. To assess other potential influences upon lung

function, the home environment was recorded with questions identifying location via the first three figures of the postcode, documenting the presence of household adult smoking and identifying socio-economic status using the index of multiple deprivation. Their health status was assessed with a short questionnaire identifying previous medical history against the pre-determined exclusion criteria:

- No previous hospital admission for a respiratory condition
- No physician diagnosis of asthma at any time
- No history of chronic cough (defined as greater than 6 weeks in length), during the last one year
- No history of recurrent wheeze (defined as greater than two episodes of wheeze during the previous one year)
- Born at a gestation of less than 37 weeks
- Born following intrauterine growth restriction
- A previous medical history that includes congenital heart disease

4.3.1 Assessment of Lung Clearance Index:

MBW was performed on a single test occasion using the EasyOne Pro LAB and LCI analysed using the inbuilt software. Detailed instruction was given on the technique with explanation and demonstration as required. 100% oxygen medical gas supply was used. Distraction was provided via a tablet playing an age suitable video audible through over-ear, noise-cancelling headphones. LCI was calculated from a mean of three acceptable washouts, two washouts

were used if the patient was unable to perform three. Participants were coached in between washout trials to improve quality. Participants were encouraged to perform repeated washouts until the adequate number of trials of acceptable quality had been reached.

The LCI test session was stopped upon successful completion of the test, upon request of the subject/family, or when, in the judgement of the operator, no result was likely to be achieved from further washout attempts. Each LCI washout was performed according to manufacturer instruction with these standards adhering to previously published protocols on the acquisition of multiple breath nitrogen washout (52,190). Following multiple breath washout, each subject was instructed in and performed spirometry using the EasyOne Pro LAB according current standards (130).

Further details on the measurement of LCI is described in Chapter 3, page 103

4.3.2 Sample size and power calculation:

Recruitment of participants was opportunistic as previously described. Recruitment was challenged due to a curtailed time frame and limitations on participant movement due to the COVID-19 pandemic.

There is limited data which can be used to support formal power calculations. Previous studies have used sample sizes of 30-34 to demonstrate differences between groups (external to birth weight, sex, height and maternal smoking) (115,148). This aims to achieve 80% power with a two sided significance level of 0.05 to detect a difference of 10% in LCI.

4.3.3 Reporting of ethnicity in research:

The study recorded self-reported ethnicity of participants using the United Kingdom Census list of ethnic groups (188). Research on lung function in the general population has not reported race/ethnicity regularly or reliably and it is not clear if race/ethnicity is even the correct category of data to record (144,196). Self-reporting of race/ethnicity is recommended by the ATS/ERS and was the deciding factor on how ethnicity data was collected for this data set. However, this method is not ideal as it can be influenced by the categories offered to respondents and subject reporting can change with time and location (144).

The ideal method of recording such important information is not clear and there are important issues within lung function research including the rationale for categorisation of race/ethnicity, recording the impact of socioeconomic disparity and the recording the impact of systemic barriers in healthcare, that are equally, if not more important in having clinically meaningful results (144,197,198).

4.4 Results

4.4.1 Clinical characteristics of children participating:

Seventy-two participants were recruited who all attempted to perform lung clearance index. The mean age was 9.4 (1.4) years and 37 were female (51%) (Table 4.1). Recruitment is shown in the consort diagram below (see Figure 1.1. Participants screened were excluded primarily due to participant unwillingness to complete the study tests that were described to

them as part of screen. (Several reasons were given for this including the test sounding worrying, implications of COVID, but mostly, there were logistical concerns with the time required to perform the study visit).

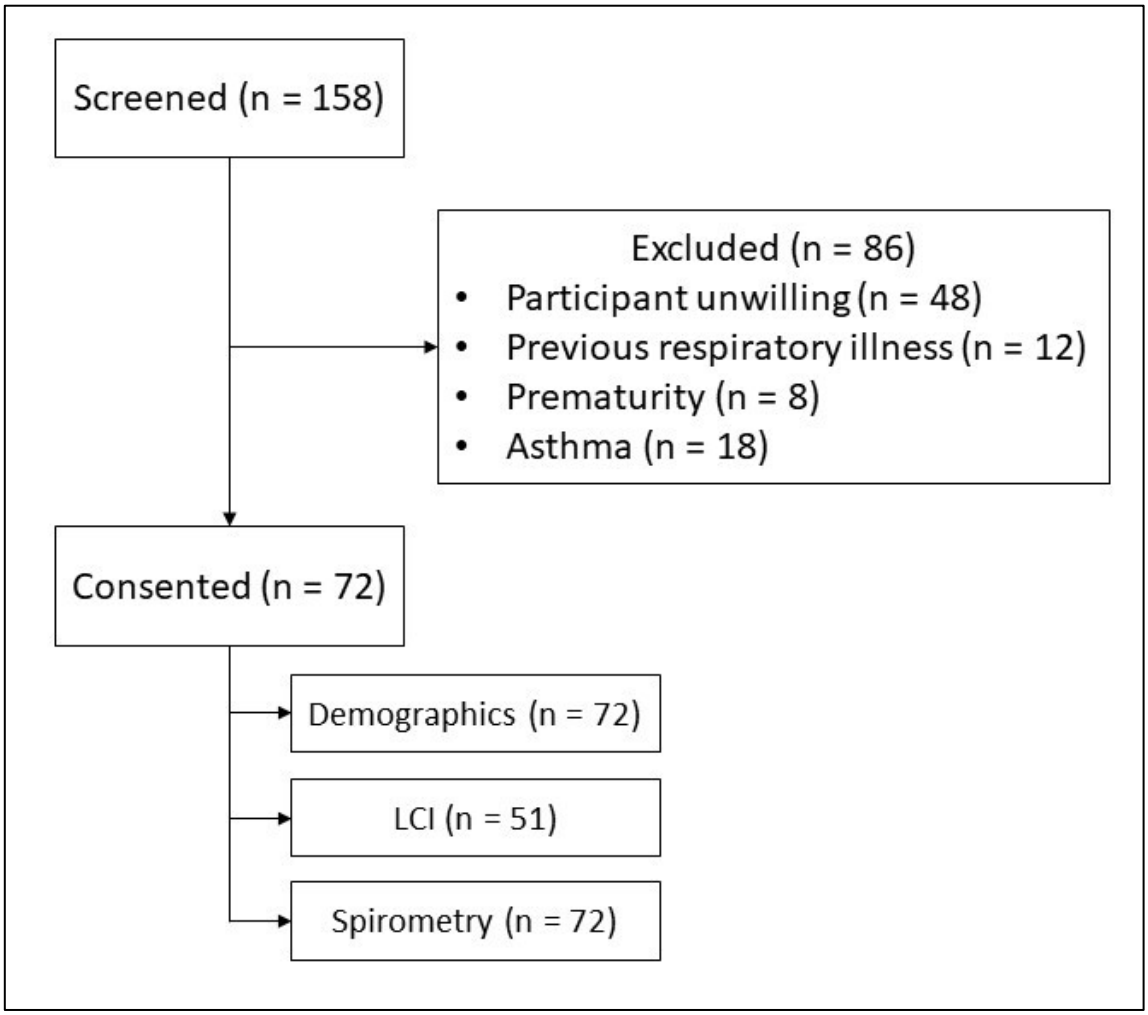

Figure 4.2: CONSORT diagram of participant screening, consent and study tests

	Healthy Children (n=72)
Age, years (mean, SD, range)	9.4, 1.5, 6.0-11.8
Female	37, 51%
Height, cm (mean, SD)	138.0, 10.4
Height, centile (mean, SD)	62.0, 25.6
Weight, kg (mean, SD)	36.1, 13.0
Weight, centile (mean, SD)	62.0, 28.1
Ethnicity	
White	35, 48.6%
Mixed/Multiple ethnic groups	5, 6.9%
Asian / Asian British	27, 37.5%
Black / African / Caribbean / Black British	2, 2.8%
Other ethnic group	3, 4.1%

Table 4.1: Demographic details of healthy participants

Number and percentage unless otherwise stated. SD: standard deviation, FEV_1: forced expiratory volume in one second, FVC: forced vital capacity

All subjects were able to perform spirometry; FEV_1 mean 98.2% (1.82L), range 79% - 127% (0.98L – 2.84L), FVC mean 98.4%(2.08L), range 72% - 127% (1.07L – 3.09L). The largest group were White British children (34 participants, 47.2%, all participants identifying as White English, Irish, Scottish, Welsh or British), followed by those from Indian Subcontinent (26 participants, 36.1%, all identifying as Indian, Pakistani, or Bangladeshi). 2 patients (2.8%)

were of Black African ethnicity, 5 (7.0%) were of mixed or multiple ethnic groups and 3 (4.1%) were of Arab ethnicity. The ethnic groups 'White British' and 'Indian Subcontinent' were used as geographically appropriate combinations to be able form groups for comparison and have been used for ethnic comparisons in previous research (199–201). Many of the participants lived in an urban major conurbation (79.2%) and 12.5% lived in a household with an adult who smoked.

Healthy Children (n=72)	
White	
English/Welsh/Scottish/Northern Irish/British	34 (47.2%)
Irish	0 (0%)
Gypsy or Irish Traveller	0 (0%)
Any other white background	1 (1.4%)
Black/African/Caribbean/Black British	
African	2 (2.8%)
Caribbean	0 (0%)
Any other Black/African/Caribbean background	0 (0%)
Mixed/Multiple ethnic groups	
White and Black Caribbean	2 (2.8%)
White and Black African	1 (1.4%)
White and Asian	2 (2.8%)
Any other mixed/multiple ethnic background	0 (0%)
Asian/Asian British	
Indian	22 (30.6%)
Pakistani	3 (4.1%)
Bangladeshi	1 (1.4%)
Chinese	1 (1.4%)
Any other Asian background	0 (0%)
Other ethnic group	
Arab	3 (4.1%)
Any other ethnic group	0 (0%)

Table 4.2: Complete ethnic group data for all participants

4.4.2 Lung Clearance Index:

All participants successfully completed spirometry. 51 (70.8%) performed at least two acceptable washouts and therefore produced a valid LCI result. The mean LCI for this healthy

population was 7.19 (0.6 SD), in keeping with previous nitrogen washout performed using ndd EasyOne Pro LAB (Figure 4.1 and Table 4.3).

	This thesis data	Oestreich et al (192)	Poncin et al (113)	Zwitserloot et al (115)	Isaac et al (193)
Mean LCI	7.19	7.37	6.78	7.2	7.4
Standard Deviation	0.6	1.25	0.63	Not published	0.6
LCI Range	6.13 – 8.45	Not published	Not published	6.8-7.5	Not published
Age	6.0 – 11.8 (range)	8.6 – 13.9 (range)	8.5 – 13.9 (IQR)	6.1 - 17.1 (range)	5 – 17.8 (range)
Participant Number	51	8	50	23	45

Table 4.3: Normative data for LCI using ndd EasyOne Pro LAB

This data contributes the greatest number of participants in a tight age group with the mean LCI extremely close to the aggregate mean of the highlighted studies above. This comparison is approximate and does not allow confirmation of the normative range due to the differences in age range and small total numbers. It is however supportive of this data

	Healthy children (n=72)			
		(White British) n=34	*(Indian Subcont.) n=26*	*P value*
Age, years (mean, SD)	9.4, 1.46	*9.60 (1.57)*	*9.28 (1.40)*	*0.41**
Male	35, 48.6%	*19, 55.9%*	*12, 46.2%*	*0.60#*
Height, cm (mean, SD)	138.0, 10.4	*138.40 (10.9)*	*138.44 (11.26)*	*0.97*
Weight, kg (mean, SD)	36.1, 13.0	*36.16 (14.5)*	*35.26 (10.96)*	*0.92*
Household smoking	9, 12.5%	*7 (20.5%)*	*0 (0%)*	***0.02#***
Index of Multiple Deprivation	15288 (10653)	*14982 (9415)*	*15727 (11084)*	*0.48*
Ethnicity				
White	35, 48.6%			
Mixed/Multiple ethnic groups	5, 6.9%			
Asian / Asian British	27, 37.5%			
Black / African / Caribbean / Black British	2, 2.8%			
Other ethnic group	3, 4.1%			
Rural/Urban Location				
A1: Urban major conurbation	57 (79.17%)	*23 (67.6%)*	*24 (92.3%)*	***0.03#***
C1: Urban (City and Town)	8 (11.11%)	*5 (14.8%)*	*1 (3.85%)*	*1.00#*
D1: Rural (Town and fringe)	3 (4.17%)	*3 (8.8%)*	*0 (0%)*	*0.25#*
E1: Rural Village	2 (2.78%)	*2 (5.9%)*	*0 (0%)*	*0.50#*
F1: Rural hamlet and isolated dwelling	1 (1.39%)	*1 (2.9%)*	*0 (0%)*	*1.0#*
Unknown	1 (1.39%)	*0 (0%)*	*1 (3.85%)*	*0.43#*
Spirometry				
FEV_1% (mean, SD)	98.2%, 10.6	*98.4 (10.3)*	*98.77 (11.84)*	*0.99*
FVC% (mean, SD)	98.4%, 11.5	*98.6 (12.3)*	*98.73 (11.65)*	*0.99*
LCI (mean, SD)	7.19, 0.6	*7.04 (0.64)*	*7.20 (0.55)*	*0.33*

Table 4.4: Demographic data for healthy participants.
Mean (SD) and Mann-Whitney test unless otherwise specified. FEV_1: forced expiratory volume in one second, FVC: forced vital capacity. FEV_1 and FVC reported as percent predicted, GLI reference data [13]. *Unpaired t-test, #Fischers exact test

4.4.3 Feasibility and practicality of lung clearance index:

Six participants (8.3%) were able to perform only one acceptable washout, and 15 participants (20.9%) were not able to perform any. Those who were unable to complete any successful washouts had a mean of 6.6 attempts (range 2 to 9), with a mean age of 9 (1.4) years (Table 4.5).

Acceptable washouts	0	1	2	3	P Value
Number (%)	15 (20.8%)	6 (8.3%)	29 (40.3%)	22 (30.6%)	
Age (mean, SD)	9.1 (1.4)	9.1 (1.4)	9.1 (1.5)	10.1 (1.3)	p=0.052
Attempts (mean, range)	6.6 (2-9)	6.7 (6-8)	7.7 (5-10)	6.6 (4-9)	p=0.8
Number of washout attempts before 1st success (mean, range) (* test abandoned)	N/A*	3.8 (2-6)	3.5 (1-8)	2.9 (1-7)	p=0.2

Table 4.5: Demographic and washout information grouped by number of acceptable washouts.
Groups analysed by Kruskal-Wallis test.

4.4.3.1 Successful vs. unsuccessful participants:

When comparing participants who were successful (two or more acceptable washouts) vs. those who were unsuccessful (none or one acceptable washouts), there was no difference in their age (p=0.44), their lung function assessed by spirometry (FEV_1 and FVC, p=0.17 and p=0.22

respectively), the length of time taken to complete the test (minutes) (p=0.36), the total number of trials completed (p=0.53), the participants height (p=0.87) or weight (p=0.47), nor the Index of Multiple Deprivation (p=0.09). Group difference assessed by unpaired t test.

It was noted that success of achieving an LCI result increased over the course of the study. We divided the study cohort in to two equal, 36 subject groups (early and late) in the chronological order of subject recruitment; there was a significant difference in the success rates between the groups (19 of 36, 52.7% vs. 32 of 36, 88.8% respectively; p=0.001). There was no significant difference between these groups of participants' age, spirometry (FEV_1 and FVC), the number of trials performed or LCI, but there was a difference between the early and late groups in relation to the time taken to complete the test (27.1 min vs. 31.1 min respectively, p=0.02) and the proportion of White participants (66.7% vs. 27.8% respectively, p=0.0019).

4.4.3.2 Requirements for successful LCI test:

The 51 participants who were successful required a total of 351 washout attempts; mean 6.9 (1.5 SD). To be feasible, these LCI occasions were tested against a theoretical maximum LCI trial limit. For example, if limited to 6 trials, in this scenario, only 22 (30.5%) of the 72 LCI tests measured in this study would have been successfully completed (Table 4.6).

Theoretical Washout Limit (Number of washouts)	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Number of successful participants at this limit (n %)	3 (4.1%)	8 (6.9%)	22 (30.5%)	33 (45.8%)	45 (62.5%)	49 (68.1%)	51 (70.8%)

Table 4.6: The theoretical effect of limited LCI test by number of trials

The mean length of time for an individual washout trial was 106 seconds. The mean total time for a subject to complete all washouts was 29.1 (7.5) minutes inclusive of the mandatory delay time between trials to allow rebalance of the nitrogen in the participants' lungs. This mean total does not include any time for explanation, instruction and device set up or cleaning of device after completion. There was no significant difference between the time taken to test participants who were successful vs unsuccessful in producing an LCI (29.9 (7.41) vs. 27.3 (7.24) minutes respectively, p=0.36). There was a trend of greater time taken between the early and late cohorts of our sample (sample population split into early and late by time of recruitment in to trial), but this difference was not statistically significant; early 27.1 (6.8) minutes, vs late 31.1 (7.7) minutes, p=0.09 We explored a theoretical time limit for the test length on overall success (Table 4.7).

Theoretical Time Limit (minutes)	10	20	30	40	50	60
Number of successful participants at this limit (n %)	0 (0%)	0 (0%)	14 (19.4%)	39 (54.1%)	48 (66.6%)	51 (70.8%)

Table 4.7: Effect of limiting LCI test by number of trials.
Participants who successfully completed the test had within test repeatability assessed for LCI (CV 4.68%), and FRC (CV 3.21%).

Of the 43 participants who were able to produce at least one acceptable washout within their first four attempts, 40 (93%) were able to go on to complete a successful LCI test. Of the 29 participants who were not able to produce an acceptable washout within their first four attempts, only 11 (37.9%) went on to achieve a successful LCI test.

4.4.3.3 Trial Rejection:

72 participants performed 490 trials in total. 360 (73.4%) trials were rejected with the most common reason being the inability to maintain a satisfactory tidal breathing pattern (317, 64.7%). Other reasons for trial rejection included leak [23 (4.7%)], and the subject being unwilling to continue [9 (1.8%), usually due to the sensation of the nose clip or reporting a dry mouth/cough]. Following these exclusions, a further 11 (3.1%) were rejected for excessive variation in FRC from the mean as per recommended guidance (52). 15 participants could not perform any acceptable washouts, with 99 attempts between them.

There was a significant difference in the mean respiratory rate of all trials of participants grouped by the total number of successful trials they completed (0 success, 32.5/min; 1 success, 27.8/min; 2 successes, 25.2/min; 3 success 24.6/min; p=0.0002, Figure 4.3). The mean tidal volume of all trials was 370ml (105ml) with a small, although non-significant, increase in tidal volume when grouped by the total number of successful trials completed (0 success, 321ml; 1 success, 369ml; 2 successes, 380ml; 3 successes, 389ml, p=0.24).

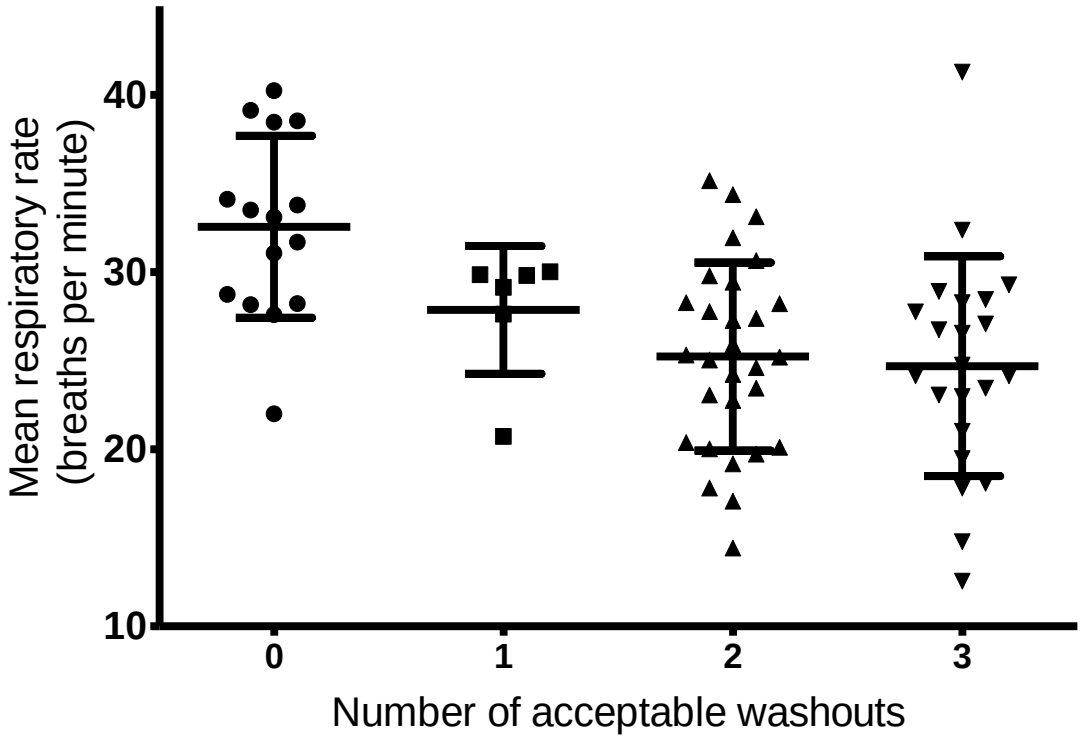

Figure 4.3: Mean respiratory rate grouped by total number of acceptable trials.
The mean respiratory rate of each subject (mean of all trials, acceptable and unacceptable) grouped by total number of acceptable trials achieved by the subject, data shown as mean (standard deviation). Analysis using Kruskal -Wallis test, p=0.0005

4.4.3.4 Consistency of LCI:

Multiple breath washout was successful in 70.8%, the coefficient of variation was 4.68% (LCI) and 3.21% (FRC). Spirometry was successful in 100%. LCI coefficient of variation was not correlated with age (p=0.8), lung clearance index (p=0.6), index of multiple deprivation (p=0.8), height centile (p=0.7) nor weight (0.1). FRC coefficient of variation was not correlated with LCI (p=0.7), index of multiple deprivation (p=0.2), height (p=0.5) and weight (p=0.5), but it was significantly correlated with age (p=0.003) see Figure 4.4. Increasing age of participant was correlated with increasing coefficient of variation of FRC (p=0.003). Correlation assessed with Spearman r correlation coefficient.

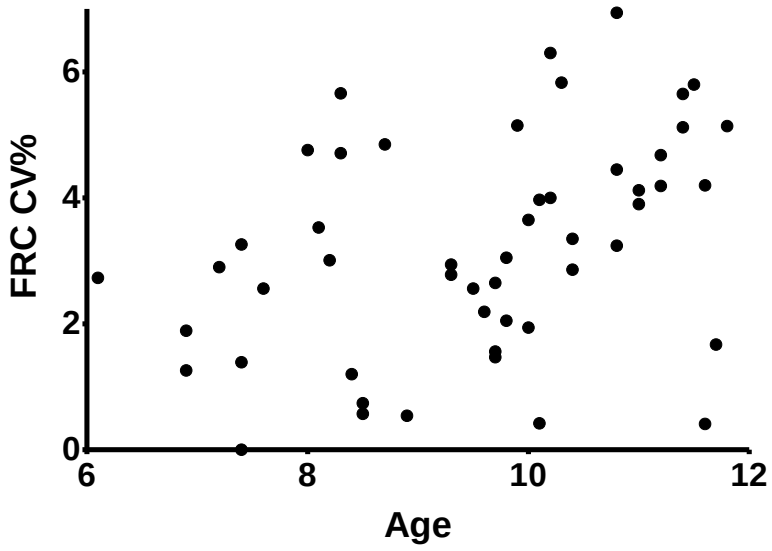

Figure 4.4: Coefficient of variation (CV%) of FRC with age Spearman r p=0.003

4.4.4 Lung Clearance Index in participants from different ethnic groups:

There was no difference in lung clearance index nor spirometry measures, (using percent predicted), between the 'White British' or 'Indian Subcontinent' groups; analysis of other ethnic subgroups was prohibited by the small numbers. It was noted that a greater proportion of participants from the 'White British' group lived in a household with an adult smoker see Table 4.4. There was no significant difference in LCI between groups of patients from a smoking vs. non-smoking household (Mann-Whitney t-test, p=0.55).

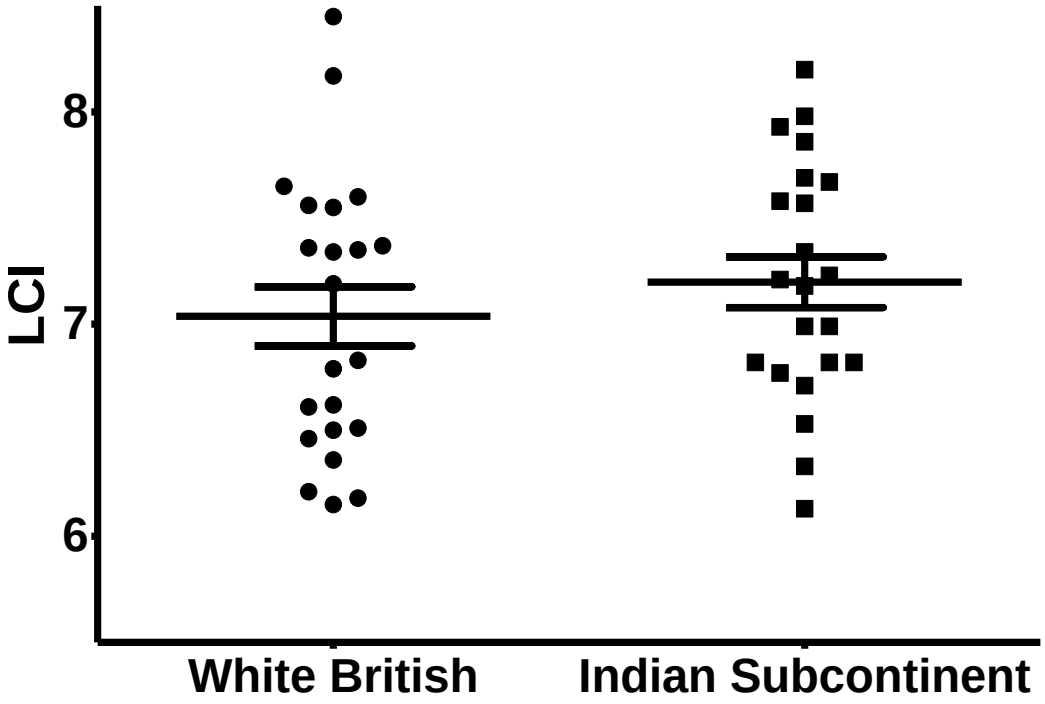

Figure 4.5: LCI from White British and Indian Subcontinent healthy children Mean, SD; 7.04, 0.6 and 7.20, 0.55 respectively

4.4.4.1 Lung Clearance Index and participant demographics:

The participants in this study are within a small age bracket (over 6 years, less than 12 years) and so there is minimal relative change in age, height and weight between participants. These demographics were assessed with simple linear regression separately for all the covariates (age, height, weight). Lung clearance index showed only a weak association for age, weight and height (0.31, p=0.02; 0.28, p=0.04; 0.29, p=0.03 respectively), see Figure 4.6.

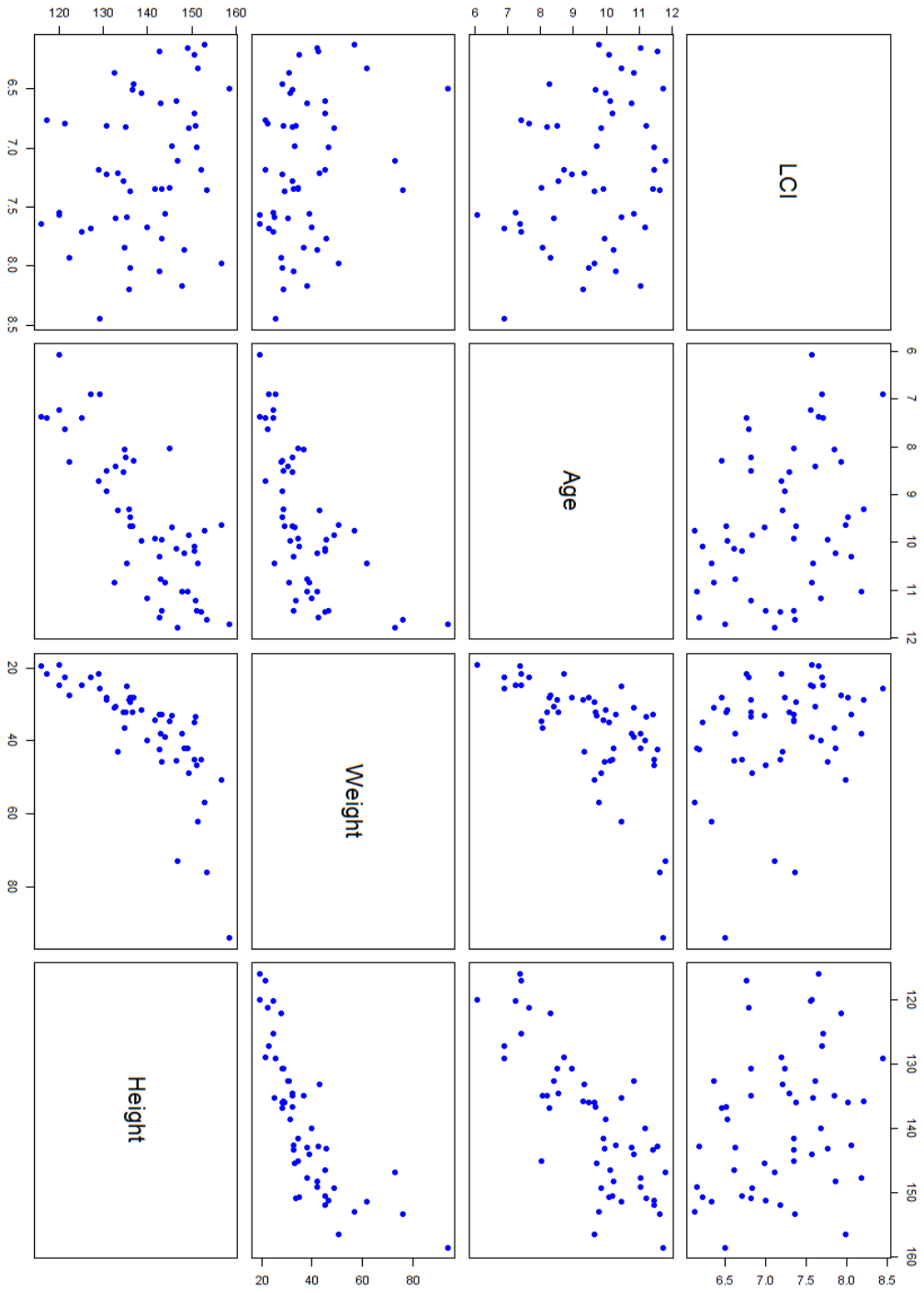

Figure 4.6: Matrix scatterplot of LCI, Age, Weight and Height
LCI: lung clearance index. Age shown as years, Weight in kilogram, Height in centimetre.

4.5 Discussion:

4.5.1 Summary of principal findings

The aim of this chapter was to demonstrate normative values for LCI in children from ages 6 years to 12 years using the EasyOne Pro LAB device and to assess differences between healthy children of differing ethnic groups. In the measurement of LCI in this cohort, I also wanted to assess the feasibility and practicality of measuring LCI using this device.

I measured LCI in 72 healthy children finding the measurement feasible, but with challenges including the length of time to measure, the inability of some children in being able to perform adequate technique and also showed the importance of operator experience. I demonstrated a range of LCI across this cohort of children with values in keeping with previously published data. Examining LCI between two groups of children from different ethnicity, I showed there was no significant difference between groups of children identifying as White British and as South Asian.

4.5.2 Normative Data:

The data presented here includes lung clearance index reported from two ethnic groups; there was no statistical difference detected between these groups although there is a suggestion of a higher mean LCI from those in the Indian Subcontinent (mean 7.20 vs. 7.04). The groups were not matched across all factors (sex, height, weight, age). The smaller sample size of the groups in this data set may have precluded a true difference being demonstrated (with 36 participants and 26 participants recruited from White British and Indian Subcontinent groups, with 22

participants from each group successfully providing data). However, the conclusion of no significant difference is in keeping with other data already published as previously mentioned and is also supported by the likely physiological factors involved.

The normative data generated in this study contributes to the general dataset available and will provide useful guidance to the community who use lung clearance index. This study demonstrated a very small, but significant decrease in LCI with increasing age, height and weight from those age 6 years up to 12 years of age with no association between LCI and sex. This trend is in keeping with other data on school aged children using nitrogen washout on the Exhalyzer D, from age 6 to 18 years, where they reasonably describe the small, maximum change in LCI due to age through this time period as being unlikely to have clinical influence, and therefore gives the ability to have a fixed upper limit of normal through this age range (101).

This trend is different to a cohort of children from birth to 19 years, using SF6 as the tracer gas with room air washout measured by mass spectrometer (118). Here, Lum et al describe that once children reached the approximate age of 6 years, there was no significant association between LCI and either height or age, again allowing for a fixed upper limit of normal for the group.

It is not clear why a significant association exists in our cohort between LCI and subject demographics (age, height and weight). The association of subject demographics have been reported previously in school aged children using the EasyOne Pro, with a significant association found using nitrogen MBW, but not in SF_6 MBW (107,108,193).

It may be that the younger (and shorter/lighter) participants are influenced more by the noise and sensation of the EasyOne Pro and therefore more liable to altered tidal breathing patterns that can influence the washout result. Younger children, with small tidal volumes, are more likely to be influenced by the equipment dead space (rebreathing expired gases that would raise LCI). It has already been shown that children using the ndd device for MBW have higher respiratory rates and lower tidal volumes when compared to testing on other commercial devices (113,115). The mean respiratory rate for the successful trials in our cohort was 24.1(+/-5.8), which is in keeping with previously reported figures.

Further work is required to ensure normative data is suitable for the corresponding age groups.

4.5.3 Feasibility and Practicality:

This data demonstrates the feasibility and practicality of measuring lung clearance index using a readily available commercial device. The total success rate in this data set was 70.8% with the primary reason for trial rejection being the inability to maintain a pattern of tidal breathing (64.7% of trials). Individuals took 29.1 minutes to complete the test; success rates improved during the study.

4.5.3.1 Comparison of success and repeatability with published data:

The total success rate described is in keeping with previously described figures in the use of lung clearance index in children using a similar MBW set up. Fuchs et al demonstrated success

of 75% in healthy school age children (mean 12.5 years) using the ndd EasyOne Pro LAB device with sulphur hexafluoride as the tracer gas (108). Poncin et al (113) achieved a higher, 100%, success rate in a similar age group (mean 11.2 years) using the same device set up. In a comparison of nitrogen washout using the EasyOne Pro LAB to other devices, Isaac et al showed a range of successful test rates from 50% to 93% across a range of operators and sites (100). The coefficient of variation of LCI and FRC within test are similar to previously reported measures of inter test repeatability (4.85% and 6.22% respectively) (108).

Over time, there was significant improvement in success rate between patients during progression of the study; this is a pattern which has been seen in previous studies using the EasyOne Pro LAB, but it is not clear as to the cause of the increase (108). It likely reflects the impact of staff experience and familiarity on likelihood of success, (LCI guidance gives an emphasis on training, repeated performance and oversight and support from experienced centres) (190).

4.5.3.2 Impact of staff training:

Consensus documents and previous research have all highlighted the importance of consistent staff training and continued quality control processes. This is most important in the data acquisition and washout selection stage. Here, poor experience or inappropriate acceptance of inadequate washout trial data could skew the results, potentially with clinically significant results.

Previous work examining multi-centre lung clearance index have demonstrated protocols to minimise these effects (202). Here, in the assessment of LCI in sedated infants (generally considered to be more technically challenging than older children), standardised multiple breath washout protocol was used with consistent equipment with a dedicated training session on acquisition and washout interpretation held for all participating staff. Results from washouts were reviewed at each local hospital and anonymised for assessment at a coordinating centre for further quality control, evaluation and analysis. Reports were fed back to the study site to improve their experience in interpretation of multiple breath washout readings. They achieved a success rate of 91.8% across three sites with consistency in results and practice.

This intensive approach of central training and harmonised protocols allowed experience of a large centre to be transferred to a centre with no previous experience in multiple breath washout. The single operator collecting the data in this thesis, CH, participated in a large, multinational therapeutic trial of patients with CF (203). This required multiple breath washout data collection and was set up in a similar way. Having personal experience of this process further supports my conclusion that a similar structure would be required in the role out of LCI for clinical use.

Consideration must be given to how this is established in each centre as requirements will vary as to the frequency at which LCI is needed which will have an impact on the number of people requiring training and continued quality control feedback.

4.5.3.3 Time requirement for LCI:

We have demonstrated participants may need to perform up to ten washouts to obtain a successful test result. This is a significant investment of time and resources for participants, their families, and the staff involved. The mean test time of almost 30 minutes is considerable and aligns with previous measurements of test time with this device (107). It is longer than test time reported using the Innocor device (Cosmed, Rome, Italy) but similar to the Exhalyzer D in LCI naïve children with CF (121,154).

Using an ndd prototype system, multiple breath washout using SF_6 took between 30-40 minutes to achieve a successful lung clearance index assessment in a study examining repeatability and reproducibility (107). Two of their 46 participants were unable to complete a lung clearance index test due to inability to cooperate during their first attempt, giving a success rate of 95.6%. These children were older than those in this work (mean age 12 years), but they were also naïve to lung function testing.

Using the Exhalyzer device, with nitrogen washout, in an assessment of practicability of lung clearance index in the clinic setting, it was demonstrated that it was realistic to acquire LCI within a defined 20 minute time frame, but only following adequate instruction and training (154). Here, children between age 4 and 16 years with CF and healthy controls were assessed and on this first occasion, 41% achieved two or more tests within the 20 minute period with the mean length of successful test being 3.3 minutes for those with CF. They describe that with sufficient training time, it would be possible to achieve a good success rate (80-85%) within a 20 minute limit. Children in this study were aged 11.9 years; it should also be noted that a proportion of the participants had CF and therefore would very likely have had experience of

lung function (mostly spirometry) prior to this attempt. That prior experience of lung function may have boosted the familiarity of interaction with lung function technicians, relaxing participants and perhaps improving compliance with instruction for LCI that is so vital for a successful test.

Finally the Innocor device, using SF_6 as the tracer gas, was assessed for feasibility in an outpatient clinic setting. This included children from age 5, up to adults aged 59. The median test time was 18.7 minutes but was even shorter in children (17.8 minutes). 8 children were unable to complete the required washouts, all adults were successful.

4.5.3.4 Summary:

In this chapter, I have described the experience of healthy, lung function naïve, participants completing an LCI test with one of the strengths of the data reported being the significant level of detail on the characteristics of both successful and unsuccessful LCI measurements. The operator completed a comprehensive training programme and prior to this study also participated in the data acquisition of lung clearance index in a large, international drug trial in cystic fibrosis with central oversight of the washout data.

A further limitation was that LCI was performed on a single occasion, so we were unable to assess success rates in longitudinal measures. However, as all participants were naïve to lung function testing, the success rates shown here would likely represent the initiation of a monitoring programme in a naïve clinical population.

Lung Clearance Index can appear to be a simple to perform test for a patient and has been applied to several different diseases and a range of patient groups (112). There is particular emphasis on its use in children due to the non-invasive nature of the test and the ability as a sensitive measure of disease (69). For the test to become useful in a real-world clinical setting it must be easy to use for the operator, have a good success rate and be practical in its operation.

One barrier to success in this study was the high proportion of trial rejections due to non-tidal breathing patterns. The EasyOne Pro LAB uses a gas bias flow system to provide valve-controlled washout gas which gives a subtle physical and audible sensation to the user at the onset of each breath. This may contribute to some children having difficultly achieving the desired technique due to the positive feedback with respiration. Significant efforts were employed to coach and distract the subject to mitigate this effect in accordance with established practice and guidance, but despite these efforts, many children continued to have difficulty (190,195,204). This specific feature of the EasyOne Pro LAB device must be considered during its operation.

Success rates could be increased by repeated subject performance. Evidence suggests that this may be the case in both the EasyOne Pro LAB device and with other devices (107,110,151). It is anticipated that as a clinical team develop experience with LCI on their chosen device, and participants perform repeat assessments, that feasibility and success rates improve although this has not been reliably demonstrated. Staff training, experience and support is undoubtedly

vital. It is likely that hospital sites will need to have small teams who are able to perform LCI regularly although this may also affect access to the test.

For a test to be usable within the same clinic visit within which the LCI is measured, analysis and calculation of LCI must be performed contemporaneously with the result delivered to the clinician promptly. It is not clear if this would be practicable and this has also been previously reported as a concern (112).

4.6 Conclusion:

Lung clearance index can be performed in children with no previous experience of lung function using the ndd EasyOne Pro LAB device. Operator familiarity is essential as are clear methods to assist in the delivery of a tidal breathing pattern including coaching and distraction. Multiple washout attempts may be required to deliver adequate results and tests may last beyond 50 minutes in a healthy cohort and may be longer is those with lung disease; this information will allow teams to manage participants' and families' expectations. Some participants may be unable to perform the test and time should be dedicated to instruction and practice if possible.

Data of healthy children from different ethnic backgrounds demonstrates no significant difference in lung clearance index and supports the limited data already published in this area. We highlight the importance of accurate reporting of ethnicity, race and ancestry in order to accurately represent the populations studied in work developing normative lung function data.

Chapter 5: Lung Clearance Index in children with non-cystic fibrosis, non-primary ciliary dyskinesia bronchiectasis

5.1 Introduction:

Lung clearance index has been shown to be of value in the assessment of lung disease in children such as cystic fibrosis (55,69) and primary ciliary dyskinesia (PCD) (86,87). The potential of LCI, is as a sensitive marker of disease, in a safe, repeatable, non-invasive way to help guide patient care.

As described in section 1.1, bronchiectasis not caused by CF, nor PCD (henceforth described as bronchiectasis) is a diagnosis of increasing prevalence and significant morbidity in children and young people. In section 1.2, I highlighted the current challenge of monitoring in bronchiectasis with the illustrated potential of LCI.

This chapter examines LCI in children with bronchiectasis and the potential value as a marker of disease.

5.1.1 LCI in children with bronchiectasis

We have published a systematic review examining the role of LCI in bronchiectasis (Chapter 2 (155)). To briefly summarise, the available data for children is extremely limited. A literature search found only five paediatric studies which describe a small number of children.

The highest quality study compares LCI with spirometry in a group of 12 children with bronchiectasis (164). Here, it was shown that LCI and FEV_1 was significantly correlated along with individual signs of bronchiectasis seen on HRCT (see Table 2.4); extent of bronchiectasis, r=0.8, p=0.002; severity of bronchiectasis, r=0.7, p=0.01; airway wall thickening, r=0.7, p=0.01; and air trapping, r=0.8, p=0.0006. This is similar to data from adult studies (170,171).

This existing data does show promise in the role of LCI in children with bronchiectasis but consideration must be given to the wide range of aetiologies of bronchiectasis and there must be caution from extrapolating data from other disease groups into this cohort.

5.1.2 Other non-invasive tests for monitoring of bronchiectasis

To assess severity, monitor care, or review the impact of new or discontinued treatments, objectively measured data is helpful to the clinician. We have previously described the role of high resolution computed tomography (HRCT) (section 1.1.4.1), spirometry (section 1.1.4.2), and microbiology (section 1.1.4.3). None of these approaches are without their questions or challenges.

HRCT requires exposure to a not insignificant amount of radiation (0.57-2.79 mSv for helical scans and 0.22-0.59 mSv for axial scans (205)) . This must be considered if repeated measures of disease by HRCT are required (which would be the case for the monitoring of care). Frequent HRCT (without a clear clinical question) is also not recommended in recent international guidance (2).

Spirometry is non-invasive and repeatable but as previously demonstrated in data from populations of people with CF and in adults with bronchiectasis, it may not be sensitive enough to detect clinically relevant changes.

Microbiology data is important and illustrates both active infection and colonisation. Reliably obtaining airway specimen for culture is difficult in children, particularly those who are younger (2). This likely influences the reliability of these tests. Furthermore, it is not fully established that colonisation with pathogens contributes to progressive lung damage or if colonisation occurs in those with already accelerating disease (206).

It is for these reasons that we explore the potential of LCI in the assessment of bronchiectasis where it may play a role in providing additional information to the current battery of investigations available.

5.2 Hypothesis:

- In children with non-cystic fibrosis, non-primary ciliary dyskinesia bronchiectasis, with evidence of disease on CT imaging, that Lung Clearance Index is a more sensitive disease marker than FEV_1.

5.2.1 Aims:

1. To establish the relationship between LCI and measures of spirometry for children with bronchiectasis.
2. To establish the relationship between LCI and structural changes seen on HRCT for children with bronchiectasis.
3. To determine the sensitivity of LCI in detecting bronchiectasis otherwise diagnosed on HRCT.

5.3 Methods:

This study included children with bronchiectasis, plus a group of healthy children previously described as a comparison cohort. The group of healthy children are the same cohort described in Chapter 4:. All participants completed a single study visit where they completed lung function testing and demographic data was gathered as has been previously described in Chapter 3.

5.3.1 Nitrogen multiple breath washout

Nitrogen washout was performed using the ndd EasyOne Pro LAB device. Participants were instructed in its use and coached between trials. Participants were in the seated position with distraction via a tablet screen and headphones. Regular, tidal breathing was encouraged targeting tidal volumes between 8 and 15 ml per kg of the participants ideal weight. Washouts were completed when three consecutive breaths were completed with nitrogen concentrations less than 2.5% of the original concentration. FRC, LCI, tidal volume and respiratory rate were recorded. Attempts at nitrogen washout continued until the adequate number of acceptable washouts were achieved, or the subject reported they were unable to complete further washouts.

5.3.2 Spirometry:

Spirometry was completed as per ATS/ERS guidance using the ndd EasyOne Pro LAB. Three attempts of adequate quality were recorded with the results from the best attempt taken.

5.3.3 Recruitment:

Children with bronchiectasis (diagnosed on HRCT) were recruited from specialist bronchiectasis and general respiratory clinics at Birmingham Children's Hospital, Birmingham Children's and Women's NHS Trust. Participants were identified after clinical coding and patient database review. Patients were included if they were aged 6 years up to 12 years of age and had a radiologically (HRCT) confirmed diagnosis of bronchiectasis within the previous two years. Written consent was obtained from all participants.

Exclusion criteria:

- A respiratory exacerbation (requiring antibiotic or physiotherapy escalation) within the previous 4 weeks
- A concurrent diagnosis of:
    - neuromuscular disease
    - neurological disability
    - congenital structural airway/gut/thoracic disease
    - cystic fibrosis
    - primary ciliary dyskinesia

Inclusion criteria:

- Diagnosis of bronchiectasis demonstrated on HRCT
- HRCT performed with two years of study lung function testing

5.3.4 High Resolution Computed Tomography Imaging:

HRCT images for each patient were reviewed by two independent radiologists and were scored using a standardised scoring system, the Brody score (178). An overall score is obtained within which are specific scores for bronchiectasis, mucous plugging, peribronchial thickening, and parenchymal disease. This score includes Hyperinflation and air trapping but was not assessed in this study as many of the participants did not have expiratory images as part of their HRCT as was local practice.

HRCT scoring systems have been widely used in research, particularly within cystic fibrosis, with the Brody score being a recommended system as an endpoint is assessing lung disease related to cystic fibrosis (207). No specific scoring systems exist for non-CF, non-PCD bronchiectasis but CT remains a widely used tool to assess bronchiectasis due to its sensitivity over chest xray and lung function (208). The Brody score was chosen for its good between and within observer variability, and its previous use in large studies of subjects with bronchiectasis (171,207)

5.3.5 Sample size and power calculation

There is limited previously published data that could be used for comparison. The previous, largest study of children with bronchiectasis in the assessment of LCI contained just 12 children (164). This is representative of the challenge of assessment in children with this condition that is of low frequency.

Studies in adults with bronchiectasis (171) have used greater size of cohort to determine the relationship between HRCT and LCI; 60 patients recruited including allowing for dropouts or technical exclusions. Smaller numbers have been used in the literature when examining for differences in LCI between two groups. Zwitserloot et al (115) used the data from Raaikmakers et al (116) to calculate a requirement of 9 participants in order to detect a difference of 10%, achieving 80% with a two-sided significance of 0.05. Due to expected variation of FRC measurements, and with an approximated drop out rate of 10% they aimed

to recruit 40 participants in total (20 children and 20 adults) with result reported separately. 18 children were included in the final analysis.

This study recruited from specialist clinics, and recruitment was limited by the small number of patients with the condition and meeting all inclusion criteria.

5.4 Clinical characteristics of children participating:

Thirteen patients were recruited with bronchiectasis from specialist clinics at Birmingham Children's Hospital. The mean age was 9.9 years, 54% were male. On review of subject medical background, there were a range of causes for the bronchiectasis noted; post infection (7, 53%), immunodeficiency (1, 7.7%), juvenile idiopathic arthritis (1, 7.7%), and the cause noted as idiopathic (4, 30.8%). 9 were White and 4 were Asian.

72 healthy children were recruited: 35 male, age 9.4 (1.46 SD) years (Table 5.1). 35 were White, 5 of mixed ethnicity, 27 Asian, 2 Black and 3 of another ethnicity. This cohort of healthy children are the same group reported in Chapter 4: Lung clearance index in healthy children from a range of ethnic backgrounds on page 111.

5.5 Results:

5.5.1 Clinical characteristics of the children participating

Thirteen participants were recruited who all performed lung function tests as described in the methods section. 72 healthy children were recruited as a comparison cohort, their recruitment and results are described additionally in Chapter 4: The mean age was 9.9 (1.7) years and 7 were male (54%), see Table 5.1.

	Bronchiectasis (n=13)	Healthy Children (n=72)	P value
Age, years (mean, SD)	9.9, 1.72	9.4, 1.46	0.26*
Male	7, 54%	35, 48.6%	0.77#
Bronchiectasis aetiology (n, %)			
Post infection	7, 53.8%	NA	-
Immunodeficiency	1, 7.7%	NA	-
Juvenile idiopathic arthritis	1, 7.7%	NA	-
Idiopathic	4, 30.8%	NA	-
Height, cm (mean, SD)	137.9, 12.5	138.0, 10.4	0.78
Weight, kg (mean, SD)	33.5, 9.4	36.1, 13.0	0.43
Household smoking	3, 23.1%	9, 12.5%	0.38#
Ethnicity*			
White	9, 69.2%	35, 48.6%	-
Mixed/Multiple ethnic groups	0, 0%	5, 6.9%	-
Asian / Asian British	4, 30.7%	27, 37.5%	-
Black / African / Caribbean / Black British	0, 0%	2, 2.8%	-
Other ethnic group	0, 0%	3, 4.1%	-
Rural/Urban Location			
A1: Urban major conurbation	8 (61.54%)	57 (79.17%)	
C1: Urban (City and Town)	4 (30.77%)	8 (11.11%)	
D1: Rural (Town and fringe)	0 (0%)	3 (4.17%)	
E1: Rural Village	1 (7.69%)	2 (2.78%)	
F1: Rural hamlet and isolated dwelling	0 (0%)	1 (1.39%)	
Unknown	0 (0%)	1 (1.39%)	
IMD Score	13816 (8867)	15288 (10653)	0.71
IMD National Decile - 1	2 (15.38%)	18 (25%)	
IMD National Decile – 2	1 (7.69%)	2 (2.78%)	
IMD National Decile – 3	2 (15.38%)	4 (5.55%)	
IMD National Decile – 4	3 (23.08%)	5 (6.94%)	
IMD National Decile – 5	0 (0%)	12 (16.67%)	
IMD National Decile – 6	2 (15.38%)	3 (4.17%)	
IMD National Decile – 7	0 (0%)	7 (9.72%)	
IMD National Decile – 8	2 (15.38%)	5 (6.94%)	
IMD National Decile – 9	0 (0%)	10 (13.89%)	
IMD National Decile - 10	1 (7.69%)	6 (8.33%)	
Lung Function			
FEV_1 (mean, SD)	76.3%, 18.5	98.2%, 10.6	**p=0.0002**
FVC% (mean, SD)	84.6%, 15.7	98.4%, 11.5	**p=0.005**
LCI (mean, SD)	9.51, 2.2	7.19, 0.6	**p<0.0001**
HRCT Score, total* (mean, SD)	19.63, 12.99	NA	-

Table 5.1: Demographic details

Demographic detail for participants with bronchiectasis and healthy controls. N, % and Mann-Whitney test unless otherwise specified. FEV_1: forced expiratory volume in one second, FVC: forced vital capacity, LCI: lung clearance index, HRCT: high resolution computed tomography. *Unpaired t-test, #Fischers exact test. $Welch T-Test

5.5.2 Feasibility:

85 patients performed a total of 573 washouts; mean 6.3 (2.3 SD). 64 (75.3%) patients were able to produce a valid test (at least two acceptable washouts), (all 13, 100%, patients with bronchiectasis and 51, 70.8%, of healthy participants). Participants with bronchiectasis were more likely to complete the LCI test (Fishers exact test, p=0.03). All participants were able to perform adequate spirometry; there was no difference in spirometry in healthy participants between those able to perform a valid LCI test, and those who could not. Those with bronchiectasis did take longer to complete a test, but this was not significant; (healthy participants (34.1 min, 7.46 SD) and participants with bronchiectasis (37.0 min, 7.29 SD)). The most common reason for participants failing a washout attempt was a non-tidal breathing pattern.

5.5.3 Healthy children:

This data is also presented in detail in Chapter 4 as suggested normative data for this population. The mean LCI was 7.19 (0.6 SD). This is in keeping with previous nitrogen washout performed using ndd EasyOne Pro LAB. LCI was consistent in measurement with the coefficient of variation being 4.68%. T

Using this normative data generated by this cohort of healthy children, an upper limit of normal can be generated, defined as the mean LCI + 1.96 x standard deviation (136). This is calculated as:

$$7.19 + (1.96 \times 0.6) = 8.36.$$

This can be compared to other data generated from healthy children using the ndd EasyOne Pro LAB from Table 4.3. Poncin et al (113), upper limit of normal 8.01; Isaac et al (193), upper limit of normal 8.57. Data from Zwitserloot et al (115) cannot be used in this way without a published standard deviation. Data from Oestreich et al (192) was not used to generate an upper limit of normal due the small subject numbers and unusually large standard deviation, potentially introducing bias.

Healthy children had a range of lung clearance index of 6.13 to 8.45. One participant had an LCI that was greater than the upper limit of normal that was generated by this data but less than the upper limit of normal generated from other, similar data as noted above.

5.5.4 Children with bronchiectasis:

13 children with bronchiectasis completed lung function testing in a single visit. Lung function was significantly worse in disease compared to healthy control participants. FEV_1 76.3% vs. 98.2%, p=0.0002 see Figure 5.1; FVC 84.6% vs. 98.4%, p=0.0052, see Figure 5.2; LCI 9.51 vs. 7.19, p<0.0001, see Figure 5.3.

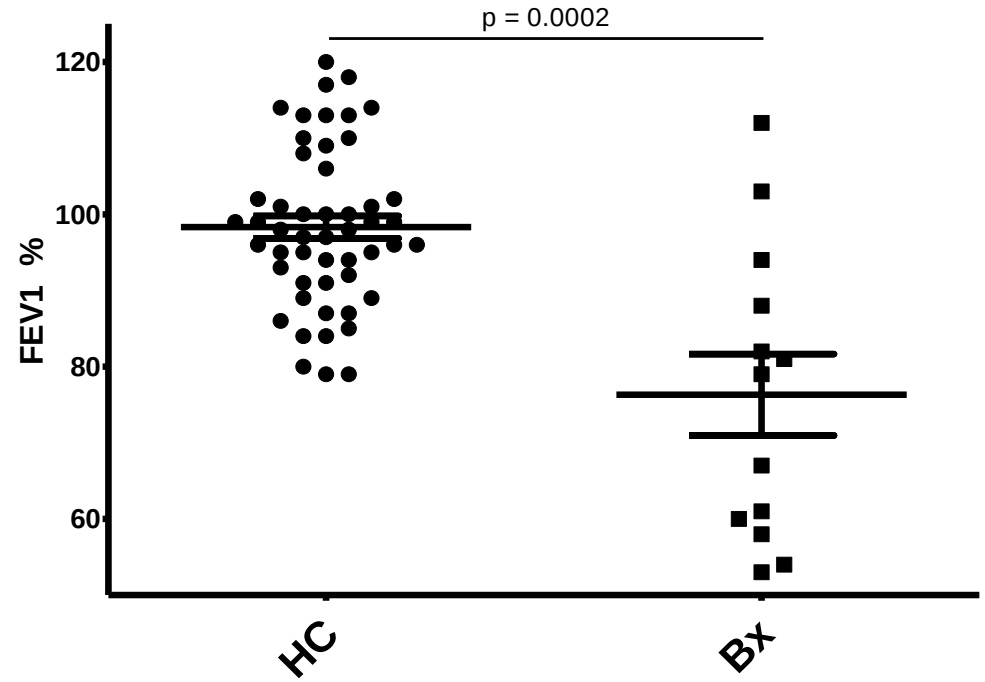

Figure 5.1: FEV_1% in healthy controls and children with bronchiectasis.
FEV_1%; percent predicted forced expiratory volumes in one second, HC; healthy controls, Bx; bronchiectasis

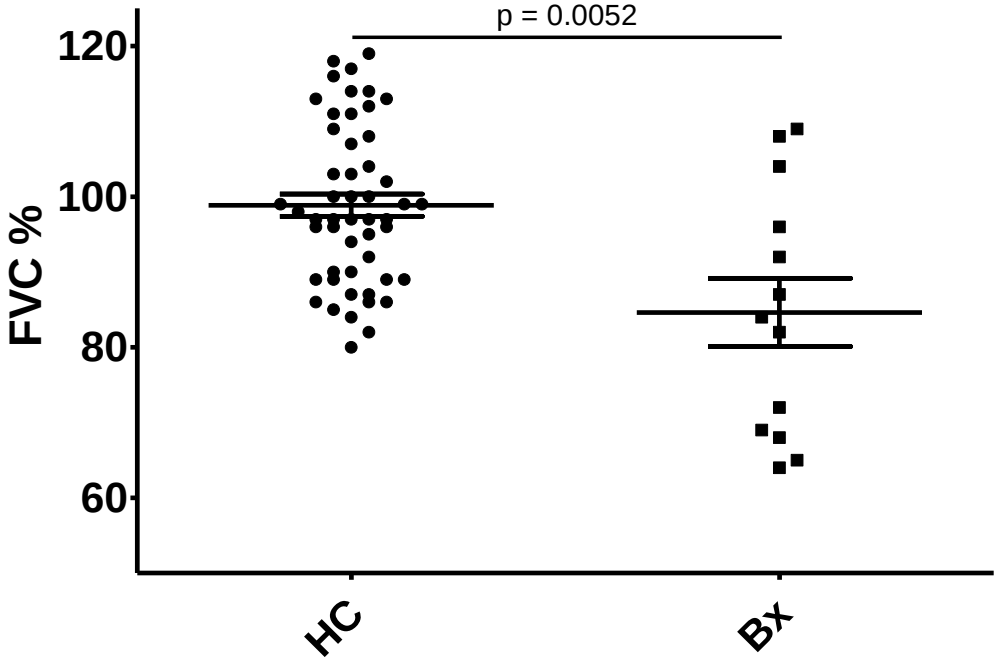

Figure 5.2: FVC% in healthy children and children with bronchiectasis.
FVC%; percent predicted forced vital capacity, HC; healthy controls, Bx; bronchiectasis

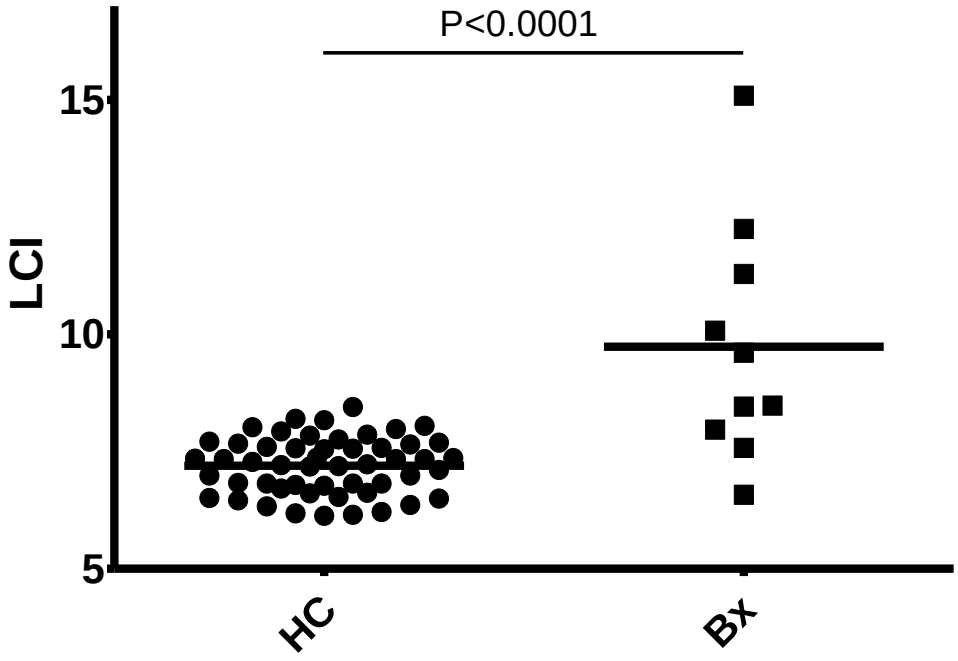

Figure 5.3: LCI in healthy controls and children with bronchiectasis
LCI; lung clearance index, HC; health controls, BX; bronchiectasis.

(There was no significant difference between the groups of healthy children and children with bronchiectasis regarding their age (p = 0.2), sex (p = 0.7), height (p = 0.7), weight (p = 0.4), household smoking (p = 0.3), or index of multiple deprivation score (p = 0.7) see Table 5.1. The largest ethnic group for each of the cohorts were of White ethnicity (healthy children 69.2%, children with bronchiectasis 48.6%).

The lung function of children with bronchiectasis demonstrated greater variation from the mean when compared to healthy children (FEV_1 standard deviation 18.5 vs. 10.6, FVC standard deviation 15.7 vs. 11.5, and LCI standard deviation 2.2 vs. 0.6 respectively).

5.5.5 High Resolution Computed Tomography Scores

Patient HRCTs was scored according to the Brody CT scoring system (178) independently by two senior paediatric radiologists. The mean total HRCT score was 19.63 (12.99), subtotal scores are presented in Table 5.2. Agreement between scorers was high when assessed by Pearson correlation; $r=0.9711$, $p<0.0001$ for total score; $r=0.8968$, $p<0.0001$ for bronchiectasis sub-score.

	Children with bronchiectasis (n=13)
Total HRCT Score	19.63, 12.99
Subtotal Scores	
Bronchiectasis	11.89, 8.74
Mucous Plugging	0.54, 13.9
Peribronchial Wall Thickening	2.96, 12.24
Parenchymal Changes	2.96, 12.2

Table 5.2: High resolution computed tomography (HRCT) scores for children with bronchiectasis.
Data presented as mean, standard deviation.

Established bronchiectasis was the most common pathology noted on imaging, with relatively little mucous plugging noted. As previously described, hyperinflation, usually a component of the Brody score, was not included in this study due to the lack of expiratory HRCT performed in this patient cohort; this therefore prevented accurate assessment of hyperinflation.

5.5.6 Lung Clearance Index and spirometry markers

LCI correlated with other spirometry markers. FEV_1 correlated the strongest; Spearman r -0.7967, p=0.0011, see Figure 5.4. FVC also correlated strongly; Spearman r -0.7582,

p=0.0027, see Figure 5.5. MMEF did not significantly correlate with LCI, Spearman r -0.5165, p=0.0707, see Figure 5.6.

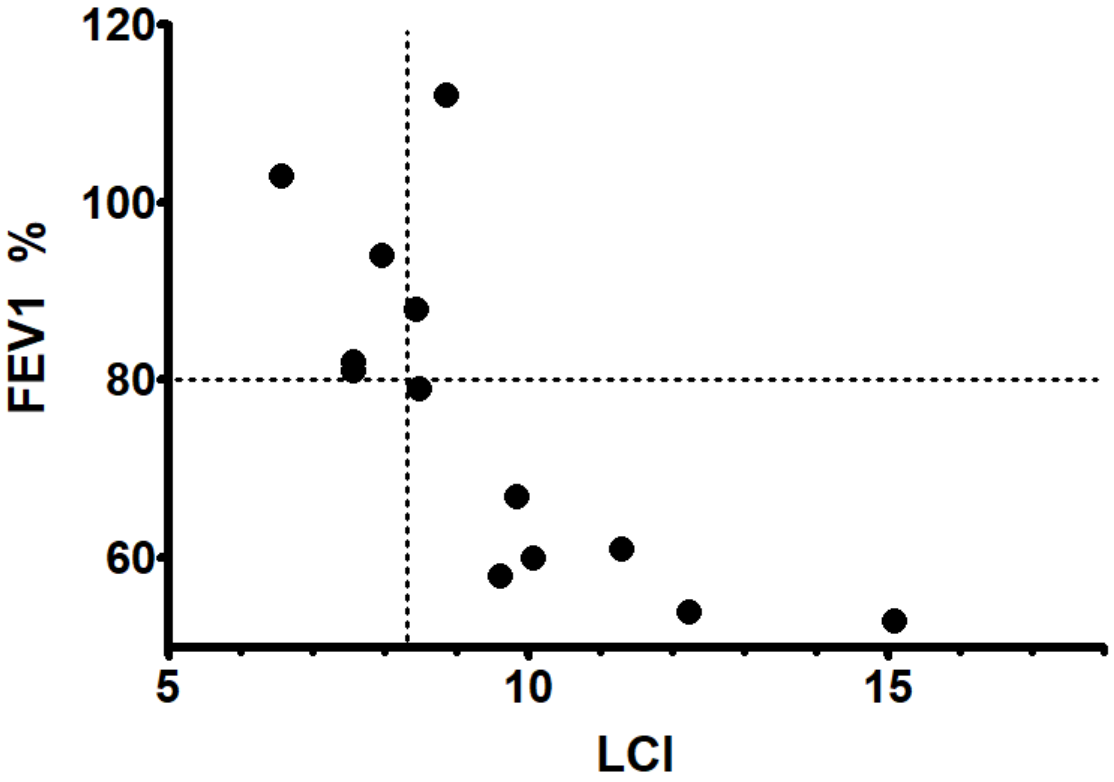

Figure 5.4: FEV_1% and lung clearance index
Normal value of FEV_1 and upper limit of normal of LCI (80% and 8.38 respectively) marked on graph. Spearman r -0.7967, p=0.0011. FEV_1: forced expiratory volume in one second; LCI: lung clearance index

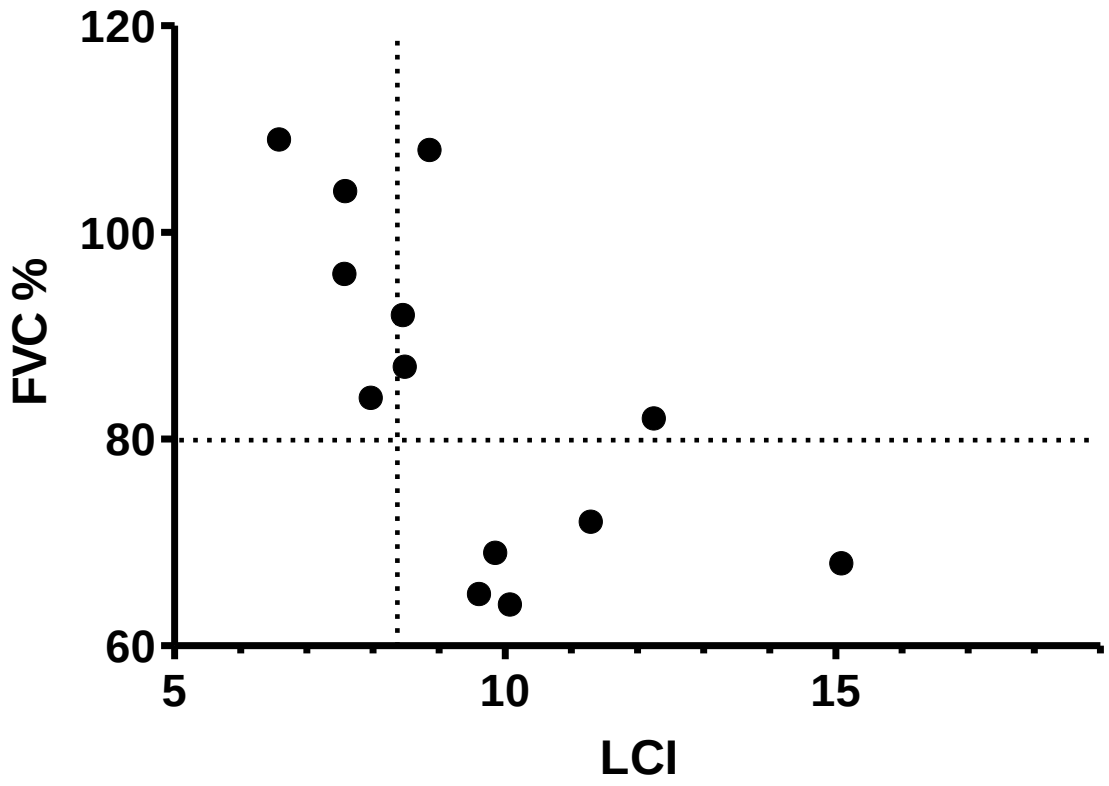

Figure 5.5: FVC% and lung clearance index
Normal value of FVC and upper limit of normal of LCI (80% and 8.38 respectively) marked on graph. Spearman r -0.7582, p=0.0027. FVC: forced vital capacity; LCI: lung clearance index

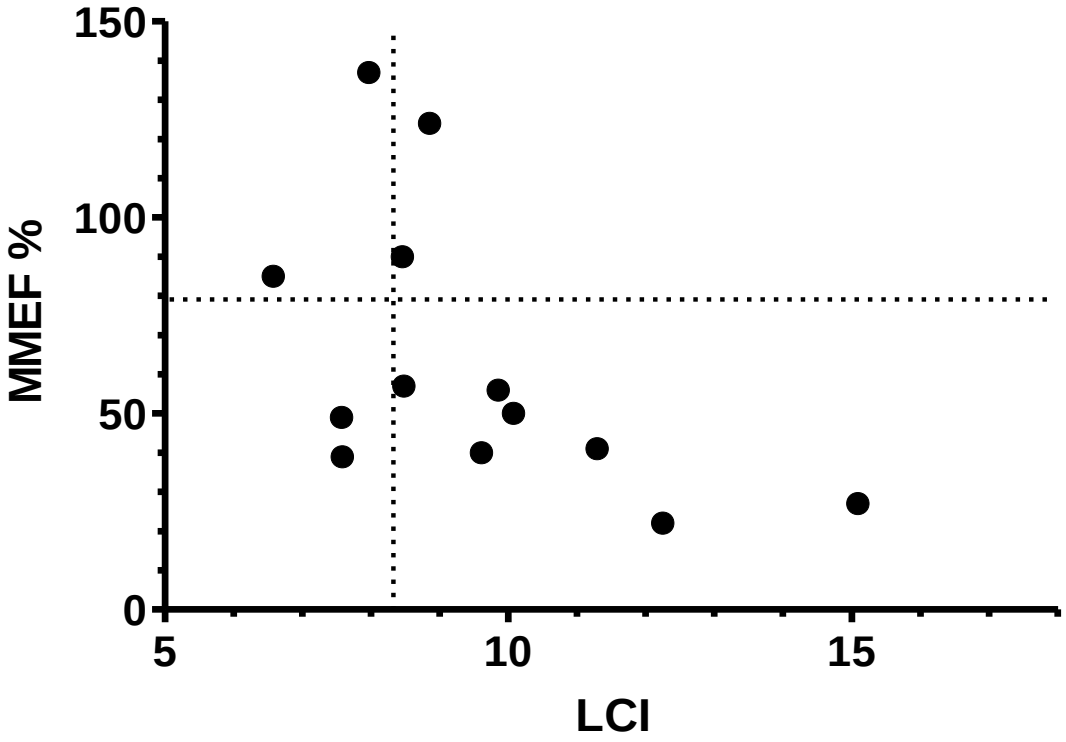

Figure 5.6: MMEF% and lung clearance index
Normal value of FVC and upper limit of normal of LCI (80% and 8.38 respectively) marked on graph. Spearman r -0.5165, p=0.0707. MMEF: maximum mid expiratory flow; LCI: lung clearance idnex

Using the definition of 80%, that is widely used (34), 6 patients had normal $FEV_{1.}$ Four of these patients had a normal LCI (using the upper limit of normal previously shown to be 8.38), but two participants had an abnormal LCI (Figure 5.4).

FVC has a similar relationship with LCI but a noted greater degree of correlation (r=-0.79 vs. r=-0.75). All participants with abnormal FVC had abnormal LCI. Eight participants had normal FVC (greater than 80%), four of these had normal LCI, four abnormal. MMEF did not significantly correlate with LCI. Additionally, in those patients with reduced MMEF, most,

but not all had abnormal LCI. Two patients with reduced MMEF had normal LCI. (In this study, 'normal' MMEF was defined as above 80% which is in keeping with other research examining small airways obstruction (although is noted to be arbitrary) (209–211). Regardless, both participants described here had very low MMEF (less than 50%) but had normal FEV1 and FVC.

5.5.7 High resolution computed tomography scores and lung function tests

LCI correlated with HRCT scores, with a rising LCI seen with increased HRCT score. Lung function assessed by spirometry showed decreasing spirometry markers (FEV_1, FVC, MMEF) correlated with rising HRCT scores (Figure 5.7, Figure 5.8, Figure 5.9, Figure 5.10). LCI correlated most strongly, followed by FEV_1, FVC and then MMEF.

Specific features seen on HRCT (sub-scores of the Brody score (178)) were found to correlate with LCI, FEV_1 and FVC (Table 5.3). LCI and FEV_1 correlated significantly with parenchymal change and bronchiectasis, FVC correlated significantly with parenchymal change, bronchiectasis and peribronchial wall thickening. MMEF did not correlate with any of these sub-scores.

	Bronchiectasis	Mucous Plugging	Peribronchial Wall Thickening	Parenchymal Changes
LCI	**0.70***	0.24	0.28	**0.61***
FEV_1	**-0.71***	-0.24	-0.49	**-0.67***
FVC	**-0.64***	-0.45	**-0.60***	**-0.60***
MMEF	-0.42	0.12	-0.24	-0.48

Table 5.3: Correlation of LCI and Spirometry with features on HRCT Spearmans r value. *p<0.05

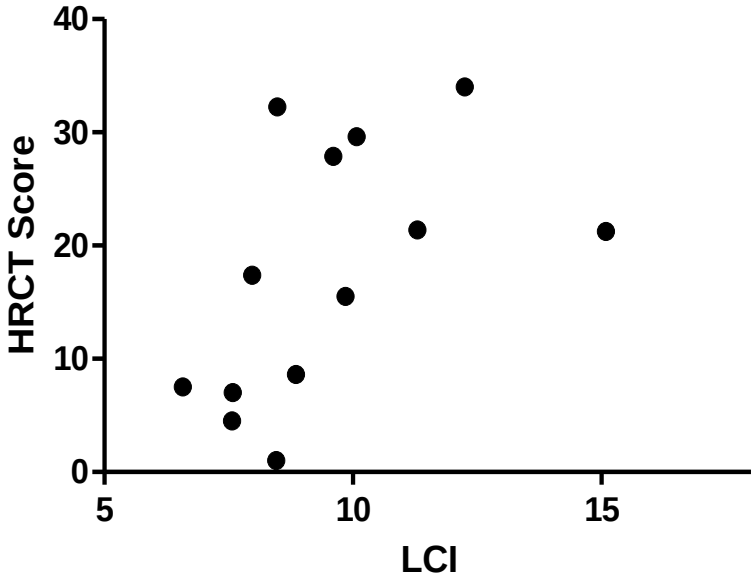

Figure 5.7: Correlation between LCI and HRCT Scores Spearman r 0.6758, p=0.0112

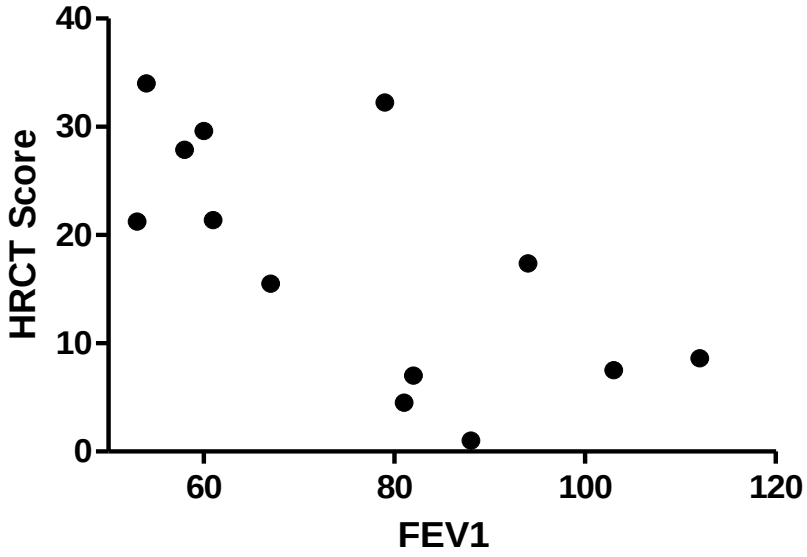

Figure 5.8: Correlation between FEV1 and HRCT Score
Spearman r -0.6648, p=0.0132

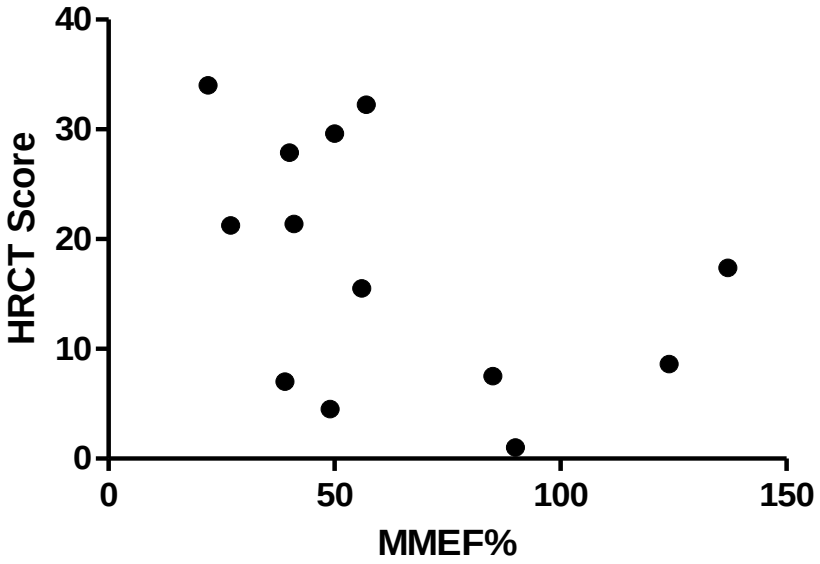

Figure 5.9: Correlation between MMEF and HRCT Score
Spearman r -0.3681, p=0.2159

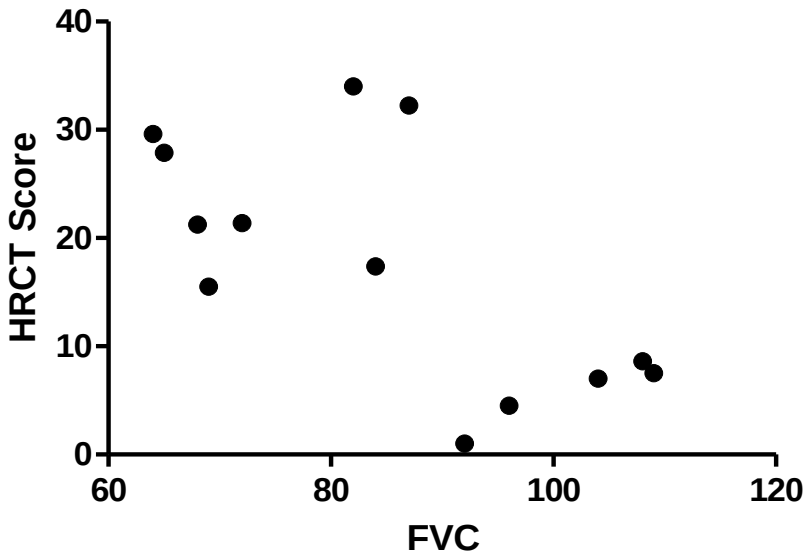

Figure 5.10: Correlation between FVC and HRCT Score
Spearman r -0.6374, p=0.0191

5.5.8 Lung Clearance Index as a marker of disease

LCI was assessed as a marker of disease using receiver operating characteristics curves (ROC) and was assessed against other markers of lung disease by calculating the area under the curve (AUC). AUC-ROC confirmed LCI as a more sensitive indicator of disease when compared with FEV_1, FVC and MMEF. (Area under the curve (AUC) LCI 0.8934 vs. FEV_1 0.8280, FVC 0.7452, MMEF 0.8024).

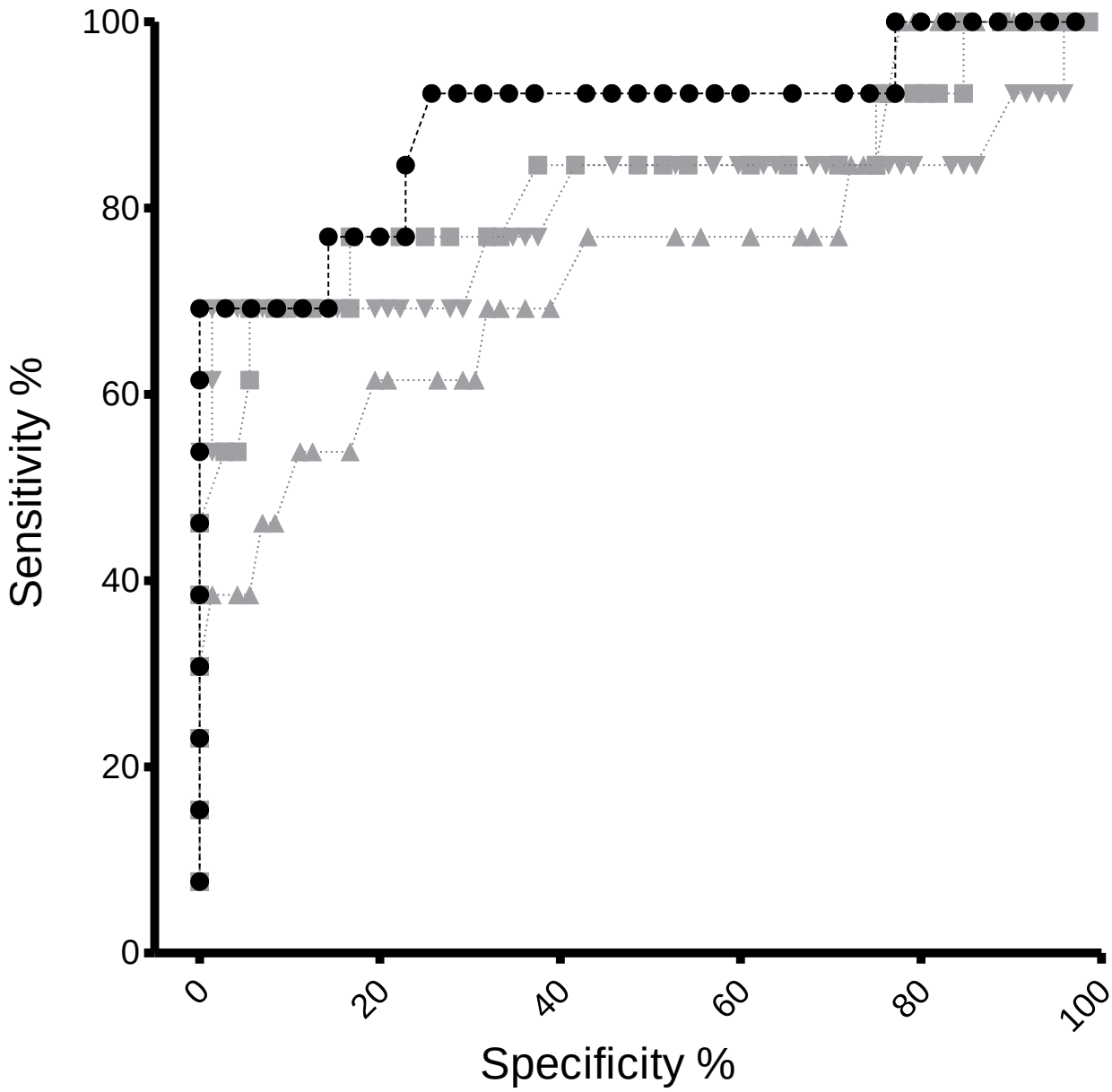

Figure 5.11: ROC curve analysis

- ***LCI, AUC 0.8934, p<0.0001,***
- ***FEV_1, AUC 0.8280, p=0.00018***
- ***FVC, AUC 0.7452, p=0.005***
- ***MMEF, AUC 0.8024, p=0.00055***

5.6 Discussion:

5.6.1 Summary of principal findings

The aim of this chapter was to report findings of data from the ndd EasyOne Pro device from a group of children with bronchiectasis and with comparison to a group of healthy children without lung disease.

The test was performed successfully by children with bronchiectasis, although the test did take longer than in children without lung disease. Those with bronchiectasis had significantly worse LCI, FEV_1 and FVC. LCI in those with bronchiectasis correlates with FEV_1, FVC and with HRCT scores. LCI was also shown to be a sensitive marker of disease and more sensitive than FEV_1, FVC or MMEF.

5.6.2 Normative Data:

In this cohort, of healthy children, the mean LCI was 7.19 (+/- 0.6) which gives an upper limit of normal of 8.38 (mean +1.96*SD). Comparison with other normative data is made more complicated by the variety of equipment and gases used to achieve MBW. This is discussed in greater detail in section 4.4 but will be covered in brief here.

It has been shown that washout using sulfur hexafluoride gives lower lung clearance index than using nitrogen with some studies achieving simultaneous measure of LCI using both techniques to demonstrate this (97,98,102). This is important as it helps to confirm that testing with either gas set up should be considered as an independent measure and the results are not interchangeable. Specific data for the ndd EasyOne Pro LAB is also available for different gas

set ups. Fuchs et al (107) describe a group of children and young adults using SF6 with a mean LCI of 6.2 (+/- 0.39). For nitrogen washout in children using the ndd EasyOne Pro LAB, the mean LCI has been shown to range from 6.78 to 7.4 (see Table 4.3) which is in keeping with the findings of this study and supports the use of the device across a range of settings. Fuchs et al (108) have explored the use of the EasyOne Pro device in a multi-centre setting (using SF6 as the washout gas) and showed good feasibility and low variability across the range of settings. This will be important for the cross centre working and support that is required to operate lung clearance index as a practical test.

The reasons for higher LCI with nitrogen washout have been explored and is likely to be multifactorial (212). Nitrogen back diffusion from the body has been shown to affect the washout readings and different groups have suggested analysis methods to take account of this feature. Furthermore, in disease, nitrogen washout from poorly ventilated lung units may contribute to the washout therefore giving a higher LCI compared to the same lung unit that may not be penetrated during the wash-in phase if using exogenous tracer gases (such as SF6). Finally, when considering the use of nitrogen as the washout gas, the impact of breathing 100% oxygen should be considered. It has long been understood that changes in the respiratory rate and other features of tidal breathing can influence lung clearance index (98,99).

5.6.3 LCI in children with bronchiectasis and comparison to published data

5.6.3.1 Raised LCI in children with bronchiectasis

LCI is raised in children with non-PCD, non-CF bronchiectasis compared to healthy children identifying it as a possible tool to support diagnosis or monitor disease. This increase in LCI is similar to findings in other pulmonary conditions that can give small airways obstruction such as cystic fibrosis and primary ciliary dyskinesia. Comparison between these cohorts of patients is challenging as both cystic fibrosis and primary ciliary dyskinesia have ranges of 'severity' of disease and therefore variation in the recorded lung clearance index. Comparison to bronchiectasis, where there is perhaps even greater range owing to the multiple aetiologies that underly the bronchiectasis, is therefore difficult.

Within studies of participants with bronchiectasis in paediatric cohorts, one study compares groups of children with bronchiectasis, healthy children and those with CF (with comparable Bhalla scores (29) of HRCT for those with lung disease). LCI, FEV_1 and peak oxygen uptake (VO_2 peak) was not significantly different between those with CF and bronchiectasis, but both disease cohorts had significantly worse LCI compared to the health cohorts.

In adults, Gonem et al (170) examined cohorts of those with bronchiectasis, healthy participants and participants with CF. Again, both cohorts of those with lung disease had worse lung function (FEV_1, FVC and LCI) when compared to the healthy cohort. However, despite being of comparable age, there was a significant difference between cohorts of patients with bronchiectasis and CF in body mass index, FEV_1, FVC and LCI, with the participants with CF having worse lung function and lower BMI. This may be due to the nature of cystic fibrosis in

that it is a progressive disease with ongoing lung damage and nutritional issues, compared to bronchiectasis where in some cases, the underlying pathology is arrested. (This is difficult to judge in this cohort as aetiology of bronchiectasis is not directly reported).

All other cohorts reported in the early systematic review, Chapter 2:, describe raised LCI in participants with bronchiectasis although there is a range, and as shown in our patient cohort, LCI can be normal despite the presence of bronchiectasis on HRCT.

5.6.3.2 LCI and relationship to spirometry metrics

LCI correlated with the spirometry metrics FEV_1 and FVC, this has not been demonstrated in a paediatric cohort before. The degree of correlation was greater for FEV_1 than for FVC (r = -0.79 vs. r = -0.75). In one other published study in adults, FEV_1 was also shown to correlate to a greater extent than FVC (172). This would be consistent with the understanding of lung clearance index in how the metric is affected by ventilation inhomogeneity, and in turn, how the causes of ventilation inhomogeneity effect FEV_1. This is expanded upon in section 1.3.2. Both FEV_1 and LCI demonstrate obstruction in the small airways and influences flow and ventilation inhomogeneity respectively.

LCI did not correlate with MMEF in this study. This is contrary to findings in one large adult study (173) where it was demonstrated that LCI correlated negatively with MMEF (r = -0.64, P<0.01). MMEF is highly dependent on the validity of the FVC measurement and the level of expiratory effort (213,214). The challenge of achieving optimal spirometry technique in

the children in this study may be impacting the ability to accurately record MMEF and therefore demonstrate correlation.

5.6.3.3 LCI and sensitivity to disease

In this study we have demonstrated the increased sensitivity of LCI to disease when compared to metrics of spirometry (FEV_1, FVC, MMEF). In the six participants who had a normal FEV_1, two had an abnormal LCI. This demonstrates patients in whom objective lung function tests using spirometry would be identified as normal but show abnormality when assessed with LCI. Both patients, who had FEV_1 of 88% and 112%, had normal FVC (92% and 112%) and normal MMEF (90% and 124%). Conversely, all patients with abnormal FEV_1 had abnormal LCI, with LCI increasing as FEV_1 decreased.

The sensitivity of LCI has been examined in studies of adults with bronchiectasis where it was also shown to be more sensitive than all spirometry markers (170,171). Gonem et al (170) demonstrated in their cohort of adults with bronchiectasis that 69% of participants had a normal FEV_1, and of that group, 40% had an abnormal LCI. They also illustrated that all those participants with an abnormal FEV_1 also had abnormal LCI. Rowan et al (171) demonstrated the greater sensitivity of LCI using ROC analysis. Here, the area under the curve for LCI was 0.96 ($p<0.0001$), greater than FEV_1 (0.82, $p<0.0001$). These findings are similar to our study where area under the curve for LCI was 0.89 ($p<0.0001$), and FEV_1 0.82 ($p=0.00018$).

Explanation for the ability of LCI to more sensitively detect disease has been explored in this work and in the literature extensively, mainly in the field of cystic fibrosis, section 1.4.1.4, (55,69). The likely rationale for this more sensitive ability in cystic fibrosis is that the early disease process in CF delivers airway obstruction in the small airways, the anatomical location that influences ventilation inhomogeneity. (Later disease and obstruction of bigger airways or more numerous small airways has more effect on air flow, therefore influences spirometry measures such as FEV_1.) This pathophysiological mechanism would also seem to be correct in bronchiectasis with regards to its effect on lung clearance index and spirometry.

5.6.3.4 LCI and high-resolution computed tomography (HRCT) images

We have shown how LCI correlates with HRCT scored using the Brody score (178) and how LCI also correlates with various components of that score, Table 5.3 and Figure 5.7. In our study, bronchiectasis and parenchymal change ('sub score' elements of the Brody score) were significantly correlated with LCI. In comparison with the published data in children, LCI has been shown to correlate with HRCT using alternate scoring systems (modified Bhalla (177)) (164). In this work by Irving et al, extent of bronchiectasis, severity of bronchiectasis, airway wall thickening, and air trapping were all significantly associated.

In an adult study assessing HRCT using the Brody score, correlation was also found between LCI and; bronchiectasis, parenchymal change, mucus plugging, and air trapping. All were found to be significantly correlated (171). Air trapping was not assessed in this study as previously addressed. It is not clear why mucus plugging was significantly correlated in this

adult study and not in our study of children. In the study by Rowan et al, participants had similarly affected lung function measured by spirometry (mean FEV_1 76.5% vs 76.3% in our study) and LCI (mean LCI 9.1 vs 9.5 in our study). HRCT in their study however was performed on the same day as the record of lung function. In our study, the mean time between lung function record and HRCT was 18 months, (range 194 days to 730 days) and so it is important to consider the possible impact of this.

Mucus plugging on HRCT has shown to be improved with treatment in cystic fibrosis (215) and the nature of this particular pathology is that secretions are mobile along the airways. It is therefore reasonable to assume that any mucus plugging identified on the diagnostic imaging in our cohort would appear differently on contemporaneous imaging as treatment was usually commenced following diagnosis. This highlights the possibility that the increased delay between imaging and lung function tests in our study do not allow for the accurate assessment of correlation between mucus plugging and lung function. The imaging in paediatric patients is performed usually for diagnosis (2). It is not recommended to perform repeat assessment unless there is a clear clinical indication. It is for this reason that the small cohort of paediatric patients with bronchiectasis did not have repeat HRCT imaging performed to coincide with the lung function tests required with this study.

It has been shown that in paediatric bronchiectasis, the extent of disease on HRCT can remain static, show improvement, or even resolve over time (216). This is important when considering the impact of the delay between imaging and lung function testing. In one study of 22 children with bronchiectasis (14), imaging was shown to be static in three (13%) cases, and improve or resolve in 14 (63%) cases. Other data has shown different outcomes with a

smaller proportion improving; in a review of repeat HRCT at 18 months in children with bronchiectasis, 38% showed improved or resolved bronchiectasis, 27% progressed and 33% were unchanged (217). These cohorts of patients were varied with a range of ages (1 year to 18 years old) and underlying causes for bronchiectasis. This does make comparison between these two published cohorts, and between the published cohorts and our data challenging.

Our mean delay between imaging and lung function assessment of 550 days (18 months) is at the lower limit of time where previous resolution has been demonstrated. There is no evidence to suggest which patients may improve and which may worsen. It is possible that HRCT changes may be different after this period of delay either improving or worsening but for important clinical reasons, the pragmatic decision to not repeat HRCT imaging in this study cohort was taken.

5.6.3.5 Clinical context of LCI in bronchiectasis

In children with bronchiectasis there is potential value in the use of lung clearance index in the assessment of disease although the data from this study, in addition to the data from the published literature, does not allow definitive clinical conclusions to be drawn about its practice.

5.6.3.5.1 What might LCI be used for in children with bronchiectasis?

5.6.3.5.1.1 Diagnosis

Lung clearance index would not be used in the diagnosis of bronchiectasis. As previously described, the diagnosis is demonstration of pathology on imaging, typically HRCT, with corresponding symptoms including chronic wet cough and sputum production. Lung clearance index has been shown in our data to be normal in some patients with more mild bronchiectasis, so a normal result does not exclude the diagnosis. A high/abnormal LCI result would not be a specific finding for non-CF, non-PCD bronchiectasis as evidence has demonstrated a raised LCI in a range of other conditions (such as CF, PCD) (71,84). A high/abnormal LCI would however highlight a patient with ventilation inhomogeneity who may need further investigation for the specific cause.

5.6.3.5.1.2 Monitoring

LCI may have a role in the monitoring of disease. The correlation of HRCT changes with LCI means we can consider if LCI could consistently be used as a measure of lung disease as a surrogate marker for HRCT changes. LCI has also been examined in its relationship to other clinically important metrics such as severity scores and symptom reporting (see section 2.9.4). LCI was shown in adults with bronchiectasis to increase with increasing Bronchiectasis Severity Index (BSI) and that it was significantly correlated with symptoms scores (171,174).

LCI has not been investigated in bronchiectasis as a tool for medium or long term monitoring and there is only limited data on short term changes in LCI related to an intervention. Again, in adults, two articles describe the change in LCI following completion of treatment for an exacerbation of bronchiectasis and pre and post physiotherapy. In both publications, they describe how the changes in LCI were small and not significantly different (172,173). This finding supports previous work in CF with variable change in LCI following physiotherapy (109). LCI does change following other therapeutic intervention in cystic fibrosis and has been demonstrated to show a significant change in LCI following initiation or discontinuation of treatment, such as, small molecule precision medication, nebulised hypertonic saline and DNase (76,218,219). LCI is being explored as an important outcome measure for studies in bronchiectasis (220) and is an outcome measure in therapeutic studies currently in progress (NCT03903913 and NCT02765295).

5.7 Conclusion:

Lung clearance index is a sensitive tool in the detection of disease in children with bronchiectasis. It is raised in disease, and correlates with changes on HRCT and with metrics of spirometry. This supports the conclusion that LCI has potential as a clinical tool in the assessment of disease in this cohort.

This study and its conclusions are limited by the relatively small sample size. Paediatric bronchiectasis remains a relatively rare condition, and few patients met the necessary inclusion criteria. Perhaps more importantly, recruitment was challenged due to a curtailed

time frame and limitations on participant movement due to the COVID-19 pandemic. These issues effected recruitment and may have not allowed for adequate assessment of the relationships between LCI and the other various measures. Reassuringly, the relationships seen in our data is consistent with previously published data but further research will be important.

Further work will also be helpful in establishing the practical use of LCI in children with bronchiectasis. The variability in relationship to exacerbation is not understood, nor is the response to therapy,

Chapter 6: Final Discussion

6.1 Principal findings

The original hypotheses of this project were;

- there are no differences in Lung Clearance Index in healthy children from differing ethnic backgrounds, and,
- in younger children with non-cystic fibrosis, non-primary ciliary dyskinesia bronchiectasis, with evidence of disease on CT imaging, that Lung Clearance Index is a more sensitive disease marker than FEV_1.

In accordance with my hypotheses, LCI was not significantly different between two cohorts of children aged 6 years to 12 years who were from a White British ethnic background and an Indian Subcontinent ethnic background. In addition, LCI was raised significantly in children with bronchiectasis when compared to healthy control participants, LCI correlated significantly with FEV_1, FVC and HRCT scored using a recognised scoring system. LCI was more sensitive to disease than both FEV_1 and FVC. These findings are supported by the work of Irving et al (164) who demonstrated correlation between HRCT scores and LCI in a group of children with bronchiectasis.

6.2 Strengths and Limitations

The time delay between imaging and recording of LCI was one of the weaknesses of my study. Repeating HRCT without a clear clinical indication is not recommended practice and could not be performed for ethically important reasons (2). Change in HRCT imaging may have occurred within the time delay between the time of image and time of recording of LCI. Future work should include LCI performed at the time of imaging in order to confirm these findings.

A further weakness of the study was the small numbers of the cohort of children with bronchiectasis and the cohorts of children from non-White British ethnicities. The 13 children with bronchiectasis were opportunistically recruited after a careful review of patient databases and imaging records. The relative rarity of paediatric bronchiectasis, and the necessary exclusions for the study, limited potential recruits. The timing of the study fell within the international COVID-19 pandemic that greatly affected usual health care practice and affected recruitment through the necessary limitations on activity within hospitals at that time. Despite the reduced number, the cohort of patients is the largest reported group of children with bronchiectasis with recorded LCI. This data is supported by previous work and is in keeping with adult data. I am therefore confident that the limited numbers would have a limited impact on the findings of the study into children with bronchiectasis.

This work is one of the largest cohorts of children with bronchiectasis who were examined with lung clearance index and provides the largest cohort when comparing LCI to imaging. This work will support future research and help develop the role of LCI in the future clinical application in this area.

Recruitment of healthy children from various ethnicities was principally restricted due to the COVID-19 pandemic. The necessity of recruitment of healthy children was in direct conflict with clinical practice guidelines at the time, those being the restriction of 'clinically unnecessary' attendances at hospital. The practicalities of the test limited its use outside the hospital setting.

This project highlighted to me and the base hospitals the importance of recruitment in to studies from a range of ethnic groups. I was fortunate to have the support of the Equality, Inclusion and Diversity group and the topic has been raised at a senior level. I am grateful the work has helped highlight an important aspect of developing high quality research.

6.3 Current literature exploring LCI in children with bronchiectasis

There is very limited data examining the utility of LCI in children with bronchiectasis. I completed a systematic review finding only five published articles in childhood with limited patient numbers, the quality of which does not allow for definitive conclusions. In adults, the evidence is better with seven articles described much larger patient cohorts. The quality of study in the adult cohort was generally significantly higher.

The main findings of the paediatric data identified raised LCI in children with bronchiectasis and found significant correlation between LCI, HRCT findings and FEV_1 (164).

This highlights the need for more data to demonstrate the utility of LCI in children. This is important due the current lack of clinical endpoints in bronchiectasis in general, but specifically in children. There is a lack of evidence supporting treatment options in bronchiectasis and this

in part may be due to the lack of an important trial endpoint. Several previous have found it difficult to demonstrate a treatment response in bronchiectasis to therapy (39,40).

In adults, the data is of better quality and several important relationships between LCI and other metrics of lung disease were highlighted. LCI is abnormal and it correlates with FEV_1 and markers of disease on CT. These findings suggest that LCI may be a marker of disease severity but the volume of data and heterogeneity of the studies limits conclusion.

Additional work is required to further explore this relationship in children and to identify the clinical relevance of LCI results and how it can best be used to guide and monitor healthcare.

6.4 LCI in healthy children from ethnic backgrounds

There is developing data for LCI across a range of devices, washout gases and age groups although this is far from being as robust as other measures of lung function, such as spirometry. There are important details to be established to overcome what is a weakness of LCI measured by multiple breath washout.

In my study I demonstrated a normal range of LCI using the ndd EasyOne Pro LAB that was in keeping with previously described details. My cohort of 51 patients able to perform LCI represents the largest group of patients contributing to a normal range for this device. The participants of this study age between 6 years and 12 years. The normative data for this relatively narrow range of participants is helpful in establishing appropriate normative data for a lung function test.

I have shown that two groups of participants who self-identified from different ethnic backgrounds had no significant difference in LCI which is in keeping with other data (148). This conclusion helps support the use of data with a population undifferentiated by ethnicity although definitive conclusion is limited due to the small numbers of participants in this study. This study also highlighted the importance of recruitment of participants from a range of socioeconomic backgrounds and ethnicities to ensure appropriateness for the general population in whom the test would be available for. A strength of this study was the range of participants from across the socioeconomic spectrum that were recruited to perform lung function.

6.5 Feasibility of performing LCI using the ndd EasyOne Pro LAB

70.8% of healthy participants successfully completed an LCI measure in their first attempt at any form of lung function. 100% of participants with bronchiectasis completed an LCI measure on their first attempt at LCI (although all had previously performed other lung function measures, most commonly spirometry). This is in keeping with previously published data (108), although better success rates up to 100% have been demonstrated (113).

The improvement in success rates over the course of my study highlights an important issue that has been discussed previously in the literature (108) and supports a key feature in the assessment of lung clearance index; the familiarity of staff and subject with the test. Personal experience with LCI test, using the ndd EasyOne Pro LAB, and using other devices that measure LCI. have illustrated to me this important point. As staff experience increases, and

with high quality training, the likelihood of good quality data being produced more consistently increases. Training by an experienced tertiary centre played an important role in the success of this study and the prospect of the test being adopted into clinical practice identifies the need for networks to exist to provide access to this limited resource.

In this study examining LCI performed for the first time by this group of participants, the time required to complete the test was considerable. The mean test time was almost 30 minutes and was in keeping with other LCI measures using the same device (107). The number of washouts required to achieve the success rate in this study was up to 10. This may be a practical barrier to performing the test in a clinical setting. Limited numbers of trained staff, and prolonged test time would restrict access to potential patients and therefore limit the potential utility of the test.

It is difficult to draw conclusions as to if the number of trials required, and therefore the total test time, would decrease with increasing staff experience and increasing participant experience. Anecdotally, I have performed LCI repeatedly in the same subject on several occasions over the course of several months. It was noticeable to me that the test was easier to complete for the patient perhaps due to degree of reduced novelty in the task they were being asked to do, plus additional familiarity with the technique. It is difficult to separate the effect of my personal increased skill over time. Further work is required to establish the what the real-world impact would be of commencing such as test.

6.6 Utility of LCI in children with bronchiectasis and future research

This study supported the hypothesis that LCI was more sensitive to HRCT changes of bronchiectasis than FEV_1 along with the other clinically important points summarise in section 6.1. This statement is made within the context of the time lapse between the HRCT imaging and the LCI being performed. To conclude; LCI may well have a future role in the monitoring of bronchiectasis, particularly in children in whom objective measures of disease are very helpful in addition to the more subjective records of symptoms and illness.

Further work should examine the value of LCI in the clinical assessment of patients under investigation for bronchiectasis. LCI performed in conjunction with HRCT at diagnosis would provide a contemporary measure of ventilation inhomogeneity that may support the findings of imaging correlation shown in this work.

Future research should consider assessment of LCI longitudinally through the course of patient care including around the commencement of discontinuation of therapies. LCI is currently being explored as a clinical outcome measure in therapeutic studies. This is another important areas in the development of evidence based treatment options for patients with bronchiectasis.

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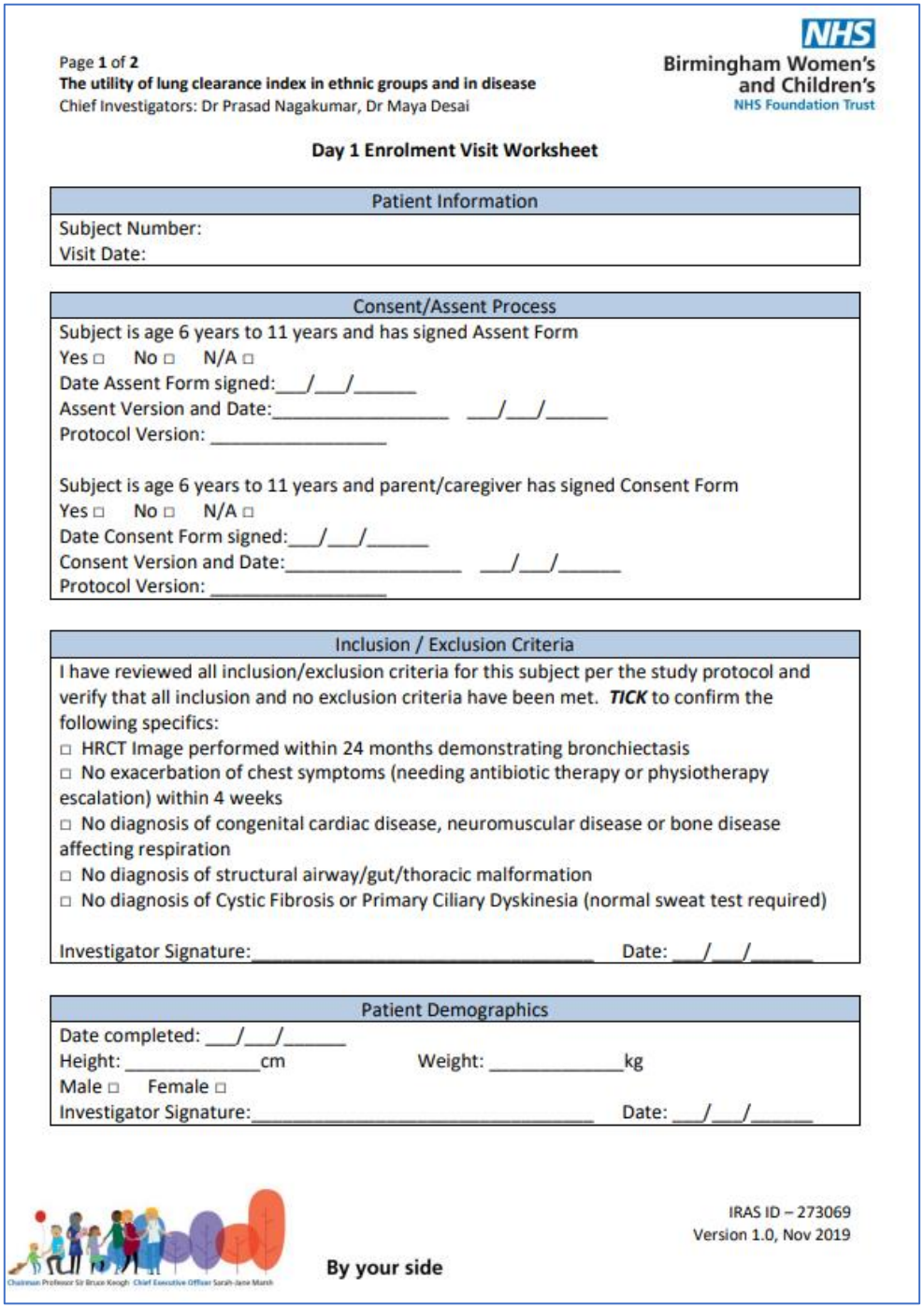
Chapter 8: Appendices:

8.1 Appendix 1

Demographic data collection worksheet (Participants with bronchiectasis, page 1)



Page **1** of **2**
The utility of lung clearance index in ethnic groups and in disease
Chief Investigators: Dr Prasad Nagakumar, Dr Maya Desai

NHS
Birmingham Women's and Children's
NHS Foundation Trust

Day 1 Enrolment Visit Worksheet

Patient Information
Subject Number: Visit Date:

Consent/Assent Process
Subject is age 6 years to 11 years and has signed Assent Form Yes □ No □ N/A □ Date Assent Form signed:___/___/_____ Assent Version and Date:_____ ___/___/_____ Protocol Version: _____ Subject is age 6 years to 11 years and parent/caregiver has signed Consent Form Yes □ No □ N/A □ Date Consent Form signed:___/___/_____ Consent Version and Date:_____ ___/___/_____ Protocol Version: _____

Inclusion / Exclusion Criteria
I have reviewed all inclusion/exclusion criteria for this subject per the study protocol and verify that all inclusion and no exclusion criteria have been met. ***TICK*** to confirm the following specifics: □ HRCT Image performed within 24 months demonstrating bronchiectasis □ No exacerbation of chest symptoms (needing antibiotic therapy or physiotherapy escalation) within 4 weeks □ No diagnosis of congenital cardiac disease, neuromuscular disease or bone disease affecting respiration □ No diagnosis of structural airway/gut/thoracic malformation □ No diagnosis of Cystic Fibrosis or Primary Ciliary Dyskinesia (normal sweat test required) Investigator Signature:_____ Date: ___/___/_____

Patient Demographics
Date completed: ___/___/_____ Height: _____cm Weight: _____kg Male □ Female □ Investigator Signature:_____ Date: ___/___/_____

Chairman Professor Sir Bruce Keogh Chief Executive Officer Sarah-Jane Marsh

By your side

IRAS ID – 273069
Version 1.0, Nov 2019

Demographic data collection worksheet (Participants with bronchiectasis, page 2)

The utility of lung clearance index in ethnic groups and in disease

Chief Investigators: Dr Prasad Nagakumar, Dr Maya Desai

NHS
Birmingham Women's and Children's
NHS Foundation Trust

Multiple Breath Washout
Date completed: ___/___/_____ Time completed: ____:____ (24hr clock)
Estimated tidal volume (10-15ml/kg):_____ to _____ml/kg
Were three acceptable trials performed? ***TICK*** Yes □ No □ N/A □
Comments:
Mean LCI 2.5:_____

Spirometry
Date completed: ___/___/_____ Time completed: ____:____ (24hr clock)
Were three acceptable trials performed? ***TICK*** Yes □ No □ N/A □
Comments:
FEV_1:_____ L _____% Predicted FVC:_____ L _____% Predicted

Ethnic Group Question

TICK the box chosen using guide sheet:

White
- □ English/Welsh/Scottish/Northern Irish/British
- □ Irish
- □ Gypsy or Irish Traveller
- □ Any other White background:

_____

Black/African/Caribbean/Black British
- □ African
- □ Caribbean
- □ Any other Black/African/Caribbean background, write in:

_____

Other ethnic group
- □ Arab
- □ Any other ethnic group, write in:

_____

Mixed / multiple ethnic groups
- □ White and Black Caribbean
- □ White and Black African
- □ White and Asian
- □ Any other Mixed/multiple ethnic background, write in:

_____

Asian / Asian British
- □ Indian
- □ Pakistani
- □ Bangladeshi
- □ Chinese
- □ Any other Asian background, write in

_____

Home Environment Questions
First three letters of postcode: _____
Does the subject live in a house with an adult who smokes: Yes □ No □

By your side

IRAS ID – 273069
Version 1.0, Nov 2019

Demographic data collection worksheet (Healthy Participants, page 1)

The utility of lung clearance index in ethnic groups and in disease
Chief Investigators: Dr Prasad Nagakumar, Dr Maya Desai

NHS
Birmingham Women's and Children's
NHS Foundation Trust

Day 1 Enrolment Visit Worksheet

Patient Information
Subject Number: Visit Date:

Consent/Assent Process
Subject is age 6 years to 11 years and has signed Assent Form Yes □ No □ N/A □ Date Assent Form signed:___ /___ /_____ Assent Version and Date:_____ ___/___/_____ Protocol Version: _____ Subject is age 6 years to 11 years and parent/caregiver has signed Consent Form Yes □ No □ N/A □ Date Consent Form signed:___/___/_____ Consent Version and Date:_____ ___/___/_____ Protocol Version: _____

Inclusion / Exclusion Criteria
I have reviewed all inclusion/exclusion criteria for this subject per the study protocol and verify that all inclusion and no exclusion criteria have been met. ***TICK*** to confirm: □ No previous hospital admission for a respiratory condition □ No physician diagnosis of asthma at any previous time □ No history of chronic productive cough (greater than 6 weeks), recurrent wheezing (greater than two episodes) within the previous 12 months □ Born at term gestation (greater than 37 weeks) □ No diagnosis of Intrauterine Growth Restriction □ No diagnosis of congenital cardiac disease, neuromuscular disease or bone disease affecting respiration Investigator Signature:_____ Date: ___/___/_____

Patient Demographics
Date completed: ___/___/_____ Height: _____cm Weight: _____kg Male □ Female □ Investigator Signature:_____ Date: ___/___/_____

By your side

IRAS ID – 273069
Version 1.0, Nov 2019

Demographic data collection worksheet (Healthy Participants, page 2)

The utility of lung clearance index in ethnic groups and in disease

Chief Investigators: Dr Prasad Nagakumar, Dr Maya Desai

NHS
Birmingham Women's and Children's
NHS Foundation Trust

Multiple Breath Washout
Date completed: ___/___/_____ Time completed: ____:____ (24hr clock)
Estimated tidal volume (10-15ml/kg):_____ to _____ml/kg
Were three acceptable trials performed? ***TICK***
Yes □ No □ N/A □
Comments:
LCI 2.5:_____

Spirometry
Date completed: ___/___/_____ Time completed: ____:____ (24hr clock)
Were three acceptable trials performed? ***TICK***
Yes □ No □ N/A □
Comments:
FEV_1:_____ L _____% Predicted FVC:_____ L _____% Predicted

Ethnic Group Question

TICK the box chosen using guide sheet:

White
- □ English/Welsh/Scottish/Northern Irish/British
- □ Irish
- □ Gypsy or Irish Traveller
- □ Any other White background:

_____

Black/African/Caribbean/Black British
- □ African
- □ Caribbean
- □ Any other Black/African/Caribbean background, write in:

_____

Other ethnic group
- □ Arab
- □ Any other ethnic group, write in:

_____

Mixed / multiple ethnic groups
- □ White and Black Caribbean
- □ White and Black African
- □ White and Asian
- □ Any other Mixed/multiple ethnic background, write in:

_____

Asian / Asian British
- □ Indian
- □ Pakistani
- □ Bangladeshi
- □ Chinese
- □ Any other Asian background, write in

_____

Home Environment Questions
First three letters of postcode: _____
Does the subject live in a house with an adult who smokes: Yes □ No □

By your side

8.2 Appendix 2: HRCT Score Spreadsheet

Bronchiectasis = presence of one or more of: bronchoarterial ratio >1, nontapering bronchus, bronchus within 1cm of pleura, bronchus abutting mediastinal pleura. Average bronchiectasis = degree of dilation most frequently seen

Complete questions below to generate multiplier score if bronchiectasis present

Patient Details		Bronchiectasis score (range 0-12)							Bronchiectasis Multiplier				
Study Number	Patient ID		Extent of bronchiectasis in central lung		Extent of bronchiectasis in peripheral lung		Bronchiectasis multiplier (right) Multiplier	Score	Sub Total		Size of largest dilated bronchus		Average size of dilated bronchi		Size Multiplier
Test1	Test12345	Right Upper	None	0	None	0	1.00	1	0	Right Upper	≤2x	1	≤2x	1	1
		Right Middle	None	0	None	0	1.00	1	0	Right Middle	≤2x	1	≤2x	1	1
		Right Lower	None	0	None	0	1.00	1	0	Right Lower	≤2x	1	≤2x	1	1
		Left Upper	None	0	None	0	1.00	1	0	Left Upper	≤2x	1	≤2x	1	1
		Lingular	None	0	None	0	1.00	1	0	Lingular	≤2x	1	≤2x	1	1
Total Score	#REF!	Left Lower	None	0	None	0	1.00	1	0	Left Lower	≤2x	1	≤2x	1	1

Mucous Plugging Central = opacity filling a defined bronchus. Mucous Plugging Periph = either dilated mucous filled bronchi or peripheral thin branching structures or centrilobular nodules in peripheral lung

Bronchial Wall Thickening = defined as a bronchial wall thickness of >2mm in hila, 1mm central lung, and 0.5mm in peripheral lung

	Mucous plugging score (range 0-6)					Peribronchial thickening score (range 0-9)					Sub Total
	Extent of mucous plugging in central lung		Extent of mucous plugging in peripheral lung		Sub Total		Extent of peribronchial thickening in central lung	Extent of peribronchial thickening in peripheral lung	Severity of peribronchial thickening		
Right Upper	None	0	None	0	0	Right Upper	None	0	Mild	1	0
Right Middle	None	0	None	0	0	Right Middle	None	0	Mild	1	0
Right Lower	None	0	None	0	0	Right Lower	None	0	Mild	1	0
Left Upper	None	0	None	0	0	Left Upper	None	0	Mild	1	0
Lingular	None	0	None	0	0	Lingular	None	0	Mild	1	0
Left Lower	None	0	None	0	0	Left Lower	None	0	Mild	1	0

Air trapping = areas of lung on expiratory images that remained similar in attenuation on inspiratory images.

	Parenchyma score (range 0-9)							Hyperinflation score (range 0-4.5)				Lobar Total
	Extent of dense parenchymal opacity		Extent of ground glass opacity		Extent of cysts or bullae		Sub Total		Extent of air trapping		Appearance of air trapping (if present)	Sub Total	
Right Upper	None	0	None	0	None	0	0	Right Upper	None	0	Subsegmental	1	0	0	0
Right Middle	None	0	None	0	None	0	0	Right Middle	None	0	Subsegmental	1	0	0	0
Right Lower	None	0	None	0	None	0	0	Right Lower	None	0	Subsegmental	1	0	0	0
Left Upper	None	0	None	0	None	0	0	Left Upper	None	0	Subsegmental	1	0	0	0
Lingular	None	0	None	0	None	0	0	Lingular	None	0	Subsegmental	1	0	0	0
Left Lower	None	0	None	0	None	0	0	Left Lower	None	0	Subsegmental	1	0	0	0
														Grand Total	0