Non-invasive Assessment of Small Airway Obstruction in Asthma

Thesis submitted for the degree of

Doctor of Philosophy

at the University of Leicester

by

Sherif Gonem BM BSc MRCP

Department of Infection, Immunity and Inflammation

University of Leicester

February 2015
Non-invasive Assessment of Small Airway Obstruction in Asthma

Sherif Gonem

Abstract
Small airway inflammation and remodelling occurs in asthma and may underpin disease persistence, since conventional inhaled treatments do not penetrate to the small airway compartment. There is an important unmet need for reliable and non-invasive methods to measure small airway obstruction. This thesis describes the development and validation of such methods, with a particular focus on the multiple breath inert gas washout (MBW) technique. *In vitro* validation of the sulphur hexafluoride wash-in technique for performing MBW demonstrated that the method yields accurate and repeatable results. We developed novel methods for analysing inert gas washout curves, and showed that the parameters derived are repeatable, capture the full information content of the curve, and may be superior to standard parameters in distinguishing between subphenotypes of airway diseases. MBW and impulse oscillometry (IOS) parameters were found to be repeatable within-visit, but IOS parameters displayed greater stability over time. IOS parameters were independent predictors of asthma symptoms, quality of life and exacerbation frequency, suggesting that IOS may add value to spirometry in the assessment of patients with asthma. The response of small airway biomarkers to an intervention was assessed within a clinical trial of a novel anti-eosinophilic agent. Significant treatment effects were observed with respect to spirometric airflow obstruction and air trapping, as well as with a number of MBW parameters. The structural correlates of ventilation heterogeneity in asthma were examined using hyperpolarised $^3$helium magnetic resonance and quantitative computed tomography. There was evidence for a structural abnormality in the pulmonary acinus in patients with asthma causing subtle alterations in diffusion within this compartment. Future studies should seek to further understand the structural basis of IOS and MBW parameters through computational modelling and the coupling of physiological measurements and functional imaging. Longitudinal studies are also required to assess the long-term significance of small airway biomarkers.
Acknowledgements

I would like to thank the patients and healthy volunteers who willingly gave up their time to make this work possible.

I would like to thank my supervisors Dr. Salman Siddiqui and Prof. Chris Brightling for their mentoring and support over the past three years, and for encouraging me to develop my own research ideas.

This work could not have been carried out without the help of many people both within and outside the Department. I would like to thank the research nurses who were involved in my studies, Beverley Hargadon, Kate Hadley, Marcia Soares, and in particular Amisha Singapuri and Michelle Bourne for going above and beyond the call of duty on countless occasions to help deliver our interventional trial.

I would like to thank my colleagues Rachid Berair, without whose help the interventional trial would not have been the success it was, and Ruth Hartley for performing the CT analysis. I would also like to thank Sushiladevi Natarajan, Alys Scadding, Steven Corkill and Dhananjay Desai for their help with patient characterisation and physiological measurements, and Nisha Rana for performing the induced sputum cell counts.

I would like to thank the many external collaborators I have worked with over the past three years, in particular Per Gustafsson for his help with multiple breath inert gas washout, and for kindly accommodating me at his house in Skövde while we validated our system; Florian Singer for his help with the lung phantom validation experiments; John Owers-Bradley and his team Iain Ball, Steven Hardy and Niels Buhl for performing the magnetic resonance measurements reported in this thesis; and Alex Horsley for kindly providing me with his multiple breath washout data in cystic fibrosis patients.
I would like to acknowledge the funding I have received from the AirPROM consortium, Novartis, Chiesi and Roche, and to thank our industry contacts Richard Kay and Rino Costanza.

Finally, I would like to thank my family for their love, encouragement and support, in particular my parents Nabil and Hoda, my sisters Sarah and Rania, my wife Shaymaa and my daughter Mariam.
**Statement of work personally performed**

I have been intellectually involved in the development of all the studies reported in this thesis, including writing scientific protocols and assisting with ethics applications and amendments. I performed all statistical analysis of data presented in this thesis, interpreted the data and wrote the resulting manuscripts. I travelled to Skövde, Sweden to undertake validation of our multiple breath washout system using a lung model built by Dr. Per Gustafsson. I performed these validation experiments under Dr. Gustafsson’s supervision and carried out all the data extraction and analysis. I independently developed novel multiple breath washout parameters and validated these using our own multiple breath washout data and raw data kindly provided to me by Dr. Alex Horsley in patients with cystic fibrosis. I performed 50% of our multiple breath washout tests and analysed 100% of the washout curves reported in this thesis. I took a major role in the clinical characterisation of patients in each of my studies, including the collection of clinical and demographic details, physical examination, and the performance of spirometry, impulse oscillometry, sputum induction, and measurement of exhaled nitric oxide. I was the main sub-investigator on the clinical trial of a CRTH2 receptor antagonist, and was in charge of the day-to-day running of the trial, together with the research nurses, and coordinated the recruitment effort.
Publications arising from this thesis

Original research articles

**Gonem S** et al. Randomized controlled trial of the prostaglandin D2 receptor antagonist QAW039 in persistent eosinophilic asthma. *Manuscript in preparation.*


Review articles


Abstracts and prizes

As a result of the work presented in this thesis I have presented 13 abstracts at European Respiratory Society and British Thoracic Society meetings. In 2014 I was awarded the ERS Excellence Grant in Clinical Physiology and Exercise, and won the Best Abstract Competition award.
### Contents

1 **Introduction**

1.1 Overview............................................................................................................. 18

1.2 Small airway disease in asthma................................................................. 20

1.3 Non-invasive physiological tests of small airway obstruction
   1.3.1 Spirometry.................................................................................................. 24
   1.3.2 Lung volumes.............................................................................................. 27
   1.3.3 Forced oscillation technique...................................................................... 28
   1.3.4 Multiple breath inert gas washout............................................................ 34

1.4 Imaging techniques for the assessment of small airway structure
   1.4.1 Morphometry of the human airway tree............................................... 43
   1.4.2 Quantitative computed tomography...................................................... 44
   1.4.3 Hyperpolarised noble gas magnetic resonance imaging..................... 45

1.5 Aims and hypotheses....................................................................................... 48

2 **Methods**

2.1 Spirometry........................................................................................................ 51
2.2 Measurement of lung volumes using body plethysmography.................... 51
2.3 Single breath determination of carbon monoxide uptake in the lung......... 52
2.4 Impulse oscillometry....................................................................................... 52
2.5 Multiple breath inert gas washout................................................................. 54
2.6 Validation of multiple breath washout technique using a lung model....... 61
2.7 Development of novel multiple breath washout parameters..................... 65
2.8 Quantitative computed tomography............................................................. 78
2.9 Hyperpolarised $^3$helium diffusion magnetic resonance.......................... 78
3 Studies

3.1 Validation of a photoacoustic gas analyser for the measurement of functional residual capacity using multiple breath inert gas washout........... 86

3.2 Specific ventilation inequality and dead space components of lung clearance index in patients with cystic fibrosis and non-cystic fibrosis bronchiectasis………………………………………….. 100

3.3 Between-visit variability of small airway obstruction markers in patients with asthma……………………………………………………………. 125

3.4 Clinical significance of small airway obstruction markers in patients with asthma……………………………………………………….. 133

3.5 Characterisation of acinar airspace involvement in patients with asthma using hyperpolarised $^3$He magnetic resonance and quantitative computed tomography…………………………………………160

3.6 Randomised controlled trial of the prostaglandin D2 receptor antagonist QAW039 in persistent eosinophilic asthma………………… 179

4 Conclusions

4.1 Summary of findings…………………………………………………………...204

4.2 Future work………………………………………………………………….. 206
# List of tables

3.1 List of multiple breath washout validation experiments performed with results………………………………………………………… 93

3.2 Demographic and physiological data across healthy controls, cystic fibrosis patients and non-cystic fibrosis bronchiectasis patients…… 107

3.3 Correlations between multiple breath washout parameters………………..113

3.4 Physiological parameters in patients with and without chronic bacterial colonisation………………………………………………. 115

3.5 Within-visit repeatability of multiple breath washout parameters in cystic fibrosis and non-cystic fibrosis bronchiectasis……… 116

3.6 Between- and within-visit variability of physiological variables in patients with asthma……………………………………………… 130

3.7 Sample size calculations for impulse oscillometry and inert gas washout parameters……………………………………………… 132

3.8 Physiological interpretation of airway obstruction markers……………… 137

3.9 Demographic and clinical data across asthma severity groups……………… 143

3.10 Physiological data across asthma severity groups…………………………144

3.11 Correlations between clinical outcome measures and physiological variables………………………………………………………… 146

3.12 Linear regression models assessing the contributions of physiological variables to ACQ-6 and AQLQ(S) scores…………………148
3.13 Demographic and physiological variables in exacerbation-prone and non-exacerbation-prone patients with asthma…… 149

3.14 Demographic and clinical characteristics of participant groups in magnetic resonance study…………………………………………………………167

3.15 Physiological data across participant groups in magnetic resonance study…………………………………………………………169

3.16 Baseline Characteristics of Subjects in the Intention-to-Treat Population…………………………………………………………188

3.17 Outcome Measures at Baseline and Post-Treatment in the Per-Protocol Population…………………………………………………………195

3.18 Outcome Measures at Baseline and Post-Treatment in the Intention-to-Treat Population…………………………………………………………198
List of figures

1.1 Resistance and reactance within the frequency and time domains…………30

1.2 Inert gas washout curve from a healthy subject………………………….36

1.3 Graph of SF₆ concentration against expired volume during a
single exhalation…………………………………………………………...40

2.1 Innocor photoacoustic gas analyser……………………………………56

2.2 Patient interface for the performance of
multiple breath inert gas washout………………………………………56

2.3 Calculation of phase III slope parameters……………………………..60

2.4 Schematic diagram of lung model for the validation of
multiple breath washout technique…………………………………….62

2.5 Photograph of lung model for the validation of
multiple breath washout technique…………………………………….63

2.6 Exponential decay curves………………………………………………67

2.7 Washout curves from a healthy subject and a patient with
cystic fibrosis fitted to a two-phase exponential decay model……………69

2.8 Simulated washout curves……………………………………………71

2.9 Magnetic resonance pulse sequences for measuring
diffusion in the lungs………………………………………………….83

3.1 Inert gas washout curve of an acrylic glass lung model………………….90
3.2  Bland-Altman plots of calculated versus measured functional residual capacity .........................................................94

3.3  Error in lung clearance index against calculated functional residual capacity .........................................................96

3.4  Multiple breath washout parameters across groups ................. 109

3.5  Correlations between lung clearance index and FEV$_1$ (% predicted) .......... 112

3.6  Receiver operating characteristic curves of lung clearance index and FEV$_1$ (% pred.) for distinguishing between control subjects and bronchiectasis patients ................................. 117

3.7  Scatterplots of forced expiratory volume in one second standardised residuals against multiple breath washout parameters in patients with non-cystic fibrosis bronchiectasis .............................................. 118

3.8  Scatterplots of forced expiratory volume in one second standardised residuals against small airway obstruction markers in patients with asthma ................................................................. 151

3.9  Pie charts showing proportions of patients with concordance or discordance between forced expiratory volume in one second and small airway obstruction markers .................................................. 154

3.10  Quantitative computed tomography densitometry between groups ........ 171

3.11  Apparent diffusion coefficients (ADC) across groups ...................... 172

3.12  Correlations between $^3$He-MR, CT and physiological variables in patients with asthma .................................................. 173
3.13 Change in apparent diffusion coefficient (%) against change in volume of gas in the lungs (%) in healthy subjects and patients with asthma………174

3.14 QAW039 study protocol………………………………………………………………………183

3.15 Number of patients who were screened, randomised and completed the study up to the post-treatment visit…………………………………………………………187

3.16 Changes from baseline to mid-treatment, post-treatment and post-washout visits with respect to main outcomes in the per protocol population…………………………………………………………193

3.17 Changes from baseline to post-treatment and post-washout visits with respect to lung clearance index and resistance at 5Hz in the per protocol population…………………………………………………………194
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACQ</td>
<td>Asthma Control Questionnaire</td>
</tr>
<tr>
<td>ADC</td>
<td>Apparent diffusion coefficient</td>
</tr>
<tr>
<td>AHR</td>
<td>Airway hyperresponsiveness</td>
</tr>
<tr>
<td>AQLQ</td>
<td>Asthma Quality of Life Questionnaire</td>
</tr>
<tr>
<td>ATS</td>
<td>American Thoracic Society</td>
</tr>
<tr>
<td>AX</td>
<td>Reactance area</td>
</tr>
<tr>
<td>BDP</td>
<td>Beclometasone dipropionate</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BTPS</td>
<td>Body temperature and pressure, saturated</td>
</tr>
<tr>
<td>CDI</td>
<td>Convection-dependent inhomogeneity</td>
</tr>
<tr>
<td>Cet</td>
<td>End-expiratory inert gas concentration</td>
</tr>
<tr>
<td>CEV</td>
<td>Cumulative expired volume</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>CoV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CRTH2</td>
<td>Chemoattractant Receptor-homologous molecule expressed on Th2 cells</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>DCDI</td>
<td>Diffusion-convection-dependent inhomogeneity</td>
</tr>
<tr>
<td>DLco</td>
<td>Diffusing capacity of the lung for carbon monoxide</td>
</tr>
<tr>
<td>DS&lt;sub&gt;eq&lt;/sub&gt;</td>
<td>Equipment dead space</td>
</tr>
<tr>
<td>EPAP</td>
<td>Expiratory positive airway pressure</td>
</tr>
<tr>
<td>ERS</td>
<td>European Respiratory Society</td>
</tr>
<tr>
<td>FAO</td>
<td>Fixed airflow obstruction</td>
</tr>
<tr>
<td>FeNO&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Fractional exhaled nitric oxide at 50ml/s</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Forced expiratory volume in one second</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Forced expiratory volume in three seconds</td>
</tr>
<tr>
<td>FID</td>
<td>Free induction decay</td>
</tr>
<tr>
<td>FOT</td>
<td>Forced oscillation technique</td>
</tr>
<tr>
<td>FRC</td>
<td>Functional residual capacity</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>FRC&lt;sub&gt;calc&lt;/sub&gt;</td>
<td>Functional residual capacity calculated from dimensions of lung model</td>
</tr>
<tr>
<td>FRC&lt;sub&gt;mbw&lt;/sub&gt;</td>
<td>Functional residual capacity calculated using multiple breath inert gas washout</td>
</tr>
<tr>
<td>FRC&lt;sub&gt;pleth&lt;/sub&gt;</td>
<td>Functional residual capacity measured with body plethysmography</td>
</tr>
<tr>
<td>fSAD</td>
<td>Functional small airway disease</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>GINA</td>
<td>Global Initiative for Asthma</td>
</tr>
<tr>
<td>HU</td>
<td>Hounsfield Units</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass correlation coefficient</td>
</tr>
<tr>
<td>ICS</td>
<td>Inhaled corticosteroid</td>
</tr>
<tr>
<td>IOS</td>
<td>Impulse oscillometry</td>
</tr>
<tr>
<td>IPAP</td>
<td>Inspiratory positive airway pressure</td>
</tr>
<tr>
<td>K&lt;sub&gt;CO&lt;/sub&gt;</td>
<td>Carbon monoxide transfer coefficient</td>
</tr>
<tr>
<td>LABA</td>
<td>Long-acting β-agonist</td>
</tr>
<tr>
<td>LCI</td>
<td>Lung clearance index</td>
</tr>
<tr>
<td>LCI&lt;sub&gt;ds&lt;/sub&gt;</td>
<td>Dead space component of lung clearance index</td>
</tr>
<tr>
<td>LCI&lt;sub&gt;ideal&lt;/sub&gt;</td>
<td>Ideal lung clearance index</td>
</tr>
<tr>
<td>LCI&lt;sub&gt;vent&lt;/sub&gt;</td>
<td>Specific ventilation inequality component of lung clearance index</td>
</tr>
<tr>
<td>MBW</td>
<td>Multiple breath inert gas washout</td>
</tr>
<tr>
<td>MCID</td>
<td>Minimal clinically important difference</td>
</tr>
<tr>
<td>MLD E/I</td>
<td>Mean lung density expiratory/inspiratory ratio</td>
</tr>
<tr>
<td>MMEF</td>
<td>Maximal mid-expiratory flow</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>P&lt;sub&gt;15&lt;/sub&gt;</td>
<td>Fifteenth lower percentile of inspiratory lung density</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>PRM</td>
<td>Parametric response map</td>
</tr>
<tr>
<td>PsA</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>R20</td>
<td>Resistance at 20Hz</td>
</tr>
<tr>
<td>R5</td>
<td>Resistance at 5Hz</td>
</tr>
<tr>
<td>RB1</td>
<td>Right upper lobe apical segmental bronchus</td>
</tr>
<tr>
<td>Rc</td>
<td>Central airway resistance</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>RL</td>
<td>Total lung resistance</td>
</tr>
<tr>
<td>RMS</td>
<td>Respiratory mass spectrometer</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operating characteristic</td>
</tr>
<tr>
<td>Rp</td>
<td>Peripheral airway resistance</td>
</tr>
<tr>
<td>RV</td>
<td>Residual volume</td>
</tr>
<tr>
<td>$S_{\text{acin}}$</td>
<td>Acinar ventilation heterogeneity</td>
</tr>
<tr>
<td>SAO</td>
<td>Small airway obstruction</td>
</tr>
<tr>
<td>$S_{\text{cond}}$</td>
<td>Conductive ventilation heterogeneity</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Spin echo</td>
</tr>
<tr>
<td>SIII</td>
<td>Phase III slope</td>
</tr>
<tr>
<td>SnIII</td>
<td>Concentration-normalised phase III slope</td>
</tr>
<tr>
<td>SR</td>
<td>Standardised residual</td>
</tr>
<tr>
<td>STE</td>
<td>Stimulated echo</td>
</tr>
<tr>
<td>SVC</td>
<td>Slow vital capacity</td>
</tr>
<tr>
<td>TH$_2$</td>
<td>T-helper 2</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lung capacity</td>
</tr>
<tr>
<td>TO</td>
<td>Turnover</td>
</tr>
<tr>
<td>VA</td>
<td>Alveolar volume</td>
</tr>
<tr>
<td>VC</td>
<td>Vital capacity</td>
</tr>
<tr>
<td>VD$_{\text{anat}}$</td>
<td>Anatomical dead space</td>
</tr>
<tr>
<td>VD$_{\text{resp}}$</td>
<td>Effective respiratory dead space</td>
</tr>
<tr>
<td>VH</td>
<td>Ventilation heterogeneity</td>
</tr>
<tr>
<td>VT</td>
<td>Tidal volume</td>
</tr>
<tr>
<td>X5</td>
<td>Reactance at 5Hz</td>
</tr>
</tbody>
</table>
1 Introduction

1.1 Overview

Asthma is a chronic inflammatory airway disease that is estimated to affect 300 million people worldwide\(^1\). The reported prevalence has been increasing for a number of decades, in association with worldwide trends towards urbanisation and the adoption of Western lifestyles\(^1\). Asthma is estimated to cause 250,000 deaths\(^2\) and the loss of 15 million disability-adjusted life years each year\(^1\). The economic burden of asthma is no less severe, with direct and indirect costs totalling approximately €17.7 billion each year in Europe\(^3\). Severe asthma imposes a disproportionate economic burden, accounting for 50% of these costs but only 10-20% of patients with asthma\(^3\).

The Global Initiative for Asthma (GINA, 2012) defines asthma as follows\(^2\):

‘Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment.’

This definition emphasises the variable nature of airflow obstruction in asthma. However, it is known that patients with asthma may manifest incompletely reversible airflow obstruction akin to that seen in chronic obstructive pulmonary disease (COPD), and that this is associated with increased morbidity\(^4\). Indeed, it was suggested in 1961 that asthma and COPD were variants of the same disease\(^5\), a proposition that was later termed the ‘Dutch hypothesis’. Whilst this view is controversial\(^6,7\), it is increasingly recognised that a number of patients cannot be easily categorised into classical asthma and COPD groups, and that a significant overlap exists between the conditions, particularly with respect to airway physiology\(^8\). Bronchodilator responsiveness, a classical feature of asthma, occurs in a significant proportion of patients with a diagnosis of COPD\(^9\), as does airway hyperresponsiveness\(^10\). Conversely, many patients with asthma manifest a degree of fixed airflow obstruction (FAO) and accelerated lung function decline, traits that are usually associated with COPD\(^4,11,12\). The mechanisms of
airflow limitation in asthma may include airway smooth muscle hypertrophy and airway remodelling\textsuperscript{13,14}, obstruction of the airways with mucus and inflammatory debris\textsuperscript{15}, airway closure due to surfactant dysfunction\textsuperscript{16}, or loss of alveolar attachments causing reduced lung elastic recoil\textsuperscript{17}. Risk factors for FAO in asthma include cigarette smoking\textsuperscript{12}, severe exacerbations\textsuperscript{11}, duration of asthma\textsuperscript{18}, and either eosinophilic\textsuperscript{19} or neutrophilic\textsuperscript{20} airway inflammation.

It is widely recognised that asthma is a heterogeneous disorder rather than one single well-defined condition, a concept that dates back to the description by Rackemann in 1941 of a group of patients whose asthma did not appear to be driven by an extrinsic allergen, and who were therefore labelled as having ‘intrinsic asthma’\textsuperscript{21}. In the past decade there has been a concerted effort to understand the subtypes of asthma, based upon observable clinical characteristics (phenotypes) or underlying biological mechanisms (endotypes)\textsuperscript{8,22-27}. This has been driven to a large extent by the emergence of high-cost asthma therapies that appear to be effective only in subgroups of asthma patients. For example, the anti-IL-5 monoclonal antibody mepolizumab did not produce statistically significant clinical benefits in an unselected asthma population\textsuperscript{28}, but was subsequently found to significantly reduce exacerbation frequency in patients with eosinophilic asthma\textsuperscript{29,30}. Similarly, in a trial of the anti-IL-13 monoclonal antibody lebrikizumab, patients with high serum periostin, a marker of T-helper 2 (TH\textsubscript{2}) cell inflammation, had a significantly greater improvement in forced expiratory volume in one second (FEV\textsubscript{1}) than those with low serum periostin\textsuperscript{31}.

A clinical phenotype has been defined as:
\begin{quote}
‘a single or combination of disease attributes that describe differences between individuals as they relate to clinically meaningful outcomes (symptoms, exacerbations, response to therapy, rate of disease progression, or death.)’\textsuperscript{32}
\end{quote}

According to this definition, to qualify as a clinical phenotype, a group of observable characteristics must be related to clinically important outcomes. Characteristics that do not fulfil this criterion are referred to as ‘phenotypic traits’. Well-described clinical phenotypes of asthma include early-onset atopic, late-onset eosinophilic, aspirin-intolerant and obesity-related\textsuperscript{8}. 
The small airways are usually defined as airways with an internal diameter of less than 2mm, comprising both the smaller conducting airways and the intra-acinar airways. The morphometry of the human airway tree is discussed in detail in Section 1.4.1. Whilst asthma has traditionally been thought of a disease of the larger airways, there is increasing interest in the role of small airway disease in this condition. The potential importance of the small airways in asthma is two-fold: Firstly, since in health the small airways contribute only a small proportion of total airway resistance, disease in the small airways may be widespread before abnormalities are detectable using spirometry. Therefore, tests of small airway obstruction may provide a sensitive measure of early airway damage in asthma, and may allow intervention to be instituted before the development of FAO, with its associated morbidity. Secondly, small airway disease may underlie disease persistence in asthma, since standard inhaled corticosteroids (ICS) are deposited mainly in the larger conducting airways and do not penetrate to the more peripheral regions of the lung. Persistent asthma, in which symptoms and/or airway inflammation are inadequately controlled despite topical therapy, imposes a disproportionate burden on individual patients and society, and is relatively common. Indeed, a large randomised controlled trial comparing ICS with combination ICS and long-acting β-agonist (LABA) only achieved total control of asthma at one year in 28% and 41% of patients respectively. The causes of persistence are not completely understood but include poor patient adherence, inadequate inhaler technique and corticosteroid resistance. A further possibility is that patients with persistent asthma have pathology in the small airways that standard topical therapy cannot reach. Thus, the detection of small airway disease may indicate the need for systemic therapies and/or small particle inhalers to be introduced.

1.2 Small airway disease in asthma

It has been suggested that small airway disease may define a distinct clinical phenotype of asthma. Evidence for small airway involvement in asthma comes from post-mortem specimens, lung biopsies (surgical or transbronchial), and direct measurement of peripheral airway resistance using semi-invasive physiological methods such as the wedged bronchoscope technique. Carroll et al examined post-mortem lung specimens from patients who had died of asthma (fatal asthma), those who had a history of asthma
but died of a non-respiratory cause (non-fatal asthma) and control subjects who died suddenly with no history of respiratory disease\textsuperscript{47,48}. They found that airway remodelling occurred throughout the bronchial tree in both fatal and non-fatal asthma cases\textsuperscript{47}. In particular, airway wall areas in the small membranous bronchioles (perimeter $<2\text{mm}$) were significantly greater in both fatal and non-fatal asthma cases than in control subjects. Airway inflammation was also observed in both large and small airways\textsuperscript{48}. Lymphocytes were distributed within both large and small airway walls, in both fatal and non-fatal asthma cases, to a significantly greater extent than in controls. Eosinophils were distributed within both large and small airway walls, but to a greater extent in fatal asthma compared to non-fatal asthma or controls. Similarly, Faul et al observed an inflammatory infiltrate of CD8$^+$ T-cells, eosinophils and macrophages in both the proximal and distal airways of patients who died suddenly of asthma\textsuperscript{49}. However, it is possible that the pathology seen in fatal asthma, which is relatively uncommon, may not be representative of the majority of asthma cases.

Surgical lung biopsies taken from patients with asthma showed increased numbers of T-cells and eosinophils in both large and small airway walls compared to non-asthmatic controls\textsuperscript{50}. Moreover, in patients with asthma, the number of activated eosinophils was greater in airways with an internal perimeter of $<2\text{mm}$ compared with those with an internal perimeter $>2\text{mm}$, suggesting that eosinophilic airway inflammation in asthma is more severe in the peripheral than the central airways. Using transbronchial biopsies obtained at 4:00 am and 4:00 pm, Kraft et al showed that patients with nocturnal asthma exhibited greater numbers of alveolar tissue eosinophils\textsuperscript{51} and CD4$^+$ T-cells\textsuperscript{52} at 4:00 am than patients with non-nocturnal asthma. Moreover, patients with nocturnal asthma had greater numbers of tissue eosinophils and macrophages at 4:00 am compared to 4:00 pm\textsuperscript{51}. Wenzel et al observed greater numbers of neutrophils in transbronchial biopsies from patients with oral glucocorticoid-dependent severe asthma than in healthy control subjects\textsuperscript{53}. Balzar et al reported a significantly higher density of inflammatory cells in small airways compared to medium or large airways, in an analysis of paired endobronchial and transbronchial samples from 12 patients with severe oral corticosteroid-dependent asthma\textsuperscript{54}. Hauber et al showed that treatment with a small-particle ICS could attenuate eosinophilic inflammation in both the central and peripheral airways, with a concomitant increase in neutrophils\textsuperscript{55}. The same group subsequently showed that a 6-week course of small-particle ICS reduced expression of
alpha-smooth muscle actin in the peripheral airways, but did not attenuate collagen deposition or transforming growth factor-β expression. However, assessing small airway disease using transbronchial biopsies is not without limitations, most notably that non-diagnostic biopsies (ie. not containing viable small airways) are relatively common. From a total of 29 transbronchial biopsies performed by Balzar et al, only 45% contained airway tissue, and 28% contained small airways. Moreover, transbronchial biopsy carries a small but significant risk of causing bleeding or pneumothorax.

Peripheral airway resistance (Rp) was first measured by Macklem and Mead in 1967, using a retrograde catheter, in open-chested living dogs and excised lungs from a variety of species including humans. Their results suggested that Rp constitutes a relatively small proportion of total lung resistance (RL) in health. However, the retrograde catheter technique is too invasive to be used in living humans. The wedged bronchoscope technique for measuring Rp was introduced by Wagner et al in 1990. These investigators wedged a flexible bronchoscope into a segmental right upper lobe bronchus and passed a double-lumen catheter through the instrument port of the bronchoscope. Air was passed through one lumen of the catheter at three different flow rates, whilst pressure was measured using a transducer positioned within the second lumen. Rp was defined as pressure divided by flow, averaged over the three flow rates measured. Since the bronchoscope was tightly wedged, air could not escape proximally, and therefore must have been exiting the lung segment distal to the bronchoscope via collateral channels leading to adjacent lung segments. Rp measured using this technique therefore represents the resistance of both the airways distal to the bronchoscope and the collateral pathways leading out of the lung segment. The procedure was performed in six healthy volunteers and nine patients with asthma. The patients with asthma were found to have markedly raised Rp compared to healthy subjects, despite only minor differences in spirometry. The same technique was later used to show that peripheral airway responsiveness to histamine is increased in patients with asthma compared to normal subjects, and that the peripheral airways in patients with exercise-induced asthma are responsive to cool dry air. These results suggested that the small airways make a significant contribution to airway hyperresponsiveness in asthma. Kaminsky et al later refined the wedged bronchoscope technique by analysing the decay in pressure that occurred upon cessation of airflow, as well as the plateau pressure that was reached.
once this decay ceased\textsuperscript{62}. They reasoned that cessation of airflow would cause a sudden pressure drop which would be reflective of the resistance of the small airways distal to the wedged bronchoscope, and that the subsequent slow decay in pressure would reflect the passive emptying of the distal lung segment through collateral channels. The plateau pressure would be indicative of the volume of air that was trapped in the lung segment by closure of collateral channels. In fact, the initial sudden drop in pressure was not observed in either normal or asthmatic subjects, leading the authors to conclude that Rp was related almost entirely to the resistance of collateral channels, and that the resistance of these channels was increased in patients with asthma. A subsequent study showed that in patients with nocturnal asthma, plateau pressure was higher at 4:00 am than at 4:00 pm, suggesting that there was diurnal variation in the patency of collateral channels in this group of patients\textsuperscript{63}. Three distinct types of collateral channel have been identified in human lungs\textsuperscript{64}, namely the alveolar pores of Kohn, and the epithelium-lined channels of Lambert and Martin, which respectively connect terminal bronchioles to alveoli or to other terminal bronchioles. These channels would potentially allow the movement of gas molecules by both convection and diffusion, and they may constitute important alternative pathways for ventilation in patients with emphysema\textsuperscript{64}. However, there have been no histological studies investigating how collateral channels are altered in asthma in order to account for their increased resistance.

A different technical approach to the same problem was taken by Yanai \textit{et al}\textsuperscript{65}, who wedged a catheter-tipped micromanometer of 3mm diameter into a right lower lobe segmental bronchus. Pressure changes in the central airways during tidal breathing were recorded by a transducer lying proximal to the wedged portion of the catheter, and were used to calculate central airway resistance (Rc). RL was calculated using the oesophageal balloon technique of Mead and Whittenberger\textsuperscript{66}, and Rp was obtained by subtracting Rc from RL. The procedure was performed in 5 healthy controls, 20 patients with asthma (10 of whom had FEV\textsubscript{1} < 70\% predicted), and 15 patients with COPD. The authors found that in patients with COPD, and in those with asthma and low FEV\textsubscript{1}, both RL and Rp were significantly higher compared to healthy controls, and that Rp was disproportionately raised. Specifically, Rp was approximately 25\% of RL in healthy controls, and 50\% of RL in patients with COPD, or asthma with low FEV\textsubscript{1}. The authors concluded that the peripheral airways were the major site of development of obstructive airway diseases. In a subsequent study using the same technique, the RL response to
methacholine was partitioned into Rc and Rp components in 10 patients with asthma\textsuperscript{67}. A variety of patterns of response were observed, with most patients manifesting a combination of Rc and Rp responses.

The methods described in this section may be useful for small-scale mechanistic research studies, but are too complex and invasive for routine clinical or research use. The development of methods for reliably and non-invasively measuring small airway obstruction is therefore an important unmet need in clinical respiratory physiology. A number of candidate tests have been proposed to fulfil this purpose, as discussed in the following section, with the forced oscillation technique (FOT) and multiple breath inert gas washout (MBW) being the strongest contenders at present. These methods have in common the fact that they may be influenced not only by the site of obstruction but also by the degree of heterogeneity of airway obstruction. Thus it may be predicted on theoretical grounds that the results obtained from these tests may be related to a certain extent.

1.3 Non-invasive physiological tests of small airway obstruction

1.3.1 Spirometry
Vital capacity (VC) measurements were first reported in 1846 by John Hutchinson\textsuperscript{68}, who observed in a study of 2130 individuals that VC was proportional to height and inversely related to age. The introduction of the timed spirometer by Gaensler in 1951\textsuperscript{69} allowed dynamic ventilatory capacity to be reliably measured, and the ratio of forced expiratory volume in one second (FEV\textsubscript{1}) to forced vital capacity (FVC) has stood the test of time as a measure of airflow obstruction. Flow during a forced expiration is largely effort-independent, other than during the brief period before peak flow is achieved\textsuperscript{70}, a phenomenon that contributes to the remarkable reproducibility and clinical utility of spirometric indices. The shape of a volume-time curve or flow-volume loop during a forced expiration is thus determined almost entirely by the mechanical (resistive and elastic) properties of the airways and lung parenchyma. Two physical mechanisms of expiratory flow limitation have been described\textsuperscript{71}:
i) Wave speed limitation\textsuperscript{72}: Flow through an airway cannot exceed the speed of wave propagation through the airway walls.

ii) Frictional and turbulent dissipation of gas energy

A recent mathematical modelling study\textsuperscript{73} has shown that wave speed limitation appears approximately 0.1 seconds after the onset of a forced expiration, and is the primary flow-limiting mechanism during the first 1.5 seconds of the manoeuvre. The site of flow limitation moves distally from the 4\textsuperscript{th} generation at 0.1 seconds to the 9\textsuperscript{th} generation at 1.0 seconds. Flow limitation due to frictional energy dissipation becomes the dominant mechanism from approximately 1.8 seconds, occurring in the distal airways, whilst turbulent energy losses may play a role in the transition between wave-speed and frictional flow limitation. These simulations suggest that FEV\textsubscript{1} may largely reflect the calibre of the central airways, whereas alternative spirometric indices that particularly assess events late in a forced expiration may provide an insight into small airway obstruction.

A number of investigators have studied the potential utility of alternative indices derived from volume-time curves or flow-volume loops recorded during forced expiration. The maximal mid-expiratory flow (MMEF) was formerly considered a marker of small airway obstruction\textsuperscript{74}, but its usefulness has since been called into question by its wide normal range and poor discriminatory ability\textsuperscript{75}. Indeed, since MMEF mainly reflects events occurring within the first second of a forced expiration, it would be unlikely to provide significant additional information to FEV\textsubscript{1}. More recently, it has been suggested by Morris et al that the ratio FEV\textsubscript{3}/FVC may represent a marker of early lung injury\textsuperscript{76}. These authors investigated the physiological characteristics of patients who had an isolated reduction in FEV\textsubscript{3}/FVC but with normal FEV\textsubscript{1}/FVC. They found that, compared with patients who had both normal FEV\textsubscript{1}/FVC and normal FEV\textsubscript{3}/FVC, this group exhibited significantly higher residual volume (RV) and RV to total lung capacity (TLC) ratio, and significantly lower diffusing capacity of the lung for carbon monoxide. However, the group with an isolated reduction in FEV\textsubscript{3}/FVC was relatively small, comprising just 6.3% of the study population, suggesting that this pattern of lung function abnormality is relatively uncommon. Cohen et al investigated the potential role of FVC to slow inspiratory vital capacity (SVC) ratio as a marker of small airway obstruction. In a group of lung transplant recipients, the FVC/SVC ratio
was found to fall significantly from baseline in patients who developed bronchiolitis obliterans syndrome, a classical small airway disease\textsuperscript{77}. However, the median (interquartile range) percentage change in FVC/SVC in this group was only -4.4 (-1.2 to -7.5), compared to -39.0 (-36.4 to -42.1) for FEV\textsubscript{1}, calling into question the discriminatory ability of FVC/SVC as a marker of small airway obstruction. Moreover, there is no \textit{a priori} reason to suppose that the FVC/SVC ratio, being a general marker of expiratory air trapping, would selectively measure obstruction of the small airways.

A further method of analysing forced expiratory spirograms was proposed by Fish \textit{et al}\textsuperscript{78}, who postulated that each lung unit that emptied during a forced expiration could be considered to have a ‘transit time’, or emptying time, measured from the start of the expiration. If these transit times followed a given probability distribution, then a spirogram would simply represent the cumulative distribution function of this probability distribution. A number of authors investigated indices derived from the moments of the spirogram, of which the simplest was the mean transit time\textsuperscript{79-82}. However, a disadvantage of these indices was that they required the spirogram to be truncated at an arbitrary proportion of VC (eg. 75\% or 90\%) in order to allow comparison between individuals, thus leading to loss of information from the terminal portion of the spirogram\textsuperscript{83,84}.

Permutt \textit{et al}\textsuperscript{85} found that transit times tended to follow a log-normal distribution, such that log-transformed transit times were normally distributed, with a mean and standard deviation of $\mu$ and $\sigma$, respectively. Thus $\mu$ was interpreted as a measure of the ‘typical’ transit time, whilst $\sigma$ was considered to be a measure of the dispersion of transit times around this typical value. This approach is attractive since the information content contained within the shape of a spirogram can be condensed to just two parameters, from which, with the addition of a scaling parameter such as the theoretical maximum FVC, any other index can in theory be derived. Reference values for the parameters $\mu$ and $\sigma$ have been derived\textsuperscript{86}, and longitudinal changes in these indices over a four-year period were reported in a group of 225 healthy men\textsuperscript{87}. In a study of 484 male factory workers, Nakadate \textit{et al} showed that $\sigma$ was significantly raised in asymptomatic smokers compared to non-smokers, whereas conventional spirometric parameters such as FEV\textsubscript{1} did not differ between the groups\textsuperscript{88}. Miller \textit{et al} showed that both $\mu$ and $\sigma$ were significantly related to pack-years smoked in a linear regression analysis\textsuperscript{89}. However,
there is no evidence that the parameters $\mu$ and $\sigma$ can be used to distinguish between large and small airway obstruction.

### 1.3.2 Lung volumes

Whilst spirometry may be used to measure the vital capacity, the determination of absolute lung volumes requires an alternative method such as body plethysmography, helium dilution or nitrogen washout. The ratio of residual volume (RV) to total lung capacity (TLC) is a commonly utilised marker of expiratory air trapping. Although it has been suggested that air trapping may be a specific marker of small airway disease, this assertion does not have a strong theoretical basis, and it is possible that large airway obstruction may also contribute. Nevertheless, a number of studies have shown that air trapping is associated with increased asthma disease expression. Sorkness et al found that at any given level of airflow obstruction, as measured by FEV$_1$/FVC (% pred.), patients with severe asthma had more prominent air trapping, as measured by increased RV/TLC (% pred.). Mahut et al found that children who had suffered an asthma exacerbation within the previous 3 months had higher values of RV/TLC than those who had not. The oral leukotriene receptor antagonist montelukast has been shown to reduce air trapping, as measured using RV or RV/TLC, in both adults and children with asthma. Kraft et al found that improvements in asthma symptoms in patients treated with montelukast correlated with reductions in RV, but not with increases in FEV$_1$ or FEV$_1$/FVC ratio. Filippelli et al utilised optoelectronic plethysmography to investigate the relationship between breathlessness (measured on the Borg scale) and changes in both FEV$_1$ and lung volumes during a methacholine challenge test. They found that FEV$_1$ and chest wall volume were independent predictors of Borg score, but that chest wall volume was a stronger predictor, suggesting that hyperinflation and air trapping have a major influence on the sensation of breathlessness in patients with asthma.

The ratio of alveolar volume (VA) derived from a single breath helium dilution technique to TLC measured using an alternative method such as multiple breath helium dilution or body plethysmography represents the proportion of the lung volume that is unventilated following a single deep inspiration. The VA/TLC ratio is a marker of airway obstruction and gas mixing inefficiency, and has been found to have similar sensitivity and specificity for detecting obstructive airway disease (asthma or COPD) as
FEV$_1$ is likely to be reflective of the extent of patchy ventilation defects, as may be visualised using techniques such as hyperpolarised $^3$He magnetic resonance imaging (MRI). As such, it is probable that this parameter represents obstruction of both the large and small airways.

1.3.3 Forced oscillation technique

The forced oscillation technique, introduced by DuBois et al in 1956, is a method for non-invasively assessing lung mechanics by examining the relationship between pressure and flow whilst forced oscillations are delivered to the respiratory system by a loudspeaker or piston. The waveform delivered may be a sine wave at a single frequency, a combination of sine waves at multiple discrete frequencies, or an impulse which is mathematically decomposed to a continuous spectrum of frequencies (a variant known as impulse oscillometry [IOS]). The waveform delivered determines the frequencies at which the mechanical impedance of the respiratory system is measured. However, it should be noted that the impedance at a given frequency measured using different devices may not be exactly comparable, as was demonstrated in a recent multicentre study performed by Oostveen et al. FOT is typically performed using a frequency range of approximately 5Hz to 35Hz, since at lower frequencies than this the subject’s breathing harmonics are prone to interfere with the measurement. However, FOT has been successfully performed at lower frequencies during short voluntary apnoeas in well-trained participants. In the steady state, the amplitude and phase relationships between pressure and flow waves produced by a forced oscillator depend upon the mechanical impedance (incorporating resistance and reactance) of the system being oscillated. In particular, a pure resistance will cause a reduction in the amplitude of flow with respect to pressure, but pressure and flow will remain in phase with each other. A negative reactance (known as an elastance) will cause pressure to lag behind flow, whereas a positive reactance (known as an inertance) will cause pressure to lead flow. Reactance naturally increases from being negative (predominantly elastive) at low frequencies to positive (predominantly inertive) at high frequencies, whereas resistance is always positive, and in an ideal linear system is equal across all frequencies. The resonant frequency of a given mechanical system is the oscillation frequency at which reactance is zero, so that flow and pressure are perfectly in phase. Figure 1.1 shows an illustrative trace of resistance and reactance against frequency, as might be measured.
using IOS (Panel A), and a plot of resistance against time in a patient with asthma (Panel B).
Figure 1.1: Resistance and reactance within the frequency and time domains

Panel A shows a schematic diagram of a typical trace of resistance (continuous line) and reactance (dashed line) against frequency as might be measured using impulse oscillometry. Resistance is positive at all frequencies, whereas reactance is negative at low frequencies and positive at high frequencies. The reactance curve crosses the x-axis at the resonant frequency, which is 10 Hz in this example. Panel B shows an example trace of resistance at 20 Hz against time measured using impulse oscillometry in a patient with asthma, showing typical oscillations in resistance during the respiratory cycle.
Examination of the resistance spectrum across a range of frequencies often reveals that resistance is disproportionately raised at low frequencies in patients with airway obstruction, a phenomenon known as frequency-dependence of resistance\textsuperscript{101-102}. From a theoretical point of view, this may arise due to unequal ventilation time constants between parallel lung units\textsuperscript{108}, or non-linear viscoelastic properties of the airways and surrounding tissues\textsuperscript{109}. Computational models of lung impedance have shown that heterogeneous constriction of the peripheral airways would be expected to cause marked frequency-dependence of resistance between 0.1Hz and 3Hz, but that these effects are far less prominent if constriction is homogeneous or confined to the central airways\textsuperscript{110}. This suggests that low frequency FOT may be essential to detect the effects of peripheral airway constriction. However, it should be noted that this study was based upon the branching structure of a canine lung, and frequency-dependence of resistance was observed only if extreme constriction (>80% reduction in diameter) was imposed upon randomly distributed airways.

Tissue and chest wall mechanical properties may also impact upon frequency-dependence of resistance, particularly at frequencies below 5Hz. Hantos et al used the oesophageal balloon technique to partition low-frequency respiratory resistance into pulmonary and chest wall components. They found that frequency-dependence of resistance was present in healthy subjects at low frequencies (0.25Hz to 5Hz), and that this was largely attributable to non-linear mechanical properties of the chest wall\textsuperscript{104}. Navajas et al investigated the mechanical properties of isolated strips of dog lung parenchyma, and observed that lung tissue resistance displayed prominent frequency-dependence, dropping close to zero at oscillation frequencies above 2Hz\textsuperscript{109}.

A further effect of airway constriction and closure on FOT measurements is that airways distal to the site of obstruction ‘move into the shadow’ so that their capacitive properties become hidden, thus making the respiratory system appear to be stiffer\textsuperscript{111}. For this reason, airway obstruction causes low-frequency reactance to become more negative. This is commonly expressed using the reactance at 5Hz (X5), or the reactance area (AX), which is the integrated low-frequency reactance between 5Hz and the resonant frequency. Distal airway obstruction may also cause diversion of forced oscillations across the upper airway and pharyngeal walls, so that the measured resistance and reactance spectra partly reflect the viscoelastic properties of these
structures. In support of this concept, FOT performed in patients with upper airway obstruction due to tracheostenosis revealed striking frequency-dependence of resistance and a marked reduction in low-frequency reactance\textsuperscript{112}. Furthermore, the resistance at 5Hz minus the resistance at 20 Hz (R$_5$-R$_{20}$) and AX are known to be closely correlated\textsuperscript{113}, suggesting that these parameters may relate to similar structural abnormalities.

In order to remove the influence of the upper airways on FOT measurements, Kaminsky \textit{et al} delivered forced oscillations to a single segment of the lung by means of a wedged bronchoscope, in a group of healthy subjects and patients with mild asthma\textsuperscript{114}. At baseline, the mechanical impedance of the groups overlapped, although the asthma patients appeared to show a more pronounced response to methacholine than the healthy controls. In contrast, measurement of the resistance of collateral channels using a wedged bronchoscope technique with a constant flow showed clear differences between healthy and asthma groups at baseline\textsuperscript{59}. These results suggested that asthma may be characterised by abnormalities of the most peripheral respiratory bronchioles and alveolar ducts, where collateral pathways are located\textsuperscript{114}. However, there is currently no direct histological evidence of abnormalities of collateral channels in patients with asthma.

The structural interpretation of FOT data is not straightforward because it may be affected by multiple factors including heterogeneous airway constriction, non-linear viscoelastic tissue properties and airway closure causing central airway shunting. In order to disentangle these effects, attempts have been made to fit FOT data to inverse models of lung impedance, in which the lungs are modelled using idealised electrical analogues with a small number of parameters. The most widely-used of these is the ‘constant phase model’\textsuperscript{115}, which consists of a Newtonian resistance (R) and inertance (I) in series with a viscoelastic tissue compartment described by tissue damping (G) and elastance (H) parameters. However, such inverse models are not without limitations, primarily because the behaviour of an organ as complex as the lung cannot be easily captured by a small number of parameters with simple physiological interpretations\textsuperscript{116}. For instance, the constant phase model described above assumes that frequency-dependence of resistance arises solely due to tissue viscoelastic properties and does not take into account the effect of heterogeneous airway constriction, as originally
described by Otis et al\textsuperscript{108}. Whilst more complex models have been proposed to take these and other effects into account\textsuperscript{117,118}, reliable parameter estimation would most likely be challenging in a clinical setting.

One promising approach to developing an understanding of the physiological basis of inverse model parameters is to utilise a combination of forward and inverse modelling. Whereas an inverse model seeks to explain measurements recorded \textit{in vivo} in terms of a simple idealised lung model, forward modelling simulates a respiratory measurement such as FOT based upon a computational model of the respiratory system which is limited in complexity only by the computational power available. The properties of the computational model may be modified at will, and the output can then be entered into the inverse model. In this way, the effects of alterations in the respiratory system upon inverse model parameters can be readily investigated, and inferences about the physiological interpretation of the parameters made. Using this approach, Lutchen \textit{et al}\textsuperscript{119} showed that heterogeneous peripheral airway constriction caused a disproportionate rise in the constant phase parameter G compared to the parameter H. A further innovative development in the field has been the coupling of computational modelling with FOT measurements and imaging data simultaneously, an approach known as image-functional modelling\textsuperscript{120-123}. Image-functional modelling is a procedure by which a computational model of the respiratory system is tailored so as to reproduce a given combination of ventilation defects (for instance on positron emission tomography or \textsuperscript{3}He-MRI images) and FOT measurements. Using this approach, Tgavalekos \textit{et al}\textsuperscript{120} and Campana \textit{et al}\textsuperscript{121} showed that ventilation defects and mechanical data from patients with asthma could not be accounted for by central airway constriction alone, and that the small airways must be involved. Other studies using image-functional modelling have highlighted the importance of network behaviour within the airway tree in producing observed ventilation defects and alterations in oscillatory lung mechanics\textsuperscript{122,123}. Computational modelling of airway networks has confirmed that the complex interactions between serial and parallel airways may cause catastrophic shifts in regional ventilation\textsuperscript{124,125}.

Despite uncertainties about their structural interpretation, a number of studies have examined the clinical significance of FOT parameters in patients with asthma, often making use of a commercially available IOS device. Shi \textit{et al} reported that AX and R5-
R20 were significantly higher in children with uncontrolled asthma compared to those with controlled asthma\textsuperscript{126}. Similarly, Takeda \textit{et al} investigated cross-sectional associations between asthma health status, using patient-reported outcome measures such as the Asthma Control Questionnaire, and IOS parameters\textsuperscript{127}. Using stepwise linear regression models, they ascertained that IOS parameters such as R20, R5-R20 and X5 were independent predictors of asthma health status over and above FEV\textsubscript{1}.

\subsection*{1.3.4 Multiple breath inert gas washout}

Multiple breath inert gas washout is a technique for quantifying ventilation heterogeneity (VH), the uneven distribution of ventilation, through analysis of the efficiency and pattern with which an inert non-absorbed tracer gas is washed out of the lungs during tidal breathing\textsuperscript{128}. MBW was introduced in 1950 by Robertson \textit{et al}\textsuperscript{129}, and is steadily progressing from being a research tool to a clinical test of pulmonary function, particularly in the fields of paediatric respiratory medicine and cystic fibrosis (CF). A comprehensive standardisation document for the performance of inert gas washout has been recently published\textsuperscript{130}. MBW may be performed using an exogenous tracer gas, such as helium or sulphur hexafluoride (SF\textsubscript{6}), in which case a wash-in phase is required followed by washout with room air, or alternatively resident lung nitrogen is utilised as the tracer gas, in which case no wash-in phase is required and the nitrogen is washed out using 100\% oxygen. The technique requires that expired inert gas concentration and respiratory flows are measured accurately and with good temporal resolution, and that these signals are precisely aligned. A number of MBW methods have been reported, using either custom setups or commercially available devices. Inert gas concentration may be measured directly using a respiratory mass spectrometer\textsuperscript{129}, nitrogen analyser\textsuperscript{131} or photoacoustic gas analyser\textsuperscript{132}, or indirectly using measurements of molar mass\textsuperscript{133}, or O\textsubscript{2} and CO\textsubscript{2} concentrations\textsuperscript{134}, to derive N\textsubscript{2} concentrations. Respiratory flows may be measured using a pneumotachometer\textsuperscript{131,132} or an ultrasonic flow sensor\textsuperscript{133,134}.

Figure 1.2 shows a typical washout curve in a healthy subject, using 0.2\% SF\textsubscript{6} as the inert tracer gas. Panel A shows the raw trace of SF\textsubscript{6} concentration against time, while Panel B shows the end-expiratory SF\textsubscript{6} concentration (C\textsubscript{et}) of each breath of the washout. As the washout proceeds, C\textsubscript{et} decays in a roughly exponential manner until it reaches
1/40\textsuperscript{th} of the initial SF\textsubscript{6} concentration (ie. 0.005\%), at which point the washout experiment is by convention stopped. The units of the x-axis in Panel B are ‘turnover number’ (TO), where TO is the cumulative expired volume (CEV) measured in multiples of the functional residual capacity (FRC). The TO unit is used since it corrects for variations in both tidal volume and FRC. The most commonly reported MBW parameter is the lung clearance index (LCI)\textsuperscript{135}, which is the number of lung turnovers taken to wash out the inert gas to 1/40\textsuperscript{th} of its initial concentration. Gas mixing inefficiency caused by VH results in a prolongation of the washout curve and hence an increase in LCI.
Figure 1.2: Inert gas washout curve from a healthy subject

Panel A shows a raw plot of measured SF$_6$ concentration against time, with each vertical peak representing a single expiration. Panel B shows the end-expiratory SF$_6$ concentration ($C_{et}$) of each breath of the same washout test.
Whilst LCI has the virtue of simplicity, it is biased to a certain extent by variations in tidal volume (VT), anatomical dead space (VDanat) and FRC\textsuperscript{136}, and therefore the ‘ideal LCI’ for any given patient is not a fixed value, but instead varies between approximately 4.5 and 6, depending on the FRC, VT and VDanat. An alternative index, the mixing ratio\textsuperscript{136}, is the ratio of the actual number of breaths taken to wash out the inert gas to 1/40\textsuperscript{th} of its initial concentration to the ideal number of breaths assuming perfect gas mixing. This is equivalent to the ratio of the actual value of LCI to its ideal value, since LCI and breath number are related to each other by a constant term in any given patient. Therefore, the ideal value of mixing ratio is always 1, with values higher than this indicating increasing VH. Despite its theoretical advantages, mixing ratio has not been widely used, most likely due to its increased complexity. Mixing ratio is calculated using the following formula:

$$\text{Mixing ratio} = \frac{n \times \ln \left( \frac{\text{FRC}}{\text{FRC} + \text{VT} - \text{VDanat}} \right)}{\ln(0.025)}$$

Where \(n\) = number of breaths taken for \(\text{C}_{\text{et}}\) to reach 1/40\textsuperscript{th} of its initial value.

The mechanisms of VH have been elucidated largely on the basis of mathematical modelling studies, pioneered by Paiva and Engel in the 1970s and 1980s\textsuperscript{137-140}. As air is drawn into the lungs during inspiration it moves through the larger conducting airways by bulk flow (convection). As the inspired air reaches more distal airways and the total cross-sectional area of the airways rises, the convective velocity falls and the movement of gas molecules by diffusion becomes more significant. The convection-diffusion front is that region of the airway tree at which convective and diffusive flows are of approximately equal magnitude. More distal to the convection-diffusion front, convective flows are negligible, and equilibration of concentration gradients occurs primarily by diffusion. In healthy lungs, the convection-diffusion front is thought to occur in the region of the acinar entrance\textsuperscript{141}. However, the precise location is dependent upon the inert tracer gas used. In particular, gas diffusion rate is inversely proportional to the square root of the molar mass, and therefore the convection-diffusion front is more proximal for lighter gases such as helium, compared to heavier gases such as SF\textsubscript{6}\textsuperscript{129}. 
VH occurs due to two primary mechanisms\textsuperscript{142}:

i) Convection-dependent inhomogeneity (CDI) – This refers to unequal ventilation between relatively large lung units subtended by conducting airways, with associated flow asynchrony such that the least well ventilated lung units empty later in expiration.

ii) Diffusion-convection-dependent inhomogeneity (DCDI) – This refers to asymmetries of airway volume or cross-sectional area occurring in the region of the convection-diffusion front, and arises due to a complex interaction between convection and diffusion involving ‘diffusive pendelluft’.

Two-compartment model simulations of gas mixing have shown that LCI may be increased by either CDI or increased respiratory dead space\textsuperscript{143}. As a point of clarification, the respiratory dead space in the setting of inert gas washout has a different physiological significance compared to the normal situation in which the exchange of O\textsubscript{2} and CO\textsubscript{2} across the alveolar-capillary membrane is being considered. In both settings, a component of the respiratory dead space is the anatomical dead space, the volume of the conducting airways that do not participate in gas mixing or gas exchange. In the context of normal gas exchange, the remainder of the respiratory dead space (often referred to as the alveolar dead space) comprises the theoretical volume of ventilated gas (per tidal breath) that is wasted due to ventilation-perfusion mismatch. However, in the context of inert gas washout, ventilation-perfusion relationships are of no significance, since the gas of interest is by definition not absorbed across the alveolar-capillary membrane. Intriguingly, analysis of LCI washout curves suggests that even in this context, the effective respiratory dead space ($V_{D_{\text{resp}}}$) can be increased over and above the anatomical dead space. The origin of this additional dead space is poorly understood, but it is believed to be related to complex interactions between convection and diffusion occurring in the region of the convection-diffusion front\textsuperscript{143}. The construction of a computational model of convection and diffusion within a simulated human acinus that could explain this phenomenon would be an important advance in the field. Throughout this thesis, the term ‘respiratory dead space’, or its abbreviation $V_{D_{\text{resp}}}$, is used to signify the perfusion-independent effective respiratory dead space that occurs within the context of inert gas washout. $V_{D_{\text{resp}}}$ may be estimated from the SF\textsubscript{6} expirogram using the Bohr equation\textsuperscript{130}, but one disadvantage of this method is that a
different value may be obtained on each expiration. In Section 2.7, an alternative method for determining $V_{D_{\text{resp}}}$ using a two-compartment lung model is described.

The CDI component of increased LCI may be estimated by measures of the so-called ‘curvilinearity’ of the curve, or the degree to which it deviates from the single exponential decay pattern that would be expected if all lung compartments were homogeneously ventilated. Examples of such indices include the slope index$^{142}$ and Curv$^{143,144}$. Verbanck et al.$^{143}$ utilised Curv because this parameter was thought to be relatively independent of $V_{D_{\text{resp}}}$, in contrast to LCI. They did not attempt to isolate the respiratory dead space effect from LCI.

An alternative method of analysing washout curves is to examine changes in expired inert gas concentration within breaths, rather than simply treating each breath as a single point. Figure 1.3 shows an expirogram of inert gas concentration against expired volume over the course of a single exhalation during a MBW test in a healthy subject. Phase I represents expired air from the anatomical dead space that does not take part in gas mixing, and thus the inert gas concentration during this phase is negligible. Phase II represents the arrival at the mouth of the first portions of alveolar air, with a corresponding sharp increase in inert gas concentration. Phase III (alveolar phase) represents pure alveolar air, following the complete clearance of the anatomical dead space. In some cases, a sharp increase in inert gas concentration is seen at the end of phase III, which is thought to correspond to the onset of airway closure in the basal lung segments, and is designated phase IV$^{145}$. The expirogram can also be utilised to calculate the anatomical dead space using the Fowler or Langley methods$^{130}$, as illustrated in Figure 1.3.
Figure 1.3: Graph of SF₆ concentration against expired volume during a single exhalation

Expirogram of SF₆ concentration against volume in a healthy subject. SF₆ concentration is indicated by the heavy continuous line and cumulative expired quantity of SF₆ is indicated by the heavy dashed line. Thin continuous lines indicate linear extrapolations through phase III and through the linear portion of the graph of expired quantity of SF₆. The Langley dead space is the volume indicated by the intersection of the latter line with the x-axis. The thin vertical dashed line is positioned such that areas p and q are equal. The Fowler dead space is the volume indicated by the intersection of this line with the x-axis. Figure redrawn from Reference 130 using data collected in our laboratory.
In the absence of VH, the phase III segment would be expected to be horizontal. An increased phase III slope indicates VH due to either CDI or DCDI. Specifically, mathematical modelling studies have predicted that CDI should cause a progressive linear increase in the phase III slope (normalised to the mean inert gas concentration over the course of phase III), whilst DCDI should cause an increase in the phase III slope of the first breath of the washout test, reaching a maximum after approximately five breaths\textsuperscript{142}. This model underlies the derivation of the indices $S_{\text{cond}}$ and $S_{\text{acin}}$\textsuperscript{131}, which represent VH due to CDI and DCDI, respectively.

Verbanck \textit{et al}\textsuperscript{131} performed MBW using a fixed tidal breathing protocol, in which subjects were encouraged to maintain a constant $V_T$ of 1 litre, using a visual guide. Since not all groups of patients are able to achieve this (e.g. children, or patients with severe airways disease), and because phase III slopes may be influenced by $V_T$, Aurora \textit{et al} have proposed an empirical correction to account for within- and between-subject variability in $V_T$, namely that phase III slopes should be multiplied by the tidal volume of the corresponding breath before calculating $S_{\text{cond}}$ and $S_{\text{acin}}$\textsuperscript{146}. These tidal volume-corrected values may be designated $S_{\text{condVTc}}$ and $S_{\text{acinVTc}}$, respectively\textsuperscript{147}. The Aurora correction is recommended for use in children, or in patients who cannot maintain a constant tidal volume of 1 litre\textsuperscript{130}, although it is not yet fully validated. Such validation would require detailed studies of the effect of tidal volume changes on the phase III slope, in both health and disease.

Whilst phase III slope parameters are widely used, they have a number of disadvantages. Determination of the phase III slope for each breath is labour intensive, and can be challenging in some cases due to cardiogenic oscillations, or ambiguity with respect to the transitions between phase II and III, and between phase III and IV. Therefore, phase III slope parameters are subject to inter-observer variability, and although automated phase III slope detection has been reported\textsuperscript{148}, manual confirmation of computer-specified slopes is still required\textsuperscript{130}. Horsley \textit{et al}\textsuperscript{147} found that tidal volume-corrected $S_{\text{condVTc}}$ was poorly reproducible in healthy controls, and that the median goodness of fit ($R^2$) of the regression line upon which $S_{\text{condVTc}}$ is based was only 0.14 in healthy controls and 0.64 in patients with CF. Furthermore, in CF patients with severe VH, phase III slopes often reach an asymptote before the 6\textsuperscript{th} turnover, thus artificially reducing $S_{\text{cond}}$\textsuperscript{147}. The occurrence of this asymptote was in fact predicted by
Paiva in 1975\textsuperscript{149}. Partly in response to these findings, Verbanck \textit{et al} proposed modified phase III slope parameters (\(S_{\text{cond}*}\) and \(S_{\text{acin}*}\)), which are calculated analogously to \(S_{\text{cond}}\) and \(S_{\text{acin}}\), except that \(S_{\text{cond}*}\) is based upon SnIII values between 0 and 3 turnovers, excluding the first breath of the washout, instead of between 1.5 and 6 turnovers\textsuperscript{150}. The clinical and analytical validity of these parameters, in particular their repeatability and discriminatory ability, has not yet been determined.

A number of studies have demonstrated evidence of VH in patients with asthma\textsuperscript{151-155}. Verbanck \textit{et al}\textsuperscript{151} found that adults with asthma manifested partially reversible increases in \(S_{\text{acin}}\), but no abnormality in carbon monoxide diffusing capacity. They suggested that this combination of findings could be due to an intra-acinar process that spared the alveolar airspaces. The same group also found that \(S_{\text{acin}}\) correlated with modelled alveolar nitric oxide in patients with asthma, suggesting a link between peripheral airway function and inflammation\textsuperscript{152}. In contrast, mild asthma\textsuperscript{153} and asthma in children\textsuperscript{154} appeared to be associated mainly with conductive airway disease. MacLeod \textit{et al}\textsuperscript{155} showed that children with well-controlled asthma and normal spirometry manifested an increase in LCI compared to healthy controls, and that this was not reversible following bronchodilator administration. The authors concluded that LCI may be a marker of structural remodelling in children with asthma.

The clinical significance of VH in asthma has been investigated by a number of authors. Bourdin \textit{et al} found that single breath nitrogen phase III slope correlated with ACQ scores\textsuperscript{156} but these authors did not control for possible associations between nitrogen phase III slope and forced expiratory volume in one second (FEV\textsubscript{1}). Subsequently, Farah \textit{et al} found that \(S_{\text{cond}}\) correlated positively with five-point ACQ score (ACQ-5) at baseline, but in a multivariate analysis only FEV\textsubscript{1} was an independent predictor of baseline ACQ-5, since both \(S_{\text{cond}}\) and \(S_{\text{acin}}\) correlated negatively with FEV\textsubscript{1}\textsuperscript{157}. The same group have also shown that \(S_{\text{acin}}\), but not \(S_{\text{cond}}\), is correlated with asthma severity, as measured using the Global Initiative for Asthma (GINA) treatment steps\textsuperscript{158}, and that asthma exacerbations are associated with increases in both \(S_{\text{cond}}\) and \(S_{\text{acin}}\). Markers of VH have also been shown to predict response to inhaled corticosteroid dose titration\textsuperscript{159}, and \(S_{\text{acin}}\) has been found to improve following the replacement of standard corticosteroid inhalers with a small particle inhaler, in those patients who had a raised \(S_{\text{acin}}\) at baseline\textsuperscript{160}. 
1.4 Imaging techniques for the assessment of small airway structure

1.4.1 Morphometry of the human airway tree
The human airway tree is an asymmetrical dichotomously branching structure of approximately 23 generations, of which the first 14 generations comprise purely conducting airways (trachea, bronchi, bronchioles, terminal bronchioles), and the final 9 generations comprise airways that are alveolated and thus participate in gas exchange (transitional bronchioles, respiratory bronchioles, alveolar ducts and alveolar sacs). The pulmonary acinus is the basic gas exchanging unit, and is a portion of lung that is ventilated by a single terminal bronchiole.

Information about the morphometry of the larger conducting airways has been obtained by analysis of resin casts of whole lungs. Weibel and Gomez found that the diameters of the first 10 generations of airways reduce by a fixed ratio with each generation, approximately equal to the cube root of ½, which from a functional point of view is ideal with respect to minimising both the work needed to overcome flow resistance and the dead space volume. Horsfield and Cumming undertook a similar study, measuring the length and diameter of all airways with diameter exceeding 0.07cm in a resin cast of a single pair of human lungs, fixed at an inflation volume of 5 litres. The number of divisions between the trachea and the lobular branches ranged between 8 and 25, with a mean of 14.6, and the path length from the carina to the lobular branches ranged from 7.5cm to 21.5cm. Therefore, the proximal portion of the human bronchial tree was found to be asymmetrically dichotomous, meaning that there was wide variation in the number of divisions between the stem branch and the terminal branches.

The structure of the acinar airways has been investigated using a number of different techniques. In the study of Horsfield and Cumming, a sample of 313 ‘lobules’ (arbitrarily defined as segments of lung subtended by ‘lobular branches’, airways with diameter 0.07cm or less) was examined under a binocular dissecting microscope. The number of divisions between the lobular branches and the most distal respiratory
bronchioles ranged from 2 to 7, whilst the intralobular path length ranged from 0.2 to 0.9 cm. The branching pattern within lobules was found to be appreciably more symmetrical than that seen in more proximal airways. In a later study, the same group confirmed that the branching pattern of the intralobular airways up to the distal respiratory bronchioles closely approximated to symmetrical dichotomy, with the number of branches in each generation increasing by a factor of 2 with each division\(^\text{164}\), as compared to the factor of 1.38 that was observed in more proximal airways\(^\text{163}\). An alternative method of investigation utilised histological sections to form inferences about the three-dimensional structure of the acinar airways, including the alveolar ducts and sacs, a technique known as stereology\(^\text{165}\). Using this technique, Weibel \textit{et al}\(^\text{162}\) showed that the diameters of these airways reduce to a much lesser extent with each generation than do those of the conducting airways. From a functional point of view, this reflects the fact that diffusion rather than convection begins to play a more prominent role in gas transport once the acinar airways are reached. More detailed information about the structure of the human pulmonary acinus has been recently obtained using micro-computed tomography (CT) imaging of resected lung specimens\(^\text{166}\). This study showed that intra-acinar airways branched over up to 11 generations, with the mean airway internal diameter reducing from 0.66 mm at the terminal bronchioles to 0.34 mm at the seventh acinar generation, thereafter remaining constant. The lengths of each branch ranged from 0.52 to 0.93 mm, with no significant difference between generations. The branching angle between daughter branches ranged from 113 to 134 degrees, again with no difference between generations.

### 1.4.2 Quantitative computed tomography

Quantitative analysis of CT scans is an emerging technique for assessing both the large and small airways in patients with asthma and other airway diseases\(^\text{167}\). Airway remodelling in asthma may be visualised and quantified based upon standard metrics such as the percentage wall area of the right upper lobe apical segmental bronchus\(^\text{168}\). This bronchus is often utilised since it is usually visualised end-on on transverse CT slices, thus allowing the wall area to be accurately determined. However, a potential drawback to this method is that a single bronchus may not be representative of remodelling throughout the airway tree. More recently, simulations of airflow in CT-derived airway trees has been performed using computational fluid dynamics\(^\text{169}\),
although, the current resolution of CT is such that only the first six airway generations may be directly visualised.

A number of CT indices of air trapping have been devised, including the percentage of voxels with attenuation < -850 Hounsfield units (HU) on expiratory CT\textsuperscript{170}, the ratio of mean lung density at expiration to inspiration (MLD E/I)\textsuperscript{171}, and the difference in lung attenuation between inspiration and expiration\textsuperscript{172}. Air trapping on CT in patients with asthma has been shown to be associated with airflow limitation\textsuperscript{170,171,173,174}, as well as an increased risk of asthma-related hospitalisations and intensive care admissions\textsuperscript{170}. In one study, treatment with inhaled corticosteroids attenuated air trapping on CT, although there was no significant difference between large and small particle inhalers\textsuperscript{172}. Montelukast has also been found to reduce air trapping on CT following a four-week treatment period\textsuperscript{175}. CT imaging has recently been utilised to classify patients with asthma into clusters that may represent distinct patterns of airway remodelling\textsuperscript{176}. Galbán \textit{et al} have described a novel CT biomarker based upon the parametric response map (PRM) technique, in which individual voxels were tracked between inspiratory and expiratory CT images using image registration techniques to determine their change in attenuation, in a group of patients with COPD\textsuperscript{177}. Voxels were classified as being indicative of functional small airway disease (fSAD) if their attenuation was > -950 HU on inspiration, but < -856 HU on expiration. In contrast, emphysematous voxels had an attenuation of < -950 HU on inspiration and < -856 HU on expiration. The authors suggested that patients with COPD may progress from predominant fSAD to predominant emphysema. Studies utilising the PRM technique in asthma are currently awaited.

\textbf{1.4.3 Hyperpolarised noble gas magnetic resonance imaging}

Whilst the small airways lie beyond the resolution of current CT scanners, hyperpolarised $^3$He and $^{129}$Xe MRI techniques have been developed in the past 15 years that show considerable potential to detect microstructural abnormalities at the level of the acinar airways and alveoli\textsuperscript{178}. Hyperpolarisation of $^3$He and $^{129}$Xe is the process by which the atoms of these noble gases are imparted with a nuclear polarisation approximately 10,000 times higher than that which would be present at thermal equilibrium, thus allowing them to act as gaseous contrast media for lung MRI\textsuperscript{179}. The principal methods for hyperpolarising noble gases are spin-exchange optical pumping
and metastability exchange optical pumping. Although the majority of studies in humans have thus far utilised $^3$He, it is likely that in the future $^{129}$Xe will be more widely used, firstly because it is cheaper and more abundant, and secondly because, unlike $^3$He, it is absorbed across the alveolar-capillary membrane, and can therefore be utilised to study gas exchange as well as ventilation.\(^{179}\)

The diffusivity of any gas in an unenclosed space may be described by its free diffusion coefficient, which is approximately 0.86 cm\(^2\)/s for $^3$He and 0.14 cm\(^2\)/s for $^{129}$Xe.\(^{180}\) However, when enclosed within a structure such as an alveolus or acinar airway, diffusion is impeded, so that the apparent diffusion coefficient (ADC) of a gas is reduced from its theoretical maximum value, the free diffusion coefficient. $^3$He- or $^{129}$Xe-MRI may be utilised to measure the ADC at either short timescales of a few milliseconds (ADC\(_{\text{short}}\)) or longer timescales of up to ten seconds (ADC\(_{\text{long}}\)). Short timescale ADC measurements correspond to diffusion mostly within alveoli and individual acinar airways, and appear to be sensitive markers of pulmonary emphysema, a condition that is characterised pathologically by the destruction and enlargement of alveolar airspaces.\(^{178}\) Several studies have shown that short-range $^3$He or $^{129}$Xe ADC is elevated in both patients with emphysema,\(^{181-187}\) and in animal models of emphysema,\(^{188-191}\) in comparison with values obtained in healthy lungs. Moreover, in a number of these studies ADC\(_{\text{short}}\) was found to correlate with quantitative histological measures of emphysema such as the mean linear intercept (L\(_m\)), mean alveolar internal area and mean chord length.\(^{184,186,188-191}\) ADC\(_{\text{short}}\) correlated negatively with FEV\(_1\)/FVC in patients with emphysema and in asymptomatic smokers.\(^{192}\) The structural significance of long timescale ADC measurements is less well understood, but they may reflect the extent of collateral ventilation pathways that bypass the normal branching structure of the acinar airways.\(^{193}\) However, Verbanck and Paiva have argued that collateral ventilation may not be necessary to explain measured long timescale ADC values, and that the pattern of intra-acinar branching may be a more important factor.\(^{194}\)

A small number of studies have reported ADC measurements in patients with asthma.\(^{184,195}\) Wang et al found that both long (1.5 s) and short (1 ms) timescale ADC was markedly elevated in patients with COPD compared to healthy controls, whereas in patients with asthma, ADC\(_{\text{short}}\) was only mildly elevated and ADC\(_{\text{long}}\) was moderately
elevated compared to controls\textsuperscript{184}. Since asthma is not known to be associated with alveolar destruction, the authors suggested that the modest elevations in ADC\textsubscript{short} and ADC\textsubscript{long} observed in asthma may be due to lung hyperinflation, causing a generalised increase in alveolar airspace size. This conclusion is supported by a subsequent study which examined the effect of methacholine inhalation on ADC values in 25 patients with asthma and 8 healthy controls\textsuperscript{195}. Methacholine inhalation resulted in the formation of ventilation defects in both patients with asthma and healthy controls. Whole-lung ADC increased in patients with asthma following methacholine inhalation (0.204 cm\textsuperscript{2}/s to 0.211 cm\textsuperscript{2}/s), although this change was not statistically significant, and subsequently returned to baseline following treatment with salbutamol (0.211 cm\textsuperscript{2}/s to 0.202 cm\textsuperscript{2}/s, p < 0.01). The authors concluded that the observed changes in ventilation defect percentage and ADC\textsubscript{short} were due to bronchoconstriction and air trapping. Indeed, it is known that there is a strong relationship between ADC\textsubscript{short} and the degree of lung inflation\textsuperscript{196}, a factor that needs to be borne in mind when interpreting the structural significance of ADC in obstructive airway diseases.

Whilst ADC provides a general index of the diffusivity of \textsuperscript{3}He within the alveoli and acinar airspaces, it does not have a direct morphometric interpretation\textsuperscript{178}. Thus, more complex MR pulse sequences have been devised that allow the derivation of modelled values for parameters such as the alveolar duct outer radius and alveolar sleeve width\textsuperscript{197}. These parameters are based upon an idealised theoretical model of the acinar airways comprising a dichotomously branching network of cylindrical structures decorated by circumferential rings of alveoli, with eight alveoli per annular ring. From the values of R and h, a number of further parameters may be calculated that can be directly compared with histological measurements, including the alveolar surface area, lung volume per alveolus, number of alveoli per unit lung volume and L\textsubscript{m}. This model has been validated in explanted human lungs with varying degrees of emphysema, with a strong correlation observed between histological and \textsuperscript{3}He-MRI-derived L\textsubscript{m} measurements\textsuperscript{197}. Quirk \textit{et al} utilised the same methodology to detect microstructural changes in smokers and ex-smokers, including those with normal lung function\textsuperscript{198}. In this study, FEV\textsubscript{1}/FVC was positively correlated with alveolar sleeve width and negatively correlated with alveolar duct outer radius, suggesting that emphysema progression is associated with alveolar shallowing and alveolar duct enlargement. Studies in asthma using this technique are currently awaited.
In addition to studying the microstructure of the acinar airways, as described above, $^3\text{He}$- and $^{129}\text{Xe}$-MRI may also provide insight into regional lung ventilation. Several studies have reported the detection of ventilation defects in patients with asthma using these techniques$^{199-207}$. The number of ventilation defects correlates with spirometric airflow obstruction$^{200,201}$, and increases following methacholine or exercise challenge$^{195,200,202}$. Moreover, their location in any given patient with asthma is remarkably consistent over time$^{202,204}$, suggesting that they are associated with long-term structural remodelling of specific airways rather than being a consequence of random variability in airway smooth muscle tone. Recent studies have shown that ventilation defects are associated with areas of air trapping$^{203}$ and airway wall thickening$^{207}$ detected by CT. Tzeng et al quantified the heterogeneity of regional lung ventilation in healthy subjects and patients with asthma before and after a methacholine challenge$^{208}$. They found that methacholine challenge elevated VH in both groups, and that this could be reversed by a deep inspiration in healthy subjects, but not in asthmatics. $^3\text{He}$-MRI washout experiments have been performed in rodents$^{209}$ and humans$^{210}$, providing a regionalised analogue of standard MBW techniques. The coupling of imaging techniques and computational modelling approaches is likely to yield important insights into the structural basis of VH$^{211-214}$.

1.5 Aims and hypotheses

1.5.1 Validation of multiple breath washout technique

This thesis presents the results of MBW measurements performed using the non-resident inert tracer gas 0.2% SF$_6$, and a modified Innocor photoacoustic gas analyser, as described by Horsley et al$^{132}$. It is recommended in current guidelines that MBW systems should be validated using a realistic lung model across the range of respiratory rates, tidal volumes and lung volumes likely to be encountered in clinical practice$^{130}$. I therefore aimed to:

i) Validate our MBW system using an acrylic glass lung phantom with realistic body temperature and pressure, saturated (BTPS) conditions.
Determine the variability of repeated MBW measurements performed *in vivo* and *in vitro* in order to estimate the relative importance of biological and instrument noise.

### 1.5.2 Development of novel indices of ventilation heterogeneity

LCI may be increased by (i) unequal convective ventilation between larger lung units, or (ii) increased respiratory dead space. I aimed to develop novel MBW parameters (LCI\textsubscript{vent} and LCI\textsubscript{ds}) that would quantify the relative contributions of these mechanisms towards observed increases in LCI, particularly in patients with severe VH. With this aim in mind, I re-analysed raw washout data from 40 patients with CF, kindly provided to me by Dr. Alex Horsley (Manchester, UK). In addition, I analysed washout data from 43 patients with non-CF bronchiectasis, since VH in this patient group has not been the subject of a large number of previous studies.

I hypothesised that:

i) LCI\textsubscript{vent} and LCI\textsubscript{ds} are repeatable in patients with CF and non-CF bronchiectasis.

ii) CF and non-CF bronchiectasis are characterised by increased LCI\textsubscript{vent} and LCI\textsubscript{ds} compared to healthy control subjects.

i) LCI, LCI\textsubscript{vent} and LCI\textsubscript{ds} are related to other measures of disease severity in CF and non-CF bronchiectasis, namely the degree of spirometric airflow obstruction and the presence or absence of chronic bacterial colonisation.

### 1.5.3 Repeatability of small airway biomarkers

Before small airway biomarkers enter into widespread clinical use, it is necessary to be assured of their repeatability and stability over time. I therefore aimed to determine the within-visit and between-visit repeatability of a range of IOS and MBW parameters. Furthermore, I aimed to calculate the standard deviation of between-visit differences for each parameter, in order to facilitate sample size calculations for future interventional trials.
1.5.4  **Clinical significance of small airway obstruction markers in asthma**

A number of studies have examined the clinical significance of various putative markers of small airway obstruction. However, there has as yet been no study examining the contributions of each of these markers to clinical outcomes in a single well-characterised group of adults with asthma.

I hypothesised that small airway obstruction markers are associated with (i) increased asthma severity, as evidenced by higher treatment requirements, (ii) impaired asthma control and quality of life, and (iii) frequent exacerbations.

1.5.5  **Structural correlates of acinar ventilation heterogeneity**

The structural and anatomical correlates of MBW indices in health and disease have not been determined, and our current understanding is based largely on simplified mathematical models\(^\text{137-142}\). I aimed to utilise \(^3\)He-MR to shed light upon the structural correlates of \(S_{\text{acin}}\), a putative marker of acinar airspace disease, in patients with asthma.

I hypothesised that asthma patients with raised \(S_{\text{acin}}\) would manifest evidence of altered diffusion within the acinar airways compared to asthma patients with normal \(S_{\text{acin}}\) and healthy controls.

1.5.6  **Modification of small airway obstruction with treatment**

A small number of studies have suggested that ventilation heterogeneity in asthma may be partially reversible with treatment\(^\text{158-160}\). I aimed to assess whether small airway obstruction in stable asthma is modifiable by a systemically active agent. With this objective in mind, we undertook a randomised, double-blind, placebo-controlled trial of QAW039, a chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) receptor antagonist with anti-eosinophilic properties, in patients with persistent eosinophilic asthma. The primary outcome measure was sputum eosinophil count, and a panel of putative small airway obstruction markers were incorporated into the study design as exploratory outcome measures.

I hypothesised that treatment with QAW039 would result in improvements in markers of small airway obstruction in patients with eosinophilic asthma.
2 Methods

2.1 Spirometry
Spirometry was performed according to standard American Thoracic Society / European Respiratory Society (ATS/ERS) guidelines\textsuperscript{215}, using a Vitalograph rolling seal spirometer, connected to a mouthpiece incorporating a bacterial filter. Participants were tested in a comfortable seated position, wearing a noseclip. Following a period of quiet tidal breathing, participants were instructed to inspire to TLC and then to immediately place their lips around the mouthpiece with a good seal and expire as forcefully as possible. The operator encouraged participants to continue the forced expiration until RV was reached. At least three forced expirations were performed. Repeatability was considered acceptable if the two largest FEV\textsubscript{1} values, and the two largest FVC values differed by no more than 150 ml. If the repeatability criterion was not met, additional manoeuvres, up to a maximum of eight, were performed until the criterion was met. The largest FEV\textsubscript{1} and FVC values were recorded, even if they occurred during different manoeuvres. Predicted values for FEV\textsubscript{1} and FVC were calculated using the European Coal and Steel Community regression equations\textsuperscript{216}.

2.2 Measurement of lung volumes using body plethysmography
Body plethysmography was performed according to standard ATS/ERS guidelines\textsuperscript{90}, using a constant volume plethysmograph. Participants were tested in a comfortable seated position, wearing a noseclip, and breathing exclusively through a rubber mouthpiece connected to a pneumotachometer. Following a period of quiet tidal breathing, participants were instructed to pant gently against a closed shutter, which automatically closed at the onset of a tidal inspiration at FRC, and automatically opened following the performance of an acceptable panting manoeuvre. Thoracic gas volume and plethysmographic FRC (FRC\textsubscript{pleth}) were calculated automatically by the on-board software, using Boyle’s Law, from pressure changes in the box and at the mouth. A minimum of three technically satisfactory panting manoeuvres were performed, with acceptable repeatability criteria being a difference of no more than 5% between the highest and lowest FRC\textsubscript{pleth} values obtained. Immediately following completion of the panting manoeuvres, and without coming off the mouthpiece, participants were
instructed to inspire to TLC and then immediately perform a forced expiratory manoeuvre to RV. This was then used to calculate absolute values for TLC and RV.

2.3 Single-breath determination of carbon monoxide uptake in the lung

Carbon monoxide uptake in the lung was determined using the single-breath method, according to standard ATS/ERS guidelines. Participants were tested in a comfortable seated position, wearing a noseclip, and breathing exclusively through a rubber mouthpiece connected to a pneumotachometer. Following a period of quiet tidal breathing, participants were asked to perform a relaxed expiration to residual volume, at which point the inspired gas was switched, under the control of the operator, from room air to a gas mixture containing 0.3% carbon monoxide and 10% helium (balance air). Participants were instructed to rapidly inspire to TLC and then hold their breath at TLC for nine seconds, followed by a relaxed exhalation. Following the exhalation of 0.75 – 1L of air, so as to exclude dead space gas, a sample of alveolar gas was automatically collected for analysis of the helium and carbon monoxide concentrations. Alveolar volume (VA) was calculated automatically by the on-board software from the dilution of helium, since this is not absorbed across the alveolar-capillary membrane. The carbon monoxide transfer coefficient (KCO), effectively a rate constant for the reduction in alveolar carbon monoxide concentration during the breath-hold period, was calculated based on the combined effect of dilution and absorption on the final concentration of carbon monoxide. The diffusing capacity of the lung for carbon monoxide (DLCO) was calculated as VA multiplied by KCO.

2.4 Impulse oscillometry

2.4.1 Theoretical background

IOS is a variant of the forced oscillation technique (FOT), a method of determining the mechanical impedance of the respiratory system. FOT involves imposing an oscillatory waveform on the respiratory system at the mouth and determining the relationship between pressure and flow waves at the same location with respect to amplitude and
phase. This allows the resistance ($R$) and reactance ($X$) of the respiratory system to be determined:

$$R = \frac{A^1}{A^2} \cos(\varphi^1 - \varphi^2)$$

$$X = \frac{A^1}{A^2} \sin(\varphi^1 - \varphi^2)$$

Where $A^1$ and $A^2$ represent the amplitudes of the pressure and flow waves, respectively. The quantity $(\varphi^1 - \varphi^2)$ represents the phase difference between pressure and flow, and may range from $-\frac{\pi}{2}$ to $\frac{\pi}{2}$ radians (or $-90^\circ$ to $90^\circ$), with a positive value indicating that pressure leads flow, and a negative value indicating that pressure lags flow. Resistance is always positive, whereas reactance is negative if $\varphi^1 < \varphi^2$, positive if $\varphi^1 > \varphi^2$, and is equal to zero if $\varphi^1 = \varphi^2$.

The phase difference between pressure and flow depends upon the frequency of the forced oscillations. This is because there are two opposing properties of the respiratory system at work, namely (i) elastance, which causes pressure to lag behind flow (negative phase shift), and (ii) inertance, which causes pressure to lead flow (positive phase shift). Elastance is an expression of the elastic properties of the respiratory system, as measured at the mouth, and is analogous to the spring constant of an elastic body. In the current context, the elastic body under study is the ‘spring’ composed of tissue and air, comprising the airways, lung parenchyma and chest wall. In the context of asthma and COPD, elastance is thought to be affected mainly by airway closure, rather than changes in the elastic properties of the lung parenchyma. Inertance is an expression of the ‘inertia’ of the respiratory system, its resistance to acceleration. At low oscillation frequencies, inertia does not have a major effect, and the effect of elastance is therefore dominant, resulting in a negative reactance. At the resonant frequency, elastance and inertance are in balance, so that there is no phase difference between pressure and flow, and reactance is zero. Above the resonant frequency, inertia becomes the dominant force, and reactance is therefore positive.
If the forcing waveform delivered to the respiratory system is a simple sinusoid at a single frequency, then resistance and reactance will only be determined at this frequency. In order to obtain information about more than one frequency simultaneously, more complex waveforms incorporating multiple sinusoids at different frequencies may be used. The pressure and flow signals may be decomposed into their discrete component frequencies using a mathematical technique known as a Fourier transform, and the impedance may then be calculated at each frequency. The waveform employed in IOS consists of alternative positive and negative impulses, which are each analysed separately to obtain a resistance and a reactance at that time point, across a frequency range spanning 5Hz – 35Hz.

2.4.2 Patient testing procedure

IOS was performed in triplicate according to standard guidelines\textsuperscript{101} using the Jaeger MasterScreen Impulse Oscillometry System (Viasys Healthcare GmbH, Hoechberg, Germany). A volume calibration was performed daily using a 3 L syringe, and the accuracy of resistance measurements was confirmed daily using a standard 0.2 kPaL\textsuperscript{−1}s resistance mesh. The ambient temperature and atmospheric pressure was checked daily and entered into the system. Participants wore a nose clip and supported their cheeks whilst an impulse waveform was delivered to their respiratory system via a loudspeaker connected to a mouthpiece incorporating a bacterial filter (MicroGard, CareFusion, Basingstoke, UK) during 60 seconds of tidal breathing. Resistance at 5Hz (R5), resistance at 20Hz (R20), R5-R20, reactance at 5Hz (X5) and AX were automatically calculated by the IOS device from pressure and flow measurements recorded throughout the 60-second period. The mean value for each parameter across the triplicate measurements was recorded.

2.5 Multiple breath inert gas washout

2.5.1 Hardware specifications

MBW was performed using a modified Innocor photoacoustic gas analyser (Innovation A/S, Odense, Denmark), with 0.2% SF\textsubscript{6} as the inert tracer gas, as described by Horsley \textit{et al}\textsuperscript{132}, and according to standard guidelines\textsuperscript{130}. The Innocor device is a small, portable
unit which weighs approximately 8 kilograms and has dimensions of $35 \times 29 \times 26$ cm. It was originally designed to measure cardiac output at rest and during exercise, by measuring the difference in absorption between N$_2$O, which crosses the alveolar-capillary membrane, and SF$_6$, which does not. In addition, the device can simultaneously measure oxygen uptake and carbon dioxide production. At the heart of the Innocor device is a photoacoustic gas analyser, which is capable of measuring the concentrations of CO$_2$, SF$_6$ and N$_2$O at high accuracy and temporal resolution. It is sensitive to low concentrations of SF$_6$ within an operating range of $0 - 0.5\%^{218}$. The photoacoustic gas analyser works by exposing the gas sample to a beam of infra-red light at three separate frequencies, each of which is absorbed by one of the three compounds measured, and converted to heat. The beam of light is pulsed at three separate frequencies, resulting in cyclical heating and cooling of the measured gases, which in turn produces pressure (sound) waves at a specific frequency for each compound. These sound waves are detected by a microphone and used to determine the concentration of each compound. A separate oxygen analyser is placed in series before the photoacoustic analyser, since the photoacoustic principle cannot be used to detect monoatomic species. A photograph of the device is shown in Figure 2.1.

A number of modifications were made to the Innocor device to facilitate the performance of MBW. Firstly, the commercially supplied patient interface has an excessive dead space for inert gas washout, and was therefore replaced with a mesh-type pneumotachometer (3700 series, Hans Rudolph Inc., Kansas City, Missouri, USA), heated to 37°C using a 3850A series pneumotachometer heater (Hans Rudolph Inc., Kansas City, Missouri, USA). The patient interface, shown in Figure 2.2, comprised a rubber mouthpiece connected to a bacterial filter (Clear-Guard Midi, Intersurgical, Wokingham, Berkshire, UK), which was in turn connected to the pneumotachometer. The gas sample needle was positioned distal to the pneumotachometer mesh, resulting in a minimal post-capillary dead space of 2.65 ml. The pre-capillary dead space was calculated as 40 ml for *in vitro* tests and 54.6 ml for *in vivo* tests (due to the additional dead space of the rubber mouthpiece).
Figure 2.1: Innocor photoacoustic gas analyser

Figure 2.2: Patient interface for the performance of multiple breath inert gas washout
The flow signal was split between the Innocor device and a separate laptop computer, which was utilised to provide a visual guide to participants, so that they could adjust their tidal volume to approximately 1L. This was accomplished using a custom program, written using TestPoint (Measurement Computing Corporation, Norton, Massachusetts, USA) and provided to us by Dr. Per Gustafsson, Skövde, Sweden. A further modification made to the Innocor device was to manually bypass the oxygen analyser by diverting the internal Nafion tubing directly into the photoacoustic analyser. This had the effect of reducing both the gas analyser rise time and the flow-gas delay time.

Before the commencement of testing on each day, a volume calibration was performed using a 1L syringe at three different flow rates, in order to ensure accuracy of flow and volume measurements made by the Innocor device. A separate volume calibration, using the same 1L syringe, was performed to ensure accuracy of the TestPoint software used by participants to monitor their tidal volume.

2.5.2 Patient testing procedure

Participants were tested in a comfortable seated position, wearing a noseclip, and breathing exclusively through the rubber mouthpiece. Throughout the test, care was taken to ensure a good seal was maintained around the mouthpiece, and participants were encouraged to maintain a steady respiratory rate of approximately 12 breaths per minute, and a constant tidal volume of 1L, using a real-time visual display of inspired volume as a guide. The first stage of the test was the wash-in phase, during which participants breathed an air mixture containing 0.2% SF$_6$, via an open-circuit bypass flow system. This comprised a Douglas bag, which acted as a reservoir for the SF$_6$ air mixture, connected to a valve, such that subjects inspired from the bag, but expired air was vented to the atmosphere. During the wash-in phase, the flow of SF$_6$ air mixture was adjusted to ensure that the Douglas bag remained approximately half-full, and participants were given instructions as appropriate to ensure that they maintained the required tidal volume and respiratory rate. Wash-in was continued until the expired SF$_6$ concentration was within 0.004% of the inspired concentration for at least three consecutive breaths. Participants were then switched to breathing room air during an expiration, by swiftly removing the Douglas bag from the end of the patient interface, and asked to continue breathing at the same respiratory rate and tidal volume. The end-
tidal concentration of SF₆ in exhaled breath (Cₑₑ) was recorded during this washout phase until it fell below 1/4₀ᵗʰ of the original concentration (0.005%) for three consecutive breaths, at which point the test was terminated.

MBW tests were performed in triplicate for each participant. In order to ensure consistency of results, a preliminary analysis was performed to verify that measured FRC values for the three tests did not differ by more than 10%°. If no two tests were consistent then further washout runs were performed (to a maximum of five tests) until at least two tests were consistent. The final results reported for each participant comprised the average of the two or three consistent tests performed. If two consistent tests could not be obtained then this was recorded, and the results for that participant were not used.

2.5.3 Analysis of multiple breath washout data

The raw MBW data consisted of a flow and SF₆ signal, sampled at a rate of 100Hz. These data were transferred from the Innocor device to a laptop computer, where they were processed and analysed using custom software written with TestPoint (Measurement Computing Corporation, Norton, Massachusetts, USA), provided by Dr. Per Gustafsson. The first step in the process was to calculate the flow-gas delay time for each washout test, which was subsequently used to synchronise the flow and SF₆ concentration signals. The flow-gas delay time arises due to a combination of the gas analyser response time, which is approximately 15₄₉₃₃₉ms, and the time taken for gas to be drawn down the Nafion tubing from the patient interface to the photoacoustic analyser, at a sampling flow rate of 120 ml/minute. The flow-gas delay time was calculated using the method based upon re-inspiration of SF₆ from the post-capillary dead space. Specifically, the time point at which the post-capillary dead space had been inspired was aligned with the time point at which the inspired SF₆ concentration fell to 5₀% of its initial value. FRC was calculated by dividing the total volume of SF₆ expired by the difference between Cₑₑ at the beginning and end of the washout period. The end of the washout period for this purpose was taken to be the first expired breath in which Cₑₑ fell below 1/4₀ᵗʰ of the original SF₆ concentration. The total volume of expired SF₆ was calculated by integrating flow and SF₆ concentration over the course of each expiration, and subtracting the re-inspired SF₆ volume, which was in turn calculated by integrating flow and SF₆ concentration over the course of each
inspiration. LCI was defined as the cumulative expired volume (CEV) at the point at which $C_{et}$ fell to $1/40^{th}$ of its initial value, divided by the FRC\textsuperscript{135}. Both FRC and CEV were corrected for equipment dead space ($D_{eq}$) by subtracting this volume (54.6 ml) from the calculated FRC, and from the expiratory volumes used to calculate CEV. The anatomical dead space ($V_{D_{anat}}$) was calculated using the method of Langley\textsuperscript{130}.

Phase III slope parameters were calculated by first determining the raw phase III slopes (SIII) of each expiration of the washout, as illustrated in Figure 1.3. The start and end points of the phase III slope were adjusted manually for each breath, to ensure that phases II and IV were not included. The TestPoint program automatically produced a linear regression line of the selected portion of the expirogram, and the slope for each breath was recorded. The data were then exported as an Excel file and further analysed using Excel 2007 (Microsoft Corporation, Redmond, Washington, USA). SIII values were concentration-normalised by dividing them by the mean expired SF\textsubscript{6} concentration over the course of phase III, to yield SnIII values for each breath. The tidal volume correction of Aurora \textit{et al}\textsuperscript{146} was not used, since most patients successfully adhered to the 1L tidal volume protocol, thus making this correction unnecessary. SnIII was then plotted against turnover number for each expiration, as illustrated in Figure 2.3. $S_{cond}$ was calculated as the rate of increase of SnIII between 1.5 and 6 turnovers, whilst $S_{acin}$ was the SnIII of the first breath minus a small correction factor to account for the CDI component of the first breath. $S_{cond}^*$ and $S_{acin}^*$ were calculated analogously to $S_{cond}$ and $S_{acin}$, except that $S_{cond}^*$ was based upon SnIII values between 0 and 3 turnovers, excluding the first breath of the washout, instead of between 1.5 and 6 turnovers\textsuperscript{149}. Phase III slope parameters may be calculated for each washout test separately (Figure 2.3, Panel A), or alternatively three washout tests may be analysed in combination (Figure 2.3, Panel B). In this thesis each washout was analysed separately.
**Figure 2.3: Calculation of phase III slope parameters**

*Panels A and B show plots of normalised phase III slopes (SnIII) against turnover number (TO) in a patient with asthma. Panel A shows data from a single washout test, while Panel B shows data from three washout tests combined. S_{cond} is the slope of the best-fit regression line of the points between TO numbers 1.5 and 6. S_{acin} is given by the following equation:*

\[
S_{acin} = \text{First breath SnIII} - (S_{cond} \times \text{TO number of first breath})
\]

**Panel A**

![Plot of SnIII against TO for a single washout test]

**Panel B**

![Plot of SnIII against TO for three washout tests combined]
2.6 Validation of multiple breath washout technique using a lung model

Validation was performed using a simple one-compartment model of the lung consisting of an enclosed clear acrylic glass tank (Soloplex, Tidaholm, Sweden), partly filled with water at 37°C, as recommended in current guidelines\textsuperscript{130}. The lung model was designed by Dr. Per Gustafsson, and had previously been used to validate an alternative MBW system\textsuperscript{134}. In order to achieve BTPS conditions within the phantom lung, it was enclosed within a further acrylic glass tank containing water that was kept at a constant temperature of 37°C by a thermostat. The dimensions of the outer and inner tanks are shown in Figure 2.4, Panels A and B, respectively.

The inner tank was fitted with an off-centre vertical partition that divided it into a larger and a smaller section. The partition did not reach the base of the tank, thus allowing the two sides to communicate, but the water level was always kept above the lower end of the partition. The lid of the larger section was connected to a bi-level positive airway pressure ventilator (Vivo 30, Breas Medical AB, Mölnlycke, Sweden) which exerted alternating high and low pressures on the water surface, designated inspiratory and expiratory positive airway pressures (IPAP and EPAP), respectively. Due to the communication below the partition, this caused the water level in the smaller section to alternately rise and fall, simulating diaphragmatic movement. The IPAP setting on the ventilator determined the FRC of the phantom lung, whilst the difference between the IPAP and EPAP settings determined the tidal volume ($V_T$). The lid above the smaller section was modified to fit the patient interface described in Section 2.5, with the exception that the rubber mouthpiece was omitted and the lung model attached directly to the bacterial filter. A photograph of the lung model whilst in use is shown in Figure 2.5.
Figure 2.4: Schematic diagram of lung model for the validation of multiple breath washout technique
Figure 2.5: Photograph of lung model for the validation of multiple breath washout technique
The testing procedure consisted of the following steps:

i) The water level, IPAP setting and EPAP setting were adjusted to achieve the required FRC and Vt within the lung model. This was facilitated by a measuring scale affixed to the inside of the lung model, which was utilised to measure the water level at its highest and lowest points during the respiratory cycle. Respiratory rate was set using the ventilator controls.

ii) An air mixture containing 0.2% SF6 was passively insufflated into the lung phantom via an open-circuit bypass flow system, and expiratory SF6 concentration was monitored online using the Innocor gas analyser. The flow rate of SF6 through the open circuit was increased sufficiently to allow complete wash-in of SF6.

iii) Once complete equilibration (wash-in) had occurred, meaning that the inspired and expired SF6 concentrations were equal, the bypass flow system was removed so that the lung model inspired from ambient air. Expiratory SF6 concentration continued to be monitored during the wash-out phase, and the experiment was terminated once the expired SF6 concentration fell below 0.005% (1/40th initial value) for three consecutive breaths.

iv) Washout data were exported to a separate laptop computer, where they were analysed to derive the FRC and LCI.
2.7 Development of novel multiple breath washout parameters

2.7.1 Derivation of the novel parameters

Gustafsson et al recently reported a method for estimating the size of the “fast” (well-ventilated) and “slow” (poorly-ventilated) compartments in patients with CF using inert gas washout\textsuperscript{220}. However, their method was not specifically indexed to the LCI, and in particular did not allow estimation of the relative contributions of specific ventilation inequality and increased respiratory dead space. This section describes the development of novel MBW parameters that estimate the contributions of each of these mechanisms to increased LCI in a given patient.

In order to simulate washout curves incorporating specific ventilation inequality between lung units, it is clear that an anatomical model with at least two compartments will be required. However, before introducing the two-compartment model, it is useful to briefly describe the even simpler one-compartment model of the lung. Consider the washout of a single uniformly ventilated alveolar compartment subtended by a conducting airway. The FRC is the sum of the alveolar volume ($V_A$) at end expiration and the conducting airway volume, or anatomical dead space ($V_{D\text{anat}}$), which is considered to be fixed and to not take part in gas mixing.

Let $\text{FRC} =$ functional residual capacity $V_{D\text{anat}} =$ anatomical dead space $V_T =$ tidal volume

With each successive inspiration, the SF\textsubscript{6} concentration in the alveolar compartment will be diluted by a fixed ratio. This dilution ratio was derived by Fowler et al.\textsuperscript{221}, and is equal to:

$$\frac{\text{FRC}}{\text{FRC} + V_T - V_{D\text{anat}}}$$

Therefore, the SF\textsubscript{6} concentration in the alveolar compartment following the $n^{th}$ inspiration will be equal to the initial SF\textsubscript{6} concentration (0.2\%) multiplied by the dilution ratio raised to the power of $n$: 
This is an exponential decay curve, of which the standard form is:

\[ y = a \times e^{-kx} \]

Where

- \( a \) = y-intercept of the curve
- \( k \) = rate constant
- \( e \) = the base of natural logarithms \( \approx 2.718 \)

Assuming that the initial SF\(_6\) concentration is 0.2\%, the y-intercept parameter will be set at 0.2, and thus the only parameter that may vary is the rate constant \( k \). The x-axis units may be breath number or TO number, but TO is preferred since it smoothes irregularities in the washout curve caused by variations in tidal volume. Figure 2.6 shows example exponential decay curves with different rate constants, illustrating that a higher rate constant results in a more rapid decay.
Figure 2.6: Exponential decay curves

Exponential decay curves are shown with rate constants of 0.2 (dotted line), 0.6 (dashed line) and 1.8 (continuous line), using linear (Panel A) and semilog (Panel B) scales.

Panel A

Panel B
In order to simulate the washout of a two-compartment lung model we require a two-phase exponential decay curve, which has the following general form:

\[ y = (a \times e^{-jx}) + (b \times e^{-kx}) \]

Where

- \( j \) = fast rate constant
- \( k \) = slow rate constant
- \( a \) = weighting of fast rate constant
- \( b \) = weighting of slow rate constant

The \( y \)-intercept of this curve is equal to \( a + b \). Since in our case this is set at 0.2 (the initial SF\(_6\) concentration), we can re-write the above equation as:

\[ y = 0.2 \times (c \times e^{-jx} + [1 - c] \times e^{-kx}) \]

Where \( c \) = proportionate weighting of fast-decaying component, and \( 0 > c > 1 \)

This curve therefore has three variable parameters (or degrees of freedom), namely the fast rate constant (\( j \)), the slow rate constant (\( k \)) and the proportionate weighting of the fast-decaying component (\( c \)). The curve-fitting software Prism 6 (GraphPad Software Inc., La Jolla, California, USA) was used to fit experimental washout curves (normalised to an initial SF\(_6\) concentration of 0.2%) to the above model, which is also known as a two-phase decay model. This was performed using non-linear regression with a weighting of \( 1/y^2 \) in order to minimise the sum of relative, rather than absolute, squared residuals. This was so that data points near the start of the washout curve (where absolute \( C_e \) values were higher) did not disproportionately impact upon the model fit. The initial values for \( j \), \( k \) and \( c \) utilised in the curve fitting process were determined automatically by Prism 6, using in-built rules. Figure 2.7 shows washout curves from a healthy subject and a patient with CF fitted to a two-phase exponential decay model.
Figure 2.7: Washout curves from a healthy subject and a patient with cystic fibrosis fitted to a two-phase exponential decay model

Washout curves from a healthy subject (Panel A) and a patient with severe CF (Panel B) are fitted to a two-phase exponential decay curve, with a good model fit in both cases (goodness of fit $R^2 = 0.9973$ and 0.9775, respectively). $C_{et(norm)}$ represents end-tidal SF$_6$ concentration, normalised to a starting concentration of 0.2%.
A natural anatomical model that would be expected to behave according to the equation above comprises two lung units in parallel, each consisting of an alveolar compartment subtended by a conducting airway. The constants j and k correspond to the rate constants for the washout of the over-ventilated (fast) and under-ventilated (slow) lung units respectively, which have resting (end-expiratory) volumes of $V_{fast}$ and $V_{slow}$, respectively. The SF$_6$ concentration of the expired gas from this system is equal to the weighted mean of the SF$_6$ concentrations in each of the two alveolar compartments at end-inspiration, where the weighting is determined by the proportion of ventilation reaching each lung unit. Thus, the anatomical interpretation of the constant c, defined above, is the proportion of the tidal volume reaching the fast lung unit.

It is now possible to derive two anatomical parameters from the two-compartment model, one of which reflects specific ventilation inequality between the lung units, and the second of which reflects the effective respiratory dead space ($V_{D_{resp}}$). A natural measure of specific ventilation inequality is the ratio of the specific ventilation of the slow lung unit to that of the fast lung unit, which we refer to as the specific ventilation ratio (SVR).

Figure 2.8 shows simulated washout curves based upon this two-compartment lung model, demonstrating the effect of alterations in SVR or $V_{D_{resp}}$. In these simulations, the proportion of ventilation reaching the fast lung unit (c) was kept constant at 0.8, and the anatomical dead space was kept constant at 0.2L. The expired SF$_6$ concentration measured at the mouth during the $n^{th}$ expiration was calculated as the weighted mean of the washouts of the two compartments, and was given by the formula:

$$0.04 \times \left( \frac{V_{slow}}{V_{slow} + \frac{V_T}{5} - \frac{V_{D_{resp}}}{5}} \right)^n + 0.16 \times \left( \frac{V_{fast}}{V_{fast} + \frac{4 \times V_T}{5} - \frac{4 \times V_{D_{resp}}}{5}} \right)^n$$

Increasing SVR results in a change in the shape of the washout curve such that there is a rapid initial phase followed by a slow terminal phase, whereas increasing $V_{D_{resp}}$ results in a prolongation of the washout curve but without altering its shape. In both cases, the result is an increase in LCI.
Figure 2.8: Simulated washout curves

Panel A shows simulated washout curves with a fixed respiratory dead space of 0.4L and a specific ventilation ratio of 0.6 (continuous line), 0.15 (dashed line) and 0.05 (dotted line). Panel B shows simulated washout curves with a fixed specific ventilation ratio of 0.15 and a respiratory dead space of 0.1L (continuous line), 0.4L (dashed line) and 0.6L (dotted line).
From the definition of SVR as the ratio of the specific ventilation of the slow lung unit to that of the fast lung unit we may write:

\[
SVR = \frac{\left[ \frac{1 - c}{{V}_{\text{slow}}} \times {V}_{T} \right]}{\left[ \frac{c \times {V}_{T}}{FRC - V_{\text{slow}}} \right]} = \frac{(1 - c)(FRC - V_{\text{slow}})}{c \times {V}_{\text{slow}}} = \frac{(1 - c)(1 - W)}{cW}
\]

Where \( V_{\text{slow}} = \) volume of slow lung unit at FRC
\( W = V_{\text{slow}}/FRC \)

\[
SVR = \frac{(1 - c)(1 - W)}{cW}
\]
\[
W = \frac{1 - c}{c \times SVR - c + 1}
\]

As stated above, \( C_{\text{et(norm)}} \) against TO curves can be accurately modelled by the following equation:

\[
y = 0.2 \times (c \times e^{-jr} + [1 - c] \times e^{-ks})
\]

The subsequent analysis is simplified if this equation is expressed in terms of breath number (n) rather than TO. This change of scale is achieved by multiplying the rate constants \( j \) and \( k \) by a constant term to yield the new equation:

\[
y = 0.2 \times (c \times e^{-rn} + [1 - c] \times e^{-sn})
\]

Where \( r = j \times (V_T - D_{\text{eq}})/FRC \), and \( s = k \times (V_T - D_{\text{eq}})/FRC \)

\( D_{\text{eq}} = \) equipment dead space between the patient's mouth and the gas sampling capillary, and was equal to 0.0546 L using our inert gas washout system.

Following the \( n^{th} \) inspiration, the SF\(_6\) concentration is equal to \( 0.2 \times e^{-rn} \) in the fast compartment and \( 0.2 \times e^{-sn} \) in the slow compartment. Using the formula for the dilution ratio in a single compartment\(^{221} \), and assuming that both tidal volume and respiratory dead space are distributed between the over-ventilated and under-ventilated lung units in a ratio of \( c \) to \( (1 - c) \), we may write the following equations:
\[
0.2 \times e^{-rn} = 0.2 \times \left( \frac{FRC \times (1 - W)}{FRC \times (1 - W) + c \times VT - c \times V_{D_{resp}}} \right)^n
\]
\[
0.2 \times e^{-sn} = 0.2 \times \left( \frac{FRC \times W}{FRC \times W + (1 - c) \times VT - (1 - c) \times V_{D_{resp}}} \right)^n
\]

Let \( p = e^{-r} \) and \( q = e^{-s} \)

Then:

\[
p = \frac{FRC \times (1 - W)}{FRC \times (1 - W) + c \times VT - c \times V_{D_{resp}}}
\]
\[
q = \frac{FRC \times W}{FRC \times W + (1 - c) \times VT - (1 - c) \times V_{D_{resp}}}
\]

By solving these two equations in the two unknowns \( W \) and \( V_{D_{resp}} \) the following formulae are derived:

\[
V_{D_{resp}} = VT + \frac{FRC \times (pq - p - q + 1)}{(pq - pc - q[1 - c])}
\]

\[
W = \frac{cpq - cq - pq + q}{cp - cq + q - pq}
\]

Substituting:

\[
W = \frac{1 - c}{c \times SVR - c + 1}
\]

We obtain the following formula:

\[
SVR = \frac{pq - p}{pq - q}
\]
We may now utilise the values of $c$, $V_{D_{\text{resp}}}$ and SVR derived above to determine:

i) $LCI_{\text{ideal}}$ – The expected value of LCI assuming no specific ventilation inequality, and no additional respiratory dead space over and above $V_{D_{\text{anat}}}$. 

ii) $LCI_{\text{vent}}$ – The proportional increase in LCI over and above $LCI_{\text{ideal}}$, taking into account specific ventilation inequality but assuming no additional respiratory dead space.

iii) $LCI_{\text{ds}}$ – The proportional increase in LCI over and above $LCI_{\text{ideal}}$, taking into account additional respiratory dead space, but assuming no specific ventilation inequality.

$LCI_{\text{ideal}}$ and $LCI_{\text{ds}}$ are calculated using the following formulae, which are based upon the dilution ratio in a single compartment$^{221}$:

\[
LCI_{\text{ideal}} = \frac{\ln(0.025) \times (VT - DS_{eq})}{FRC \times \ln\left(\frac{FRC}{FRC + VT - V_{D_{anat}}}\right)}
\]

\[
LCI_{\text{ds}} = \frac{\ln\left(\frac{FRC}{FRC + VT - V_{D_{anat}}}\right)}{\ln\left(\frac{FRC}{FRC + VT - V_{D_{resp}}}\right)}
\]

In order to determine $LCI_{\text{vent}}$, we utilise the values of $c$ and SVR derived above, but set $V_{D_{\text{resp}}}$ to equal $V_{D_{anat}}$. We then reverse the algebraic steps described above in order to arrive at two new rate constants $j'$ and $k'$. The values of $c$, $j'$ and $k'$ are then plugged into our original equation for the washout of a two-compartment model, with $C_{et}$ on the $y$-axis and TO number on the $x$-axis:

\[
y = 0.2 \times \left(c \times e^{-j'x} + [1 - c] \times e^{-k'x}\right)
\]

Setting $y = 0.005$ (1/40th of the original inert gas concentration of 0.2) yields the equation:
\[ c \times e^{-j'x} + (1 - c) \times e^{-k'x} = 0.025 \]

This equation may be solved numerically to any desired degree of accuracy in the unknown \( x \). The solution is then divided by \( \text{LCI}_\text{ideal} \) to yield \( \text{LCI}_\text{vent} \).

### 2.7.2 Step-by-step instructions for calculating \( \text{LCI}_\text{vent} \) and \( \text{LCI}_\text{ds} \)

This section summarises the calculation of \( \text{LCI}_\text{vent} \) and \( \text{LCI}_\text{ds} \) in a step-by-step fashion using Prism 6 (GraphPad Software Inc., La Jolla, California, USA):

#### Step 1
Choose an X-Y table format and enter the data with TO number on the x-axis and \( C_e \) on the y-axis. The initial SF\(_6\) concentration (i.e., the SF\(_6\) concentration of expired air at the end of the wash-in phase) should be entered against a TO number of zero, and is thus the y-intercept of the curve. If a number of washout curves are to be analysed, it is convenient to normalise \( C_e \) values to a consistent initial SF\(_6\) concentration such as 0.2\% so that the y-intercept of the curve is always the same. For instance, if a given washout curve has an initial SF\(_6\) concentration of 0.198\%, the curve is normalised to a starting value of 0.2\% by multiplying each \( C_e \) value by 0.2/0.198.

#### Step 2
Choose the analysis method ‘non-linear regression’ and fit to the equation ‘two phase decay’. In the Constrain tab, set:

- \( Y0 – \text{Constant equal to 0.2} \)
- \( \text{Plateau – Constant equal to 0} \)
- \( \text{PercentFast – Must be between zero and 100.0} \)
- \( K\text{Fast} – \text{No constraint} \)
- \( K\text{Slow} – \text{Must be greater than 0} \)
- \( K\text{Fast} \text{ must be greater than 1.0 times } K\text{Slow} \)

In the Weights tab, choose: Weight by \( 1/y^2 \)
Press ‘OK’ to perform the analysis. This should produce values for PercentFast, KFast and KSlow. KFast and KSlow represent the rate constants for the washouts of the fast and slow lung units, respectively. These are represented as j and k respectively in the equations in Section 2.7.1. PercentFast represents the percentage of the tidal volume reaching the fast lung unit, and is equivalent to the parameter c in Section 2.7.1 (multiplied by a factor of 100, since PercentFast is a percentage while c is a proportion)

**Step 3**

Set:

\[ KFast_{(b)} = \frac{KFast \times (V_T - DS_{eq})}{FRC} \]

\[ KSlow_{(b)} = \frac{KSlow \times (V_T - DS_{eq})}{FRC} \]

**Step 4**

Set:

\[ p = e^{-KFast_{(b)}} \]

\[ q = e^{-KSlow_{(b)}} \]

\[ c = \frac{PercentFast}{100} \]

Then:

\[ SVR = \frac{pq - p}{pq - q} \]

\[ VD_{resp} = V_T + \frac{FRC \times (pq - p - q + 1)}{(pq - pc - q[1 - c])} \]

**Step 5**

LCI\textsubscript{ideal} and LCI\textsubscript{ds} are given by the following formulae:

\[ LCI_{ideal} = \frac{\ln(0.025) \times (V_T - DS_{eq})}{FRC \times \ln\left(\frac{FRC}{FRC + V_T - V_{D_{anat}}}\right)} \]
To calculate $\text{LCI}_{\text{vent}}$, utilise the values of $c$ and SVR derived in step 4, and perform the following algebraic steps:

\[
W = \frac{1 - c}{c \times \text{SVR} - c + 1}
\]

\[
p = \frac{\text{FRC} \times (1 - W)}{\text{FRC} \times (1 - W) + c \times \text{VT} - c \times \text{V}_{\text{D,anat}}}
\]

\[
q = \frac{\text{FRC} \times W}{\text{FRC} \times W + (1 - c) \times \text{VT} - (1 - c) \times \text{V}_{\text{D,anat}}}
\]

\[r = -\ln p\]

\[s = -\ln q\]

\[j' = \frac{r \times \text{FRC}}{(\text{VT} - \text{DS}_{\text{eq}})}\]

\[k' = \frac{s \times \text{FRC}}{(\text{VT} - \text{DS}_{\text{eq}})}\]

Using the values of $c$, $j'$ and $k'$ derived above, solve the following equation numerically in the unknown $x$:

\[c \times e^{-j'x} + (1 - c) \times e^{-k'x} = 0.025\]

Divide the value of $x$ obtained by $\text{LCI}_{\text{ideal}}$ to yield $\text{LCI}_{\text{vent}}$. 
2.8 Quantitative computed tomography

Volumetric whole lung scans were obtained using a Siemens Sensation 16 scanner using the following low dose protocol: 16 x 0.75 mm collimation, 1.5 mm pitch, 120 kVp, 40 mAs, 0.5 seconds rotation time and scanning field of view of 500 mm, dose modulation off. Scans were obtained at full inspiration and full expiration. Participants were coached in the breath holding technique immediately prior to scanning. Images were reconstructed with a slice thickness of 0.75 mm at a 0.5 mm interval using B35f kernel. VIDA Apollo image analysis software (VIDA Diagnostics, Coralville, Iowa) was used for quantitative analysis of lung densitometry, and the geometry of the major segmental bronchi. The main parameters extracted were:

i) Ratio of mean lung density on expiration to inspiration (MLD E/I) – a marker of expiratory air trapping

ii) Fifteenth lower percentile of inspiratory lung density ($P_{15}$) – a marker of emphysema

iii) Right upper lobe apical segmental bronchus (RB1) wall area

iv) RB1 wall percentage

v) RB1 luminal area

Computed tomography was not performed in female patients under the age of 30, and the maximum allowed dose of radiation from research CT scans for any participant was 10 mSv over a three year period.

2.9 Hyperpolarised $^3$helium diffusion magnetic resonance

2.9.1 Theoretical background

Magnetic resonance techniques are based upon the physical principle that any spinning charged particle generates a magnetic field, effectively acting as a bar magnet. The nucleus of a hydrogen atom comprises a single proton, and most clinical MR applications rely upon imaging protons in the body. However, proton imaging is not suitable for imaging the airways or measuring diffusive processes in the lungs, since the density of protons in ambient air is too low. For these applications, it is necessary for the subject to inhale a gas which acts as a magnetic dipole so that a measurable MR
signal can be produced and detected. The work presented in this thesis makes use of diffusion MR measurements using hyperpolarised $^3$He. Hyperpolarisation is the process by which atoms are imparted with an increased nuclear polarisation\textsuperscript{79}, thus increasing the signal strength that may be obtained during MR measurements.

When placed in an external magnetic field such as that generated by an MRI scanner, $^3$He nuclei will tend to line up in the direction of the external field (often designated $B_0$), which is assumed by convention to point longitudinally in the $z$-axis. The nuclei not only spin about their own axes, they also spin, or precess, around the axis of the external magnetic field. The angular frequency of this precession, known as the Larmor frequency ($\omega_0$), is linearly related to the external magnetic field strength. However, because individual nuclei are not precessing in synchrony (phase) with each other at this point, the net magnetisation vector does not precess, but simply points in the direction of $B_0$.

In order to generate a signal that may be used for imaging or other applications, it is necessary to transmit a radiofrequency (RF) pulse into the patient. The RF pulse is an electromagnetic wave, which comprises an electric and a magnetic component. The RF pulse generates a comparatively weak magnetic field along a new axis (designated $B_1$), perpendicular to $B_0$. If the frequency of the RF pulse matches the frequency of precession of the $^3$He nuclei around $B_0$, resonance will be generated, and they will begin to precess around the new axis $B_1$, a process known as flipping, while continuing to precess around the original axis at a much faster rate. This combined spiral motion is known as nutation. As the $^3$He nuclei flip, they begin to precess in phase with each other, a phenomenon known as phase coherence. The rotating magnetic field they generate induces an alternating current in coils placed around the patient. This is the basic principle by which an RF pulse may produce a detectable signal from the patient.

The angle at which the $^3$He nuclei flip (flip angle) is linearly related to both the power of the RF pulse and the duration of the pulse. A 90° pulse is one that causes the nuclei to flip fully into the transverse plane, resulting in a maximal signal. A 180° pulse causes the nuclei to flip from pointing in the direction of $B_0$ to pointing in the opposite direction, and thus does not generate an immediate signal, as there is no transverse
magnetisation. However, a 180° pulse also causes the direction of precession of the nuclei to reverse, a phenomenon that is made use of in spin echo pulse sequences.

Once the RF pulse is switched off the signal caused by transverse magnetisation begins to decay. This decaying sinusoidal signal is known as free induction decay (FID). The decay of transverse magnetisation (and thus signal) occurs at a much faster rate than the recovery of longitudinal magnetisation. This is because recovery of longitudinal magnetisation (T1 relaxation) occurs due to only one process, the relaxation of 3He nuclei back to their equilibrium state, pointing in the direction of B0. However, decay of transverse magnetisation (T2* decay) is additionally brought about by loss of synchrony (dephasing) between precessing nuclei. This dephasing occurs due to (i) interactions between the magnetic fields of neighbouring nuclei (spin-spin interactions), and (ii) inhomogeneities (imperfections) in the external magnetic field B0.

Gradient coils are components of MRI scanners that allow the generation of additional gradients in the external magnetic field in the x, y or z directions. External magnetic field gradients may be utilised to perform spatial encoding, as well as to measure diffusive processes. The timing of RF and gradient pulses may be represented on a pulse sequence diagram. The top line of a pulse sequence usually indicates the timing and flip angle of each RF pulse. Immediately below this, one or more lines indicate the application of gradients (if applicable) along the x, y or z axes, in the positive or negative direction. The final line indicates the appearance of measurable signals.

2.9.2 Pulse sequences utilised to measure diffusion in the lungs

Figure 2.9a shows a simple pulse sequence that demonstrates the phenomenon of spin echoes. This pulse sequence comprises a 90° pulse which causes flipping of 3He nuclei into the transverse plane, resulting in a rapidly decaying FID signal. This is followed, after a time period τ from the 90° pulse, by a 180° pulse, which causes the net magnetisation vector to flip into the direction opposite to B0. The 180° pulse also causes the direction of precession of 3He nuclei to reverse, so that after a further time period τ a two-sided echo of the original FID is seen, as the nuclei come back into phase. For this reason, a 180° pulse is often referred to as a refocusing pulse. However, the echo is of smaller amplitude than the original FID, as the refocusing pulse can only reverse dephasing caused by fixed magnetic field inhomogeneities, but not spin-spin
interactions. It is possible to obtain repeated echoes (an echo train) by transmitting a series of 180° pulses, each of which will be followed by an echo. The amplitude of these echoes will decay in an exponential fashion, mainly due to spin-spin interactions. This is described as T2 decay, and is intermediate in speed between T1 relaxation and T2* decay.

The diffusion of atoms in an unrestricted space may be modelled as a ‘random walk’ process, in which atoms move a certain distance in a random direction after each small time increment. The constant of proportionality in this case is known as the free diffusion coefficient ($D_0$), and is measured in units of cm$^2$s$^{-1}$. If diffusion is restricted by a physical boundary such as the walls of the alveolar airspaces, the mean square displacement will be reduced, and $D_0$ is replaced by an apparent diffusion coefficient (ADC) with the same units. ADC may be measured at short timescales (measured in milliseconds) or long timescales (measured in seconds), with longer timescales corresponding to diffusion over greater distances. Two pulse sequences were utilised in the work presented in this thesis, namely spin echo (SE) and stimulated echo (STE), which measure diffusion at short and long timescales respectively.

Figure 2.9b shows the SE pulse sequence. This is similar to the sequence shown in Figure 2.9a, except that a gradient is applied in the cranio-caudal direction before and after each refocusing pulse. Diffusion of $^3$He atoms in the time interval between the gradient pulses results in additional dephasing between spins so that the subsequent echo is attenuated. An echo train of 64 echoes is obtained, and an exponential decay curve is fitted to the envelope of the echo train, using MATLAB (Mathworks, Cambridge, UK). The rate constant of this decay curve is the ADC at 13ms.

Figure 2.9c shows the STE pulse sequence with some simplifications for clarity. An initial 90° RF pulse is applied to induce phase coherence. This is followed by a gradient pulse, so that any subsequent diffusion of $^3$He atoms results in dephasing. A second 90° pulse is applied, which causes the transverse spins to flip into the longitudinal axis. Once there, the spins maintain their phase relationships, and dephasing due to spin-spin interactions does not occur. This means that diffusion over much longer time scales can be measured. Following a time period of the order of 1s, a third 90° pulse is applied, which flips the spins that were ‘parked’ in the longitudinal axis back into the transverse
plane, but now spinning in the reverse direction. A refocusing gradient is applied, which results in an echo, the amplitude of which determines the degree of diffusion (and hence dephasing) that has occurred in the intervening period.
Figure 2.9: Magnetic resonance pulse sequences for measuring diffusion in the lungs

Panel A shows a simple spin echo pulse sequence, in which after an initial 90° pulse, a 180° refocusing pulse causes an echo of the original free induction decay. Panel B shows the spin echo diffusion pulse sequence, in which a series of refocusing pulses are applied following the initial 90° pulse. In between each refocusing pulse a diffusion-weighting gradient is applied. Panel C shows the stimulated echo diffusion pulse sequence, in which an initial 90° pulse is followed by a diffusion-weighting gradient and then a second 90° pulse, which has the effect of eliminating dephasing due to spin-spin interactions. Following a relatively long time period of the order of seconds, a third 90° pulse is applied, flipping the spins back into the transverse plane, followed by a refocusing gradient which causes an echo of the original free induction decay.
Panel B

RF pulses

\[ 90^\circ \quad 180^\circ \quad 180^\circ \quad 180^\circ \]

Gradient

ADC timescale = 13 ms

Signal

Panel C

RF pulses

\[ 90^\circ \quad 90^\circ \quad 90^\circ \]

Gradient

ADC timescale = 1 s

Signal
2.9.3 Patient testing procedure

$^3$He gas was hyperpolarised via metastable exchange optical pumping\textsuperscript{226}, using a custom-built polarisation system. Six hundred ml of gas comprising 15-30 ml hyperpolarised $^3$He mixed in $^4$He, was transported from the glass storage cell to the patient in a tedlar bag (SKC Limited, Blandford Forum, UK). MR measurements were made using a 0.15 T permanent magnet system (Intermagnetics General Corporation, New York) with a Surrey Medical Imaging Systems console (Surrey, UK). Participants were positioned in a supine position within the magnet, with a custom-built induction coil around their chest. Following a period of relaxed tidal breathing, participants inhaled the contents of the tedlar bag from FRC and breath-held for 2 – 10 seconds whilst the MR measurement was performed.
3 Studies

3.1 Validation of a photoacoustic gas analyser for the measurement of functional residual capacity using multiple breath inert gas washout

Abstract

Background
The respiratory mass spectrometer is the current gold standard technique for performing multiple breath inert gas washout (MBW), but is expensive and lacks portability. A number of alternative techniques have recently been described.

Objectives
We aimed to validate, using an in vitro lung model, an open-circuit MBW system that utilises a portable photoacoustic gas analyser, with sulphur hexafluoride (SF₆) as the inert tracer gas.

Methods
An acrylic glass lung model was utilised to assess the accuracy of functional residual capacity (FRC) measurements derived from MBW. Measurements were performed in triplicate at 20 combinations of simulated FRC, tidal volume and respiratory rate. FRC measured using MBW (FRC_{mbw}) was compared to FRC calculated from the known dimensions of the model (FRC_{calc}). MBW was also performed in 10 healthy subjects and 14 patients with asthma.

Results
The MBW system measured FRC with high precision. The mean bias of FRC_{mbw} with respect to FRC_{calc} was -0.4% (95% limits of agreement of -4.6% and 3.9%). The mean coefficient of variation of triplicate FRC measurements was 4.0% in vivo and 1.0% in vitro. MBW slightly underestimated low lung volumes and overestimated high lung volumes, but this did not cause a significant error in lung clearance index except at lung volumes below 1500 ml.
Conclusions
The open-circuit MBW system utilising SF$_6$ as the inert tracer gas and a photoacoustic gas analyser is both accurate and repeatable within the adult range of lung volumes. Further modifications would be required before its use in young children or infants.
**Introduction**

Multiple breath inert gas washout (MBW) is a technique for assessing the non-uniformity of ventilation distribution in the lungs by measuring the efficiency with which an inert tracer gas is washed out of the lungs\(^{130}\). The current gold standard MBW system is the respiratory mass spectrometer (RMS)\(^{130}\), but this is expensive and lacks portability. An alternative system based upon a modified photoacoustic gas analyser (Innocor™, Innovision A/S, Odense, Denmark) and 0.2% sulphur hexafluoride (SF\(_6\)) as the tracer gas has been developed, and shown to be both repeatable and practical\(^{132}\), but its accuracy has not been formally validated. The functional residual capacity (FRC) may be derived from a MBW by dividing the total volume of inert gas expired by the difference between the inert gas concentrations at the beginning and end of the washout period\(^{219}\). Current guidelines recommend that MBW systems are validated by determining FRC measurement accuracy, and in particular that measured FRC values should lie within 5% of known volumes, at least 95% of the time\(^{130}\). Accurate FRC determination depends critically upon correct flow and gas concentration measurements, precise synchronisation of these signals, and adequate conversion of measured flows and volumes to body temperature, pressure and water vapour saturation (BTPS) conditions. These are the same technical factors that influence the accuracy of clinically relevant parameters such as the lung clearance index (LCI). FRC is a suitable end-point for quality control and methodological validation because measured values may be readily compared to a gold standard such as the known volume of an *in vitro* lung model. Current guidelines\(^{130}\) recommend that MBW systems are validated using *in vitro* lung models incorporating realistic BTPS conditions, as previously described by Singer *et al*\(^{134}\). We aimed to utilise this lung model to validate the MBW method of Horsley *et al*\(^{132}\), as well as to compare the variability of triplicate MBW tests performed *in vitro* and *in vivo*. The primary outcome of the study was the percentage difference between measured and calculated FRC measurements using the *in vitro* lung model, with satisfactory accuracy being defined as a percentage difference of between -5% and 5% for at least 95% of measurements. Secondary outcomes were the dependence, if any, of the measurement bias on absolute FRC values or the respiratory rate, as well as the difference in measurement variability between *in vitro* and *in vivo* measurements.
Materials and methods

The Innocor photoacoustic gas analyser setup, patient testing procedure and MBW data analysis methods are described in Section 2.5. The lung model utilised to perform the validation experiments and the methodology of these experiments are described in Section 2.6. Figure 3.1 shows an example trace of SF\textsubscript{6} concentration against time recorded using the lung model (Panel A), and the corresponding plot of end-expiratory SF\textsubscript{6} concentration against turnover number (Panel B).

In vitro testing procedure

Before testing began, a volume calibration of the Innocor gas analyser was performed using a 1L syringe at low, medium and high flow rates. The gas analyser had recently been serviced, including a gas calibration. All tests were performed on the same day (17\textsuperscript{th} December 2011), and the air temperature and relative humidity inside the phantom lung were measured on three occasions during the testing period to confirm that BTPS conditions in the lung compartment were maintained. This was performed using measuring probes that were inserted through a small hole in the lung phantom lid. The hole was then sealed prior to testing.

Testing using the lung phantom model was performed according to the method described in Section 2.6., at simulated FRC values between 500 ml and 4000 ml, at 500 ml intervals, in order to simulate lung volumes of young children through to large adults. Tidal volume (V\textsubscript{T}) was set to approximately one third of FRC. Respiratory rate was set at between 12 and 24 breaths per minute; Larger respiratory rates were used with smaller lung volumes, in order to accurately simulate the physiology of young children. At FRC values of 500 ml, 1000 ml, 2000 ml, 3000 ml and 4000 ml, experiments were performed at three different respiratory rates in order to assess if accuracy was affected by changes in this parameter. Each of the 20 experiments was performed in triplicate, making a total of 60 washout runs.
Figure 3.1: Inert gas washout curve of an acrylic glass lung model

Panel A shows a raw plot of measured SF$_6$ concentration against time, with each vertical peak representing a single expiration. Panel B shows the end-expiratory SF$_6$ concentration ($C_{et}$) of each breath of the same washout test.

Panel A

Panel B
**In vivo testing procedure**

Ten healthy subjects with no history of respiratory disease and 14 patients with a clinical diagnosis of asthma were recruited. Subjects were aged over 18 years, and were never-smokers or ex-smokers with ≤ 10 pack years’ smoking history. The study protocol was approved by the National Research Ethics Committee – East Midlands Leicester (approval number 08/H0406/189) and all subjects gave their written informed consent. Patients underwent MBW in triplicate using the method described in Section 2.5.

Statistical analyses were performed using Prism version 6 (GraphPad, San Diego, California, USA). Results from the *in vitro* lung model were displayed as a Bland-Altman plot\(^{22}\) of FRC measured using MBW (FRC\(_{\text{mbw}}\)) against FRC calculated using the measuring scale affixed to the lung model (FRC\(_{\text{calc}}\)). The percentage difference between FRC\(_{\text{mbw}}\) and FRC\(_{\text{calc}}\) was also compared between washout tests with low, intermediate and high respiratory rates, using one-way analysis of variance. The mean coefficient of variation (CoV) of triplicate values was compared across the three sets of measurements (lung model, healthy subjects and patients with asthma) using one-way analysis of variance with Bonferroni correction for multiple comparisons.

**Results**

The Innocor system measured FRC with high precision. Table 3.1 lists the 60 *in vitro* experiments that were performed, with the values of FRC\(_{\text{calc}}\) and FRC\(_{\text{mbw}}\) given in each case. Figure 3.2 shows Bland-Altman plots of FRC\(_{\text{mbw}}\) against FRC\(_{\text{calc}}\), with the absolute and percentage difference between FRC\(_{\text{mbw}}\) and FRC\(_{\text{calc}}\) plotted on the y-axis in panels A and B. The mean absolute bias of FRC\(_{\text{mbw}}\) with respect to FRC\(_{\text{calc}}\) was 12.6 ml, and the 95% limits of agreement were -68.6 ml and 93.7 ml. The mean percentage bias of FRC\(_{\text{mbw}}\) with respect to FRC\(_{\text{calc}}\) was -0.4%, and the 95% limits of agreement were -4.6% and 3.9%. There was a significant positive correlation between the mean of FRC\(_{\text{mbw}}\) and FRC\(_{\text{calc}}\) and the percentage difference between these values (Pearson correlation coefficient = 0.82, p < 0.0001). The equation of the regression line was y = 0.001462*x – 3.653. The regression line crossed the x-axis at a lung volume of 2499
ml, which was therefore the point of zero bias. The mean bias at a lung volume of 500 ml was -3.9% and at a lung volume of 4000 ml was 1.9%. Respiratory rate did not have an independent effect on the bias. The mean bias of FRC\textsubscript{mbw} with respect to FRC\textsubscript{calc} in washout runs with low, intermediate and high respiratory rates was -0.3%, -0.9% and -0.3% respectively, with no statistically significant difference between the sets of washout experiments.
Table 3.1: List of multiple breath washout validation experiments performed with results

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT (ml)</td>
<td>FRC&lt;sub&gt;calc&lt;/sub&gt; (ml)</td>
</tr>
<tr>
<td>224</td>
<td>16</td>
</tr>
<tr>
<td>205</td>
<td>20</td>
</tr>
<tr>
<td>187</td>
<td>24</td>
</tr>
<tr>
<td>243</td>
<td>16</td>
</tr>
<tr>
<td>261</td>
<td>24</td>
</tr>
<tr>
<td>336</td>
<td>20</td>
</tr>
<tr>
<td>448</td>
<td>16</td>
</tr>
<tr>
<td>523</td>
<td>12</td>
</tr>
<tr>
<td>504</td>
<td>16</td>
</tr>
<tr>
<td>467</td>
<td>20</td>
</tr>
<tr>
<td>541</td>
<td>12</td>
</tr>
<tr>
<td>1027</td>
<td>12</td>
</tr>
<tr>
<td>990</td>
<td>20</td>
</tr>
<tr>
<td>1046</td>
<td>16</td>
</tr>
<tr>
<td>485</td>
<td>12</td>
</tr>
<tr>
<td>1008</td>
<td>12</td>
</tr>
<tr>
<td>448</td>
<td>12</td>
</tr>
<tr>
<td>1008</td>
<td>12</td>
</tr>
<tr>
<td>971</td>
<td>16</td>
</tr>
<tr>
<td>952</td>
<td>20</td>
</tr>
</tbody>
</table>

VT = tidal volume; FRC<sub>mbw</sub> = functional residual capacity measured using multiple breath washout; FRC<sub>calc</sub> = functional residual capacity calculated from known dimensions of lung model.
Figure 3.2: Bland-Altman plots of calculated versus measured functional residual capacity

Bland-Altman plots of FRC_{mbw} against FRC_{calc} are shown, with absolute differences in Panel A and percentage differences in Panel B. % Difference is calculated as 100\*(FRC_{mbw} - FRC_{calc}) divided by the mean of FRC_{mbw} and FRC_{calc}. Dotted lines represent 95% limits of agreement. Best-fit linear regression line is shown in Panel B (Pearson correlation coefficient = 0.82, p < 0.0001). Washout run respiratory rates are represented 12 (●), 16 (■), 20 (▲) and 24 (▼).

Panel A

Panel B
Figure 3.3 shows the error in LCI, defined as measured LCI minus calculated LCI (LCI_{calc}), against FRC_{calc}, where:

\[
\text{LCI}_{\text{calc}} = \frac{(\text{calculated } V_T - \text{equipment dead space}) \times \text{number of breaths}}{\text{FRC}_{\text{calc}}} 
\]

LCI was measured accurately at lung volumes at or above 1500ml, with an error of less than 0.4 units in each case. However, at lung volumes of 500ml or 1000ml, LCI was in some cases over-estimated by up to 1.1 units.

The healthy participants comprised four women and six men with a mean (standard deviation [SD]) age of 46.4 (19.4) years, while the patients with asthma comprised seven women and seven men with a mean (SD) age of 59.0 (11.9) years. The mean (SD) FRC_{mbw} was 2796 (896) ml in healthy controls and 2555 (794) ml in patients with asthma. The mean (SD) LCI was 7.07 (1.07) in healthy controls and 8.12 (1.46) in patients with asthma. The mean (range) CoV of triplicate FRC_{mbw} measurements was 1.0% (0.4 – 2.2%) in vitro, 4.0% (1.9 – 5.3%) in healthy controls, and 2.9% (0.4 – 5.4%) in patients with asthma. These values were significantly different across the three sets of measurements (p < 0.0001). In particular, the mean CoV was significantly lower in vitro than in both healthy (p < 0.0001) and asthma (p < 0.001) groups, but did not differ significantly between the healthy and asthma groups. The difference in mean CoV between in vitro and healthy group measurements was 3.0% (95% confidence interval of difference: 2.5 – 3.5%), while the difference in mean CoV between in vitro and asthma group measurements was 1.9% (95% confidence interval of difference: 1.0 – 2.7%). The in vitro CoV of FRC_{mbw} was not significantly related to the respiratory rate. The mean (range) CoV was 1.1% (0.5 – 2.2%) with a respiratory rate of 12 breaths/minute, 1.0% (0.8 – 1.3%) with a respiratory rate of 16 breaths/minute, and 0.9% (0.4 – 1.4%) with a respiratory rate of 20 or 24 breaths per minute (no significant difference between groups of tests). The mean (range) CoV of triplicate LCI measurements was 1.2% (0.0 – 4.0%) in vitro, 4.6% (0.4 – 12.1%) in healthy controls, and 3.3% (0.3 – 7.0%) in patients with asthma.
Figure 3.3: Error in lung clearance index against calculated functional residual capacity
Discussion

In this study, we utilised a one-compartment acrylic glass lung model under BTPS conditions to validate a practical and portable MBW system that uses an Innocor photoacoustic gas analyser, with SF$_6$ as the inert tracer gas. Of note, this open-circuit system is distinct from the closed-circuit setup that was found to have poor intra-subject variability and patient acceptability by Pittman et al.$^{228}$ We found good agreement between FRC measured using Innocor (FRC$_{mbw}$) and FRC calculated from the known dimensions of the lung model (FRC$_{calc}$), with a mean bias of FRC$_{mbw}$ with respect to FRC$_{calc}$ of -0.4%, and 95% limits of agreement of -4.6% and 3.9%, comfortably below the recommended maximum error of 5%.$^{130}$ An identical lung model has been previously used to validate a commercially available open-circuit nitrogen MBW system (Exhalyzer D$^{TM}$, Eco Medics AG, Duernten Switzerland)$^{134}$. These authors found that for lung volumes above 500 ml, there was a mean bias of 0.4%, with 95% limits of agreement of -4.0% and 4.7% respectively. We therefore conclude that the accuracy of FRC measurements using Innocor and Exhalyzer D is similar within the FRC range of 500 ml to 4000 ml. We found that the mean coefficient of variation of triplicate FRC measurements was 1.0% in vitro and 4.0% in vivo, suggesting that the majority of between-measurement variability in vivo was caused by biological rather than technical factors. Singer et al.$^{134}$ obtained similar values using the Exhalyzer D system, namely 1.4% and 4.5% respectively, suggesting that the two systems perform approximately equally with respect to within-visit repeatability.

The Innocor-based system appeared to slightly underestimate FRC when lung volumes were small and overestimate it when volumes were large. We speculated that this may have been due to cyclical heating and cooling of the pneumotachometer during the respiratory cycle, resulting in non-linearity of flow measurement. On closer examination of our data, we ascertained that the source of this FRC-dependent bias was the correction for re-inspired SF$_6$, which appeared to over-compensate at low lung volumes, resulting in artificially low FRC values. In particular, inspiratory flows were over-estimated at low lung volumes, particularly below an FRC of 1500 ml, resulting in an over-estimation of re-inspired SF$_6$ volume. Of note, this FRC-dependent bias was not seen with the Exhalyzer D$^{134}$, which utilises an ultrasonic flowmeter. However, the error in FRC measurements with Exhalyzer D increased with increasing respiratory
rate, an effect that we did not observe with the Innocor-based system. We furthermore examined the accuracy of LCI measurements performed using Innocor at different lung volumes and found that at lung volumes of 1500 ml or above, there was good agreement between measured and calculated LCI, whereas at lung volumes below 1500 ml, LCI was often over-estimated, by up to 1.1 units. This suggests that at lung volumes corresponding to older children or adults, the small bias observed in FRC does not significantly affect LCI measurements.

The open-circuit Innocor-based system described in this chapter is practical and convenient, and could potentially be utilised in clinical practice and multi-centre trials in both older children and adults. However, our *in vitro* results at low lung volumes suggest that the system would require further modification before it could be used reliably in young children and infants. Such modification would be likely to include the replacement of the pneumotachometer with a smaller model that has a lower flow range. Moreover, Innocor employs side-stream sampling of gas at a flow rate of 120 ml/minute, which may have a significant influence at low tidal volumes. Furthermore, the response time of the photoacoustic analyser is relatively slow (154 ms), which may be particularly relevant at fast respiratory rates, as seen in young children. These latter two issues would require further technical development by the manufacturers of Innocor. There are a number of additional improvements that could be made relatively easily to further increase the general applicability of this technology to the performance of MBW. Foremost among these is that data analysis is currently performed off-line, which is relatively time consuming. It would however be straightforward to incorporate FRC and LCI calculations into the on-board Innocor software in the future. Ideally, this on-board software would also include a user-friendly patient interface to allow tidal volume to be targeted by the patient to a set value. Our current system requires the patient to target their tidal volume using a numerical display on a separate laptop computer. A further limitation of the current Innocor setup is the requirement of SF₆, which is restricted in some countries as it acts a greenhouse gas.

In conclusion, the open-circuit MBW system utilising SF₆ as the inert tracer gas and an Innocor photoacoustic gas analyser is both accurate and repeatable in adults, and is comparable in these respects to the Exhalyzer D MBW system. These results provide increased confidence in previous and future research studies conducted using the
Innocor-based system, and suggest its potential to develop into a commercially available MBW platform. Further modifications to the system would be required to facilitate its use in young children and infants.
3.2 Specific ventilation inequality and dead space components of lung clearance index in patients with cystic fibrosis and non-cystic fibrosis bronchiectasis

Abstract

Background
Lung clearance index (LCI) is a measure of abnormal ventilation distribution derived from the multiple breath inert gas washout (MBW) technique. We aimed to determine the clinical utility of LCI in non-CF bronchiectasis, and to assess two novel MBW parameters that distinguish between increases in LCI due to specific ventilation inequality (LCI_{vent}) and increased respiratory dead space (LCI_{ds}).

Methods
Forty-three patients with non-CF bronchiectasis and 18 healthy control subjects underwent MBW using the sulphur hexafluoride wash-in technique, and data from 40 adults with CF were re-analysed. LCI_{vent} and LCI_{ds} were calculated using a theoretical two-compartment lung model, and represent the proportional increase in LCI above its ideal value due to specific ventilation inequality and increased respiratory dead space, respectively.

Results
LCI was significantly raised in patients with non-CF bronchiectasis compared to healthy controls (9.99 versus 7.28, p < 0.01), and discriminated well between these two groups (area under receiver operating curve = 0.90, versus 0.83 for forced expiratory volume in one second [% predicted]). LCI, LCI_{vent} and LCI_{ds} were repeatable (intracllass correlation coefficient > 0.75), and correlated significantly with measures of spirometric airflow obstruction.

Conclusion
LCI is repeatable, discriminatory, and is associated with spirometric airflow obstruction in patients with non-CF bronchiectasis. LCI_{vent} and LCI_{ds} are a practical and repeatable alternative to phase III slope analysis and may allow a further level of mechanistic
information to be extracted from the MBW test in patients with severe ventilation heterogeneity.
Introduction

Non-cystic fibrosis (CF) bronchiectasis is a chronic suppurative lung disease caused by a range of underlying conditions, which is increasing in prevalence, and which imposes a significant burden of morbidity and healthcare costs. In the United States alone, annual healthcare costs for bronchiectasis are estimated as $630 million. The causes of non-CF bronchiectasis are diverse, and include autoimmune disease, primary ciliary dyskinesia, allergic bronchopulmonary aspergillosis, immune deficiency and childhood respiratory infection. Regardless of the underlying cause, the pathogenesis is thought to involve a vicious cycle of bacterial colonisation, neutrophilic airway inflammation, airway damage and mucus stasis. The evidence base for the treatment of non-CF bronchiectasis lags well behind that of CF, but this is expected to change in the near future as a number of non-CF bronchiectasis research registries and clinical trials are actively enrolling patients at present. Such clinical trials will require robust physiological outcome measures in order to provide objective measures of improvement in lung function.

Multiple breath inert gas washout (MBW) is a technique for quantifying ventilation heterogeneity, the uneven distribution of ventilation. This is an early feature of obstructive airway diseases such as asthma, chronic obstructive pulmonary disease and cystic fibrosis (CF). A comprehensive standardisation document for the performance of inert gas washout has been recently published. Lung clearance index (LCI) is the most commonly reported MBW parameter, and is defined as the cumulative expired volume at the point where end-tidal inert gas concentration falls below 1/40th of the original concentration, divided by the functional residual capacity (FRC). LCI has been shown to be both discriminatory and repeatable in patients with CF, and is increasingly being used as an outcome measure in clinical trials of CF therapies. A recent study has shown that LCI is repeatable in patients with non-CF bronchiectasis, and correlates with computed tomography bronchiectasis severity scores.

Although LCI has been shown to be a robust and repeatable measurement in patients with CF and non-CF bronchiectasis, it also represents a simplification of the washout process since it is essentially determined by data points at the start and end of the
washout curve only. From a theoretical standpoint, LCI may be increased by two distinct mechanisms, namely (i) unequal convective ventilation between relatively large lung units subtended by proximal conducting airways (convection-dependent inhomogeneity), and (ii) increased respiratory dead space, which is thought to be underpinned by diffusion-dependent gas mixing inefficiencies (diffusion-convection-dependent inhomogeneity). The only published method for separating out these mechanisms is the analysis of phase III slopes, yielding the parameters $S_{\text{cond}}$ (conductive ventilation heterogeneity index) and $S_{\text{acin}}$ (acinar ventilation heterogeneity index). This technique was developed from elegant clinical and modelling studies in healthy adult subjects. However, the use of these parameters is problematic in patients with the most severe ventilation heterogeneity, such as those with advanced CF lung disease, both from a practical standpoint (the requirement for controlled 1L breaths), and because the modelling may not be directly applicable in those with severe ventilation heterogeneity. To overcome this, modified versions of these parameters ($S_{\text{cond}}^*$ and $S_{\text{acin}}^*$) have recently been proposed for use in such patients. There remains a need however for a reliable and repeatable method of extracting mechanistic information from washout curves, which has been developed for, and can be applied in, those with more severe disease.

The aim of this study was firstly to determine whether or not ventilation heterogeneity is a significant feature of non-CF bronchiectasis, and whether LCI may have potential as an outcome measure in this group of patients. Furthermore, we aimed to extend currently available measures of ventilation heterogeneity by developing novel parameters that would distinguish between specific ventilation inequality (LCI\textsubscript{vent}) and increased respiratory dead space (LCI\textsubscript{ds}) as a cause of increased LCI. LCI\textsubscript{vent} and LCI\textsubscript{ds} would be expected to probe similar mechanisms of ventilation heterogeneity to $S_{\text{cond}}$ and $S_{\text{acin}}$, respectively, but without the potential drawbacks of phase III slope analysis, and with the advantage of being directly linked to LCI.

We hypothesised that:

i) Non-CF bronchiectasis is characterised by increased LCI, LCI\textsubscript{vent} and LCI\textsubscript{ds} compared to healthy control subjects.

ii) LCI is related to other measures of disease severity in CF and non-CF bronchiectasis such as the presence or absence of chronic bacterial colonisation.
iii) LCI is repeatable in patients with non-CF bronchiectasis, and is superior to spirometry for distinguishing between patients with non-CF bronchiectasis and healthy controls.

**Methods**

**Subjects**
Forty-three adult patients with non-CF bronchiectasis were recruited from the respiratory out-patient clinics at Glenfield Hospital. Bronchiectasis was diagnosed by high resolution computed tomography, and all scans were reported by a Consultant Radiologist to confirm the diagnosis. Eighteen healthy non-smoking control subjects with no history of respiratory symptoms were recruited through local advertising. The study was approved by the National Research Ethics Committee (East Midlands – Leicester), and all participants gave their written informed consent. As a disease comparator group, MBW data from 40 adults with CF who took part in a previous observational study$^{132}$ were re-analysed. This study was approved by the Lothian Research and Ethics Committee and all participants gave their written informed consent.

**Clinical characterisation of bronchiectasis patients**
Demographic details and a full medical history were obtained from each patient. Sputum samples were obtained for bacterial culture, and sputum culture results during the previous year were recorded to assess for chronic bacterial colonisation, defined as isolation of the same microorganism on sputum culture on at least two occasions during the previous year. Participants underwent spirometry and measurement of lung volumes using helium dilution according to American Thoracic Society / European Respiratory Society guidelines$^{90,215}$.

**Multiple breath washout test**
MBW was performed in triplicate at a single visit, as described in Sections 2.5.1 and 2.5.2. Participants with non-CF bronchiectasis maintained a steady respiratory rate of
approximately 12 breaths per minute, and a constant tidal volume of 1L throughout the test. Patients with CF in the previously published cohort performed washout tests during relaxed tidal breathing. Washout curves were analysed as described in Section 2.5.3 to yield FRC, LCI, \( \frac{S_{\text{cond}}}{S_{\text{acin}}} \) and \( \frac{S_{\text{cond}*}}{S_{\text{acin}*}} \). The novel parameters \( \text{LCI}_{\text{vent}} \) and \( \text{LCI}_{\text{ds}} \) were calculated as described in Section 2.7.

**Statistical analysis**

Statistical analyses were performed using SPSS Version 20 (IBM Corporation, Somers, New York, USA) and Prism 6 (GraphPad Software Inc., La Jolla, California, USA). Between-group comparisons were performed using Student’s T test or one-way analysis of variance for continuous data and the Chi-squared test for proportions, with the threshold for statistical significance set at \( p < 0.05 \). Repeatability of MBW parameters was assessed using the intraclass correlation coefficient (ICC) across triplicate measurements, using a two-way mixed model. Correlations between variables were assessed using Pearson’s correlation coefficient (R). A generalised linear model was used to assess whether the relationship between LCI and spirometric airflow obstruction differed between the two disease groups. Areas under receiver operating characteristic (ROC) curves were used to assess the discriminatory ability of physiological parameters.

**Results**

**Clinical characteristics**

The cohort of patients with non-CF bronchiectasis comprised 19 men and 24 women with a mean (standard deviation [SD]) age of 67.4 (7.3) years. The group included 25 never smokers, 17 ex-smokers and 1 current smoker. The median (range) pack-year smoking history of the ex- and current smokers was 17.5 (1 – 140). Out of the 43 patients, a previous history of tuberculosis was elicited in 2 patients, childhood pneumonia in 14 patients and pertussis in 22 patients. Eleven patients had a history of asthma, and four had a formal diagnosis of allergic bronchopulmonary aspergillosis. Nineteen patients had symptoms of gastroesophageal reflux disease and two had inflammatory bowel disease. Twelve patients had an inflammatory arthritis and one had yellow nail syndrome. Twelve patients were chronically colonised with *Haemophilus*.
influenzae, three patients with *Pseudomonas aeruginosa*, two patients with *Staphylococcus aureus* and two patients with coliforms.

The CF group comprised 20 men and 20 women with a mean (SD) age of 28.7 (9.8) years. Three CF patients were ex-smokers (pack-year histories of 5, 15 and 24 years). Fifteen patients had chronic *Pseudomonas aeruginosa* colonisation as defined by Lee *et al* [238], 29 had pancreatic insufficiency and 6 had diabetes mellitus. Nineteen patients had a severe genotype, defined as having a class I or II mutation on both chromosomes, and 16 had a mild genotype, defined as having a class III, IV or V mutation on at least one chromosome. The genotype was incomplete in 5 patients.

**Group comparisons**

Table 3.2 shows physiological data across all three groups. Patients with bronchiectasis and CF both displayed spirometric airflow obstruction, with significantly reduced forced expiratory volume in one second/forced vital capacity (FEV₁/FVC) ratio compared to healthy controls. LCI, LCIₜᵥ and LCIₜₛ were all significantly greater in bronchiectasis patients compared to controls, and significantly greater in CF patients compared to both controls and bronchiectasis patients, as shown in Figure 3.4 (Panels A, B and C respectively).
Table 3.2: Demographic and physiological data across healthy controls, cystic fibrosis patients and non-cystic fibrosis bronchiectasis patients

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (n = 18)</th>
<th>CF patients (n = 40)</th>
<th>Non-CF bronchiectasis patients (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.3 (3.9)</td>
<td>28.7 (1.5) ***</td>
<td>67.4 (1.1) <strong><strong>,</strong></strong></td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>50</td>
<td>50</td>
<td>44</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.8 (1.2)</td>
<td>22.9 (0.5) **</td>
<td>27.1 (0.7) ****</td>
</tr>
<tr>
<td>FEV₁ (% pred.)</td>
<td>113.3 (4.8)</td>
<td>65.9 (3.4) ****</td>
<td>82.0 (3.8) *<em><strong>,</strong></em></td>
</tr>
<tr>
<td>FVC (% pred.)</td>
<td>117.2 (5.6)</td>
<td>84.5 (3.0) ****</td>
<td>96.1 (3.4) <strong>,</strong></td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>80.9 (1.0)</td>
<td>65.9 (1.8) ****</td>
<td>68.4 (1.7) ****</td>
</tr>
<tr>
<td>FRCₘₙₖₜ (L)</td>
<td>2.52 (0.19)</td>
<td>1.99 (0.09) *</td>
<td>2.48 (0.10) **</td>
</tr>
<tr>
<td>LCI</td>
<td>7.28 (0.27)</td>
<td>13.17 (0.56) ****</td>
<td>9.99 (0.31) <strong><strong>,</strong></strong></td>
</tr>
<tr>
<td>LCIₙₜ (L)</td>
<td>1.20 (0.02)</td>
<td>1.65 (0.04) ****</td>
<td>1.42 (0.03) <strong><strong>,</strong></strong></td>
</tr>
<tr>
<td>LCIₙₜ (L)</td>
<td>1.13 (0.01)</td>
<td>1.40 (0.03) ****</td>
<td>1.27 (0.02) <strong><strong>,</strong></strong></td>
</tr>
<tr>
<td>LCIₙₜ /LCIₙₜ</td>
<td>1.06 (0.02)</td>
<td>1.18 (0.01) ****</td>
<td>1.12 (0.01) <em>,</em>*</td>
</tr>
<tr>
<td>S₉₉₉ (L⁻¹)</td>
<td>0.033 (0.007)</td>
<td>0.131 (0.010) ****</td>
<td>0.064 (0.007) ****</td>
</tr>
<tr>
<td>S₉₉₉ (L⁻¹)</td>
<td>0.118 (0.014)</td>
<td>0.509 (0.056) ****</td>
<td>0.373 (0.036) **</td>
</tr>
<tr>
<td>S₉₉₉ (L⁻¹)</td>
<td>0.097 (0.009)</td>
<td>0.308 (0.034) ****</td>
<td>0.107 (0.010) ****</td>
</tr>
<tr>
<td>S₉₉₉ (L⁻¹)</td>
<td>0.090 (0.012)</td>
<td>0.446 (0.054) ****</td>
<td>0.355 (0.037) **</td>
</tr>
</tbody>
</table>
Legend for Table 3.2

CF = cystic fibrosis; BMI = body mass index; FEV\textsubscript{1} = forced expiratory volume in one second; FVC = forced vital capacity; FRC\textsubscript{mbw} = functional residual capacity from multiple breath washout; LCI = lung clearance index; LCI\textsubscript{vent} = specific ventilation inequality component of lung clearance index; LCI\textsubscript{ds} = dead space component of lung clearance index. Data expressed as mean (standard error) or percentages. Groups compared using one-way analysis of variance with Bonferroni correction for multiple comparisons for parametric data, and the Chi-squared test for proportions. Significant differences across groups denoted †(p < 0.05), ‡(p < 0.01), ‡‡(p < 0.001) or ‡‡‡(p < 0.0001). Significant differences compared to control group denoted *(p < 0.05), **(p < 0.01), ****(p < 0.001) or *****(p < 0.0001). Significant differences between bronchiectasis and CF groups denoted ††(p < 0.05), †††(p < 0.01), ††††(p < 0.001) or †††††(p < 0.0001).
**Figure 3.4: Multiple breath washout parameters across groups**

*Error bars indicate means ± standard errors of the mean.*

**Panel A**

![Graph showing multiple breath washout parameters across groups.](image)

**Panel B**

![Graph showing multiple breath washout parameters across groups.](image)
Panel C

![Graph showing data points for Healthy, Bronchiectasis, and CF conditions with p-values indicating statistical significance.](image-url)
Correlations between spirometric and multiple breath washout parameters

Figure 3.5 shows correlations between the FEV$_1$ (% pred.) and LCI in patients with bronchiectasis (Panel A) and patients with CF (Panel B). In both cases, there was a highly significant ($p < 0.0001$) negative correlation between FEV$_1$ (% pred.) and LCI. However, the slope of the relationship between the two variables differed significantly between the groups. Patients with CF had a 0.13 unit increase in LCI for every 1 percentage point reduction in FEV$_1$ (% pred.), whereas patients with bronchiectasis had a 0.05 unit increase in LCI for every 1 percentage point reduction in FEV$_1$ (% pred.) ($p < 0.0001$). LCI$_{vent}$ and LCI$_{ds}$ correlated highly significantly with FEV$_1$ (% pred.) in both patients with non-CF bronchiectasis ($R = -0.63$ for LCI$_{vent}$, $R = -0.60$ for LCI$_{ds}$, $p < 0.0001$ for both analyses) and patients with CF ($R = -0.78$ for LCI$_{vent}$, $R = -0.76$ for LCI$_{ds}$, $p < 0.0001$ for both analyses). Table 3.3 shows correlations between MBW parameters in both patient groups. There were significant correlations between LCI$_{vent}$ and LCI$_{ds}$ in both patient groups ($R = 0.80$, $p < 0.0001$ for non-CF bronchiectasis; $R = 0.89$, $p < 0.0001$ for CF).
Figure 3.5: Correlations between lung clearance index and FEV$_1$ (% predicted)

Correlations are shown for patients with non-cystic fibrosis bronchiectasis (Panel A) and cystic fibrosis (Panel B). Best-fit linear regression lines are shown, together with Pearson correlation coefficients.

Panel A

Panel B
Table 3.3: Correlations between multiple breath washout parameters

<table>
<thead>
<tr>
<th></th>
<th>LCI</th>
<th>LCI&lt;sub&gt;vent&lt;/sub&gt;</th>
<th>LCI&lt;sub&gt;ds&lt;/sub&gt;</th>
<th>S&lt;sub&gt;cond&lt;/sub&gt;</th>
<th>S&lt;sub&gt;acin&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td></td>
<td>.96**</td>
<td>.93**</td>
<td>-.11</td>
<td>.67**</td>
</tr>
<tr>
<td>LCI&lt;sub&gt;vent&lt;/sub&gt;</td>
<td>.95**</td>
<td></td>
<td>.89**</td>
<td>-.12</td>
<td>.65**</td>
</tr>
<tr>
<td>LCI&lt;sub&gt;ds&lt;/sub&gt;</td>
<td>.90**</td>
<td>.80**</td>
<td>-.01</td>
<td>.68**</td>
<td></td>
</tr>
<tr>
<td>S&lt;sub&gt;cond&lt;/sub&gt;</td>
<td>.14</td>
<td>.08</td>
<td>.15</td>
<td>-.11</td>
<td></td>
</tr>
<tr>
<td>S&lt;sub&gt;acin&lt;/sub&gt;</td>
<td>.63**</td>
<td>.58**</td>
<td>.72**</td>
<td>.09</td>
<td></td>
</tr>
</tbody>
</table>

LCI = lung clearance index; LCI<sub>vent</sub> = specific ventilation inequality component of lung clearance index; LCI<sub>ds</sub> = dead space component of lung clearance index. Pearson’s correlation coefficients shown, for non-cystic fibrosis bronchiectasis patients in bottom triangle and cystic fibrosis patients in top triangle. Significant correlations denoted * (p < 0.05) or ** (p < 0.01).
Multiple breath washout parameters and chronic bacterial colonisation

Table 3.4 shows MBW and spirometric indices in patients with CF in the presence and absence of chronic *P. aeruginosa* colonisation, and in patients with non-CF bronchiectasis in the presence and absence of chronic bacterial colonisation. LCI\textsubscript{ds} was significantly raised in CF patients with chronic *P. aeruginosa* colonisation compared to those without chronic colonisation (1.49 vs 1.34, *p* = 0.004).

Within-visit repeatability and discriminatory ability

Table 3.5 shows the repeatability of MBW parameters in patients with bronchiectasis and CF. Intraclass correlation coefficients exceeded 0.75 for LCI, LCI\textsubscript{vent} and LCI\textsubscript{ds} in both disease groups. S\textsubscript{acin} and S\textsubscript{acin}* displayed moderate or good repeatability, but S\textsubscript{cond} and S\textsubscript{cond}* were poorly repeatable in both disease groups. Figure 3.6 shows ROC curves illustrating the discriminatory ability of LCI and FEV\textsubscript{1} (% pred.) for distinguishing between healthy controls and patients with non-CF bronchiectasis. The area under the ROC curve was 0.90 for LCI and 0.83 for FEV\textsubscript{1} (% pred.). Areas under the ROC curve for other MBW parameters were: LCI\textsubscript{vent} 0.88; LCI\textsubscript{ds} 0.89; S\textsubscript{cond} 0.76; S\textsubscript{acin} 0.91; S\textsubscript{cond}* 0.50; and S\textsubscript{acin}* 0.92.

Figure 3.7 shows graphs of FEV\textsubscript{1} standardised residuals against LCI (Panel A), LCI\textsubscript{vent} (Panel B) and LCI\textsubscript{ds} (Panel C) in patients with non-CF bronchiectasis. The lower limit of normal for FEV\textsubscript{1} was defined as 1.645 standard deviations below the predicted value, while the upper limits of normal for LCI, LCI\textsubscript{vent} and LCI\textsubscript{ds} were defined as the mean + 1.645 standard deviations in the healthy control group. Thirty out of 43 patients had an FEV\textsubscript{1} within the normal range, and of these, LCI, LCI\textsubscript{vent} and LCI\textsubscript{ds} were high in 12, 10 and 10 patients, respectively. Conversely, there were no patients who had an FEV\textsubscript{1} below the normal range who did not also have a raised LCI and LCI\textsubscript{vent}, and only one patient who had an FEV\textsubscript{1} below the normal range with a normal LCI\textsubscript{ds}.
Table 3.4: Physiological parameters in patients with and without chronic bacterial colonisation

<table>
<thead>
<tr>
<th></th>
<th>Non-cystic fibrosis bronchiectasis</th>
<th>Cystic fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No chronic colonisation (n = 26)</td>
<td>Chronic colonisation (n = 17)</td>
</tr>
<tr>
<td><strong>FEV₁ (% pred.)</strong></td>
<td>86.1 (5.3)</td>
<td>75.6 (4.9)</td>
</tr>
<tr>
<td><strong>FVC (% pred.)</strong></td>
<td>101.7 (4.6)</td>
<td>87.5 (4.1)*</td>
</tr>
<tr>
<td><strong>FEV₁/FVC (%)</strong></td>
<td>68.6 (2.4)</td>
<td>68.1 (2.4)</td>
</tr>
<tr>
<td><strong>TLC (% pred.)</strong></td>
<td>95.3 (3.1)</td>
<td>93.9 (4.0)</td>
</tr>
<tr>
<td><strong>FRC&lt;sub&gt;mbw&lt;/sub&gt; (L)</strong></td>
<td>2.38 (0.12)</td>
<td>2.62 (0.16)</td>
</tr>
<tr>
<td><strong>LCI</strong></td>
<td>10.02 (0.36)</td>
<td>9.95 (0.57)</td>
</tr>
<tr>
<td><strong>LCI&lt;sub&gt;vent&lt;/sub&gt;</strong></td>
<td>1.42 (0.03)</td>
<td>1.41 (0.05)</td>
</tr>
<tr>
<td><strong>LCI&lt;sub&gt;ds&lt;/sub&gt;</strong></td>
<td>1.27 (0.02)</td>
<td>1.26 (0.03)</td>
</tr>
<tr>
<td><strong>S&lt;sub&gt;cond&lt;/sub&gt; (L⁻¹)</strong></td>
<td>0.058 (0.010)</td>
<td>0.072 (0.010)</td>
</tr>
<tr>
<td><strong>S&lt;sub&gt;acin&lt;/sub&gt; (L⁻¹)</strong></td>
<td>0.429 (0.053)</td>
<td>0.288 (0.038)</td>
</tr>
<tr>
<td>*<em>S&lt;sub&gt;cond&lt;/sub&gt;</em> (L⁻¹)**</td>
<td>0.102 (0.014)</td>
<td>0.115 (0.016)</td>
</tr>
<tr>
<td>*<em>S&lt;sub&gt;acin&lt;/sub&gt;</em> (L⁻¹)**</td>
<td>0.412 (0.053)</td>
<td>0.268 (0.041)</td>
</tr>
</tbody>
</table>

PsA = *Pseudomonas aeruginosa*; FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; TLC = total lung capacity; FRC<sub>mbw</sub> = functional residual capacity using multiple breath washout; LCI = lung clearance index; LCI<sub>vent</sub> = specific ventilation inequality component of lung clearance index; LCI<sub>ds</sub> = dead space component of lung clearance index.

Data expressed as mean (standard error). Colonised and non-colonised groups within each disease cohort compared using Student’s T test. Significant differences between groups denoted *(p < 0.05) or ***(p < 0.01)*.
Table 3.5: Within-visit repeatability of multiple breath washout parameters in cystic fibrosis and non-cystic fibrosis bronchiectasis

<table>
<thead>
<tr>
<th></th>
<th>ICC in healthy controls</th>
<th>ICC in non-CF bronchiectasis</th>
<th>ICC in CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>0.90</td>
<td>0.86</td>
<td>0.93</td>
</tr>
<tr>
<td>LCI_{vent}</td>
<td>0.71</td>
<td>0.91</td>
<td>0.89</td>
</tr>
<tr>
<td>LCI_{ds}</td>
<td>0.47</td>
<td>0.79</td>
<td>0.88</td>
</tr>
<tr>
<td>S_{cond}</td>
<td>0.00 *</td>
<td>0.15</td>
<td>0.19</td>
</tr>
<tr>
<td>S_{acin}</td>
<td>0.69</td>
<td>0.63</td>
<td>0.88</td>
</tr>
<tr>
<td>S_{cond} *</td>
<td>0.56</td>
<td>0.10</td>
<td>0.41</td>
</tr>
<tr>
<td>S_{acin} *</td>
<td>0.44</td>
<td>0.65</td>
<td>0.90</td>
</tr>
</tbody>
</table>

ICC = intraclass correlation coefficient; SD = standard deviation; CF = cystic fibrosis; LCI = lung clearance index; LCI_{vent} = specific ventilation inequality component of lung clearance index; LCI_{ds} = dead space component of lung clearance index.

*Negative ICC reported as zero.
Figure 3.6: Receiver operating characteristic curves of lung clearance index and FEV\textsubscript{1} (% pred.) for distinguishing between control subjects and bronchiectasis patients

Receiver operating characteristic (ROC) curves are shown for lung clearance index (LCI) (Panel A) and forced expiratory volume in one second percent predicted (FEV\textsubscript{1} [% pred.]) (Panel B). Areas under ROC curves are 0.90 for LCI and 0.83 for FEV\textsubscript{1} (% pred.).

Panel A

Panel B
Figure 3.7: Scatterplots of forced expiratory volume in one second standardised residuals against multiple breath washout parameters in patients with non-cystic fibrosis bronchiectasis

$LCI = $ lung clearance index; $LCI_{vent} = $ specific ventilation inequality component of lung clearance index; $LCI_{ds} = $ dead space component of lung clearance index; $FEV_1 = $ forced expiratory volume in one second; $SR = $ standardised residuals. Dotted lines denote the lower limit of normal for $FEV_1$ and upper limits of normal for $LCI$, $LCI_{vent}$ and $LCI_{ds}$.

Panel A

Panel B
Panel C

![Chart showing data points on a scatter plot with axes labeled FEV1 SR and LCI ds. The plot displays a distribution of points with specific values.]
Discussion

We have shown that LCI, and the novel parameters LCI_{vent} and LCI_{ds}, are significantly raised in patients with non-CF bronchiectasis compared to controls, and that these parameters correlate strongly with spirometric markers of airflow obstruction. LCI, LCI_{vent} and LCI_{ds} display good within-visit repeatability in patients with non-CF bronchiectasis, and superior discriminatory ability for distinguishing bronchiectasis patients from controls compared to FEV_{1}. Moreover, these parameters are abnormally raised in a significant proportion of non-CF bronchiectasis patients with a normal FEV_{1}. These findings suggest that MBW parameters may have potential as markers of disease severity in patients with non-CF bronchiectasis, and may be indicators of incipient airflow obstruction, although longitudinal studies are required to test this hypothesis. Further studies are also required to determine the between-visit variability and minimal clinically important difference of MBW parameters in patients with non-CF bronchiectasis, as well as their responsiveness to therapeutic interventions.

Interestingly, the relationship between FEV_{1} and LCI differed significantly between patients with CF and non-CF bronchiectasis. Specifically, patients with CF had a 0.13 unit increase in LCI for every 1 percentage point reduction in FEV_{1} (% pred.), whereas patients with bronchiectasis had a 0.05 unit increase in LCI for every 1 percentage point reduction in FEV_{1} (% pred.) (p < 0.0001). In the asthma cohort presented in Study 3.4, the slope of the relationship was even shallower, with a 0.02 unit increase in LCI for every 1 percentage point reduction in FEV_{1} (% pred.). We speculate that ventilation heterogeneity is most marked in suppurative lung diseases, in which there is patchy near-complete obstruction of airways by mucus hypersecretion. The degree of lung damage and bronchiectasis in the adult CF cohort was likely to be greater than in our non-CF bronchiectasis group, some of whom had mild or localised bronchiectasis. In contrast, the remodelling changes that occur in asthma appear to cause more subtle ventilation heterogeneity.

A major aim of this study was to develop novel markers of ventilation heterogeneity that would distinguish between the two possible mechanisms of increased LCI, namely specific ventilation inequality and increased respiratory dead space. Previous studies have used measures of the curvilinearity of the washout curve as markers of specific
ventilation inequality, but these methods did not provide a formal estimate of the respiratory dead space component\textsuperscript{143,149}. Although in healthy subjects it is thought that specific ventilation inequality is the only mechanism of ventilation heterogeneity operative at the level of the proximal conducting airways, the situation is disease is far more complex. Depending on the extent of airway damage and obstruction, diffusion may not be neatly compartmentalised to the distal airways. An advantage of the current method is that it does not pre-suppose an anatomical location for the observed abnormalities in ventilation heterogeneity, but concentrates on the underlying mechanisms. This is particularly relevant when dealing with those with more severe airflow obstruction and ventilation heterogeneity. Furthermore, since the proximal and distal airways are not independent of each other, and form a complex interacting network\textsuperscript{124}, it is also unsurprising that we noted a correlation between LCI\textsubscript{vent} and LCI\textsubscript{ds} in both patient groups. LCI\textsubscript{vent} and LCI\textsubscript{ds} may however allow subtle distinctions to be made in terms of mechanisms of disease in airway diseases such as CF and non-CF bronchiectasis. Indeed, we observed that CF patients with chronic \textit{P. aeruginosa} colonisation had increased LCI\textsubscript{ds} compared to those who did not, whereas LCI\textsubscript{vent} did not differ significantly between the groups. This extends the findings of Belessis \textit{et al.}\textsuperscript{239}, who observed that LCI was higher in children with CF who had \textit{P. aeruginosa} colonisation compared to those who did not. Our results suggest that this increase in LCI may be driven predominantly by an increased respiratory dead space. Interestingly, neither MBW parameters nor FEV\textsubscript{1} (% pred.) differed significantly between non-CF bronchiectasis patients with and without chronic bacterial colonisation. Chronic colonisation in our cohort was mainly with \textit{H. influenzae} rather than \textit{P. aeruginosa}, and our data therefore concord with previous observations that \textit{H. influenzae}, unlike \textit{P. aeruginosa}, is not associated with faster lung function decline in non-CF bronchiectasis\textsuperscript{240}. The reduced FVC (% pred.) we observed in non-CF bronchiectasis patients with chronic colonisation was not associated with an abnormally low TLC (% pred.), and therefore did not represent a true restrictive deficit.

It is recognised that LCI may be influenced to a certain extent by the FRC, VT and V\textsubscript{D\textsubscript{anat}}, and is therefore not a completely unbiased measure of VH\textsuperscript{241}. We therefore derived a formula for the ‘ideal’ value of LCI for a given combination of FRC, VT and V\textsubscript{D\textsubscript{anat}}, assuming no VH (LCI\textsubscript{ideal}). We defined the novel parameters LCI\textsubscript{vent} and LCI\textsubscript{ds} as the proportional increase in LCI over and above LCI\textsubscript{ideal} due to (i) convective
ventilation heterogeneity, and (ii) increased respiratory dead space, respectively. These parameters are based upon a simple two-compartment model of the lungs. The assumptions of this model will now be discussed:

i) We modelled washout curves using just two compartments, whereas in reality the lungs consist of many thousands of convection-dependent units, forming an almost continuous distribution of washout time constants. A number of authors have investigated the theoretical and practical limits on the information that may be extracted from an inert gas washout curve. Wagner et al. showed that under ideal theoretical conditions of error-free data, continuous distributions of washout rate constants with up to four modes could be distinguished. However, subsequent investigators simulated the effect of introducing experimental error into the data, and found that even with very low levels of error, the information that was recoverable dropped dramatically. These conclusions are in accordance with our own experience, in which we found that a two-compartment model was sufficient to capture the information content of experimental washout curves. The median (interquartile range) goodness of fit of the two-compartment model was 0.995 (0.991 – 0.997), 0.985 (0.975 – 0.993) and 0.959 (0.929 – 0.984) in the healthy, non-CF bronchiectasis and CF groups, respectively. We found that in the majority of cases it was not possible to fit a three-compartment model to experimental MBW data, except in the case of CF patients with the most severe ventilation heterogeneity.

ii) Our model did not take into account the effect of ‘sequencing’, whereby poorly-ventilated lung units empty later in expiration than well-ventilated units. Otis et al. showed that the phase shift in cyclical ventilation between lung units with different time constants could result in a curious phenomenon known as convective pendelluft, in which some lung units could continue to fill while others have started emptying, and vice versa. Safonoff et al. demonstrated that the effects of this phenomenon on estimates of FRC and ‘slow’ space volume derived from inert gas washout in patients with severe airway disease were likely to be modest. However, our model could have been usefully extended by including this effect, particularly since we based our washout curves on end-tidal inert gas concentrations, which are disproportionately increased by sequencing compared to alternative points on the expirogram such as the mean expired concentration over the course of the phase III slope.
iii) Our model assumed that the respiratory dead space was distributed between the two compartments in the same proportion as the tidal volume. While it may be considered desirable to have allowed the dead space for each compartment to vary independently, it was necessary to fix the distribution of the dead space between the two compartments in some way, since otherwise it would not have been possible to distinguish between the slow washout of a compartment due to reduced ventilation or increased dead space. The assumption that dead space is distributed in the same proportion as tidal volume follows from the presumed independence of the convective and diffusive-convective mechanisms under investigation, and implies that a fixed proportion of the tidal volume in each of the two large compartments is ‘wasted’ due to anatomical dead space and diffusion-convection-dependent gas mixing inefficiencies. However, this work could have been extended by exploring the effect of different distributions of dead space, for instance by introducing a common dead space into the model.

A potential limitation of our study was that the disease groups were not matched for age with the control group. This was to a certain extent unavoidable, since patients with non-CF bronchiectasis are in general older than those with CF, and we therefore chose our control group to be approximately intermediate in age between the two disease groups. However, recently published regression equations\textsuperscript{246} indicate that the effects of this on our results were likely to be modest – in particular, LCI is expected to increase by 0.0223 units per year, so the 19-year difference in mean age between patients with bronchiectasis and healthy controls would be predicted to cause a relatively small 0.43 difference in LCI between the groups. Furthermore, the upper limit of normal of LCI derived from our healthy control data was slightly higher than that reported in previous studies using the same methodology\textsuperscript{132}, a difference that may be explained by the older age of our healthy cohort. Similarly, the values for $S_{\text{cond}}$ and $S_{\text{acin}}$ that we obtained in healthy subjects were somewhat higher than those reported by Prisk et al in a small group of healthy participants who flew on the Spacelab Life Sciences (SLS) missions\textsuperscript{247}. This may be explained by differences in both subject characteristics and methodology, since the SLS participants were younger than those in our study, and additionally washout tests in the Prisk study were performed using a mass spectrometer with 1.25% SF\textsubscript{6}. Further studies are required to derive age- and sex-dependent
normative ranges for LCI and other MBW parameters using the SF₆ wash-in method, as have been published for nitrogen washout²⁴⁶.

In conclusion, we have shown that LCI, a marker of impaired gas mixing derived from the MBW test, is significantly raised in patients with non-CF bronchiectasis, and that this elevation correlates with spirometric airflow obstruction. LCI is repeatable and discriminatory in patients with non-CF bronchiectasis, and future studies are now required to assess the prognostic significance of a raised LCI in this patient group, as well as the potential utility of this marker as an outcome measure in interventional trials. The novel parameters LCI_{vent} and LCI_{ds} are an alternative to phase III slope analysis that may allow a further level of mechanistic information to be obtained from the MBW test without any additional demand on the patient.
3.3 Between-visit variability of small airway obstruction markers in patients with asthma

Abstract

Background and aims
The forced expiratory volume in one second is often utilised as an outcome measure in clinical asthma trials, but is thought to reflect mainly large airway obstruction. Putative markers of small airway disease include measures of respiratory system resistance using impulse oscillometry (IOS), and indices of ventilation heterogeneity derived from multiple breath inert gas washout (MBW). We aimed to determine the between-visit variability of these measurements in patients with asthma in the stable state.

Methods
Eighteen patients with asthma underwent IOS and MBW at baseline, and the tests were subsequently repeated using identical methodology two weeks and three months following baseline. The intraclass correlation coefficient (ICC) and standard deviation of between-visit within-subject differences (SD) was calculated for each physiological variable, for each of the two time intervals.

Results
ICC values ranged from 0.80 to 0.91 for IOS parameters, and from 0.63 to 0.91 for MBW parameters. The SD data we have provided may be utilised to perform sample size calculations for interventional trials.

Conclusion
We conclude that IOS parameters are stable over time, and have potential as outcome measures in clinical asthma trials. MBW indices are moderately stable, but require further investigation in patients with asthma.
**Introduction**

Clinical trials in patients with airway diseases often utilise the forced expiratory volume in one second (FEV\(_1\)) as the sole physiological outcome measure. However, FEV\(_1\) is thought to be insensitive to obstruction of the smaller airways, which may be particularly relevant in asthma\(^37\). Putative markers of small airway obstruction include measures of airway resistance using impulse oscillometry (IOS)\(^113\), and indices of ventilation heterogeneity derived from multiple breath inert gas washout (MBW)\(^128\). Recently, Takeda et al have shown that IOS parameters such as R20, R5-R20 and X5 are independent predictors of asthma health status over and above FEV\(_1\)\(^127\), and Farah et al have demonstrated that MBW parameters may be responsive to asthma therapy\(^157\).

In order to conduct clinical trials using these alternative outcome measures, it is necessary to be assured of their repeatability and stability over time. Moreover, an estimate of between-visit variability in the stable state is required so that sample size calculations can be performed. Between-visit variability may arise from a number of sources including purely technical factors, differences in patient performance of the test, and true temporal variability in the degree of airway obstruction. We aimed to determine the between-visit variability of a range of IOS and MBW indices in a group of patients with asthma in the stable state. We investigated between-visit variability over two time intervals, namely two weeks and three months, in order to encompass the typical lengths of treatment period that are utilised in clinical trials.

**Methods**

We recruited 18 adults (age > 18) with moderate-to-severe asthma (Global Initiative for Asthma treatment steps 3-5), diagnosed by a specialist asthma physician in a secondary care setting, according to British Thoracic Society guidelines\(^2\,248\). The study was approved by the National Research Ethics Committee – East Midlands Leicester, and all participants gave their written informed consent. The participant group comprised nine men and nine women with a mean (standard deviation [SD]) age of 58 (11) years. All participants were treated with inhaled corticosteroids (1000 – 2000 μg per day, beclometasone dipropionate equivalent) and long-acting β\(_2\) agonists. Eleven patients
received maintenance low-dose prednisolone (5 – 15mg per day). Mean (SD) post-bronchodilator FEV$_1$ (% pred.) was 80.5 (23.0) with mean (SD) bronchodilator reversibility of 13.5% (16.0%). A previous history of atopy was documented in nine patients. Geometric mean (95% confidence interval) sputum eosinophil percentage was 2.6 (1.1 – 6.3) (upper limit of normal 2.2%)$^{249}$, and mean (SD) neutrophil percentage was 58.6 (28.2).

At each study visit, participants completed the six-point Asthma Control Questionnaire (ACQ-6)$^{250}$. Following administration of a bronchodilator (400μg via a metered dose inhaler and spacer), IOS, MBW and spirometry were performed at baseline, then at two weeks and three months following baseline. All study visits took place in the stable state, at least six weeks following any exacerbation of asthma.

IOS was performed using a Jaeger MasterScreen Impulse Oscillometry System (Viasys Healthcare GmbH, Hoechberg, Germany), according to standard guidelines$^{101}$, as described in Section 2.4.2. IOS was performed three times in succession at each visit, with each test lasting 60 seconds. R5, R20, R5-R20, X5 and AX values were obtained from each test by taking the mean value measured over the course of the 60 second test interval. MBW was performed according to current guidelines$^{130}$, using the sulphur hexafluoride (SF$_6$) wash-in method$^{132}$, as described in Section 2.5. The novel parameters LCI$_{vent}$ and LCI$_{ds}$ were calculated as described in Section 2.7.

**Results and discussion**

The intraclass correlation coefficient (ICC, SPSS Version 20, IBM Corporation, Somers, New York, USA) was calculated for each physiological variable, for the two-week and three-month time intervals, as shown in Table 3.6. Between-visit repeatability was good at both time intervals for most IOS and MBW parameters, with ICC values exceeding 0.8 in the majority of cases. However, the repeatability of S$_{cond}$ and S$_{acin}$ at 3 months was only moderate, with ICC values of 0.63 and 0.71 respectively. Within-visit repeatability was also assessed using the ICC of triplicate tests performed at baseline. All physiological measurements had high within-visit repeatability (ICC > 0.85), with the exception of S$_{cond}$. Previous investigators have also noted the poor within-visit
repeatability of $S_{\text{cond}}^{147}$, suggesting that this parameter may be less reliable than LCI and $S_{\text{acin}}$ using the current MBW methodology.

The mean, SD and 95% confidence intervals of between-visit differences are shown in Table 3.6. The SD data may be used, in conjunction with estimates of the minimal clinically important difference (MCID), to perform sample size calculations for interventional studies in patients with asthma. Given a parallel group study design in which the change in the variable of interest from baseline to follow-up is compared between an intervention and a control group using a parametric test, the required sample size may be calculated using a standard formula$^{251}$:

$$\text{Sample size} = \frac{2\sigma^2(u + v)^2}{d^2}$$

Where

- $\sigma$ = standard deviation of between-visit changes
- $u$ = one-sided percentage point of the normal distribution corresponding to $100\%$ - power (eg. if power = $80\%$, $u = 0.84$)
- $v$ = percentage point of the normal distribution corresponding to the (two-tailed) significance level (eg. if significance level = $5\%$, $v = 1.96$)
- $d$ = difference in between-visit changes to be detected

Although the MCID has not been established for most small airway outcome measures, estimates can be made in some cases from previously published data. Yamaguchi et al compared the response to small-particle and standard corticosteroid inhalers in steroid-naïve asthmatics using IOS$^{252}$. They observed a reduction in R5-R20 of 0.05 kPa·L$^{-1}$·s in the small-particle group and 0.02 kPa·L$^{-1}$·s in the standard group, thus giving a difference between groups of 0.03 kPa·L$^{-1}$·s with respect to the change in R5-R20 from baseline to follow-up. The sample size required to detect this difference following a two-week treatment period with $80\%$ power and $5\%$ two-tailed significance would be 28 per treatment arm, which would be feasible in most clinical trial settings. Table 3.7 shows, for each of the IOS and MBW parameters, the minimum between-group
difference that could be detected with respect to baseline-to-follow-up change, at 80% power and 5% two-tailed significance, with 30 patients in each arm of the study.

A potential limitation of our study was the relatively high mean age of our participants. It is possible that younger patients with asthma manifest a greater degree of variability in airway function than older patients, and further studies are required to investigate this possibility. However, the relative preponderance of middle-aged patients in our participant group is typical of previously described refractory asthma cohorts\textsuperscript{253}, and is thus representative of patients most likely to be enrolled in clinical trials. Most patients in this study had moderate or severe asthma, and the results therefore may not be generalisable to mild asthma. As expected, variability was greater at three months than at two weeks for most outcome measures. However, LCI, LCI\textsubscript{vent} and LCI\textsubscript{ds} were exceptions to this pattern. We therefore recommend that the two-week estimate of variability be used for all sample size calculations involving these indices, in order to mitigate the risk of being underpowered.

We conclude that IOS parameters are stable over time, and have potential as outcome measures in clinical asthma trials. MBW indices are moderately stable, but require further investigation in patients with asthma. Further studies are required to determine the longer-term variability of MBW and IOS parameters, as well as to establish the MCID for a number of small airway outcome measures.
Table 3.6: Between- and within-visit variability of physiological variables in patients with asthma

<table>
<thead>
<tr>
<th></th>
<th>Baseline values</th>
<th>Variability at two weeks</th>
<th>Variability at three months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>ICC*</td>
<td>Mean ± SD change from baseline (95% confidence intervals)</td>
</tr>
<tr>
<td>ACQ-6 score</td>
<td>1.61 ± 1.14</td>
<td>-</td>
<td>0.02 ± 0.64 (-1.24 – 1.28)</td>
</tr>
<tr>
<td>FEV1 (L)</td>
<td>2.21 ± 0.82</td>
<td>0.99</td>
<td>-0.04 ± 0.14 (-0.32 – 0.24)</td>
</tr>
<tr>
<td>R5 (kPa L⁻¹ s⁻¹)</td>
<td>0.52 ± 0.18</td>
<td>0.94</td>
<td>-0.02 ± 0.08 (-0.18 – 0.14)</td>
</tr>
<tr>
<td>R20 (kPa L⁻¹ s⁻¹)</td>
<td>0.39 ± 0.12</td>
<td>0.90</td>
<td>-0.01 ± 0.06 (-0.12 – 0.10)</td>
</tr>
<tr>
<td>R5-R20 (kPa L⁻¹ s⁻¹)</td>
<td>0.14 ± 0.10</td>
<td>0.97</td>
<td>-0.02 ± 0.04 (-0.09 – 0.06)</td>
</tr>
<tr>
<td>X5 (kPa L⁻¹ s⁻¹)</td>
<td>-0.19 ± 0.11</td>
<td>0.94</td>
<td>0.02 ± 0.05 (-0.08 – 0.11)</td>
</tr>
<tr>
<td>AX (kPa L⁻¹)</td>
<td>1.47 ± 1.42</td>
<td>0.98</td>
<td>-0.25 ± 0.59 (-1.40 – 0.91)</td>
</tr>
<tr>
<td>FRCmbw (L)</td>
<td>2.50 ± 0.79</td>
<td>0.98</td>
<td>-0.10 ± 0.23 (-0.55 – 0.35)</td>
</tr>
<tr>
<td>LCI</td>
<td>8.20 ± 1.48</td>
<td>0.95</td>
<td>0.14 ± 1.04 (-1.89 – 2.18)</td>
</tr>
<tr>
<td>LCIvent</td>
<td>1.28 ± 0.15</td>
<td>0.97</td>
<td>0.01 ± 0.10 (-0.19 – 0.20)</td>
</tr>
<tr>
<td>LCIds</td>
<td>1.20 ± 0.08</td>
<td>0.94</td>
<td>0.01 ± 0.05 (-0.09 – 0.10)</td>
</tr>
<tr>
<td>Scond (L⁻¹)</td>
<td>0.066 ± 0.081</td>
<td>0.23</td>
<td>0.004 ± 0.038 (-0.072 – 0.079)</td>
</tr>
<tr>
<td>Sacin (L⁻¹)</td>
<td>0.207 ± 0.127</td>
<td>0.87</td>
<td>-0.024 ± 0.067 (-0.156 – 0.108)</td>
</tr>
</tbody>
</table>
Legend for Table 3.6
SD = standard deviation; ICC = intraclass correlation coefficient; ACQ-6 = six-point Asthma Control Questionnaire; FEV\textsubscript{1} = forced expiratory volume in one second; R5/R20 = resistance at 5Hz/20Hz; X5 = reactance at 5Hz; AX = reactance area; FRC\textsubscript{mbw} = functional residual capacity measured using multiple breath washout; LCI = lung clearance index; LCI\textsubscript{vent} = specific ventilation inequality component of lung clearance index; LCI\textsubscript{ds} = dead space component of lung clearance index.
*Denotes ICC of triplicate tests performed at baseline.
†Denotes ICC of tests performed at baseline versus follow-up visits.
Table 3.7: Sample size calculations for impulse oscillometry and inert gas washout parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Δ following 2-week treatment period*</th>
<th>Δ following 3-month treatment period*</th>
</tr>
</thead>
<tbody>
<tr>
<td>R5 (kPa·L⁻¹·s)</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>R20 (kPa·L⁻¹·s)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>R5-R20 (kPa·L⁻¹·s)</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>X5 (kPa·L⁻¹·s)</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>AX (kPa·L⁻¹)</td>
<td>0.43</td>
<td>0.49</td>
</tr>
<tr>
<td>LCI †</td>
<td>0.76</td>
<td>0.76</td>
</tr>
<tr>
<td>LCI_{vent} †</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>LCI_{ds} †</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>S_{cond} (L⁻¹)</td>
<td>0.028</td>
<td>0.042</td>
</tr>
<tr>
<td>S_{ac} (L⁻¹)</td>
<td>0.049</td>
<td>0.050</td>
</tr>
</tbody>
</table>

R5/R20 = resistance at 5Hz/20Hz; X5 = reactance at 5Hz; AX = reactance area; LCI = lung clearance index; LCI_{vent} = specific ventilation inequality component of lung clearance index; LCI_{ds} = dead space component of lung clearance index.

*Denotes the minimum between-group difference with respect to baseline-to-follow-up change that may be detected given a parallel-group study design, with 30 patients in each arm, at 80% power and 5% two-tailed significance.

†Two-week estimate of variability utilised for both two-week and three-month calculations, as discussed in the text.
3.4 Clinical significance of small airway obstruction markers in patients with asthma

Abstract

Background
The role of small airway obstruction in the clinical expression of asthma is incompletely understood.

Objective
We tested the hypotheses that markers of small airway obstruction are associated with (i) increased asthma severity, (ii) impaired asthma control and quality of life, and (iii) frequent exacerbations.

Methods
Seventy-four adults with asthma and 18 healthy control subjects underwent impulse oscillometry (IOS), multiple breath inert gas washout (MBW), body plethysmography, single-breath determination of carbon monoxide uptake and spirometry. Patients completed the six-point Asthma Control Questionnaire (ACQ-6) and standardised Asthma Quality of Life Questionnaire (AQLQ(S)). Asthma severity was classified according to the Global Initiative for Asthma (GINA) treatment steps.

Results
The putative small airway obstruction markers $S_{acin}$, resistance at 5Hz minus resistance at 20 Hz (R5-R20) and reactance area (AX) were not independently associated with asthma severity, control, quality of life or exacerbations. In contrast, markers of total (R5) and mean airway resistance of large and small airways (R20) were significantly higher in the severe asthma group compared to the mild-moderate group (0.47 vs 0.37, $p < 0.05$ for R5; 0.39 vs 0.31, $p < 0.01$ for R20). The strongest independent contributors to ACQ-6 score were R20 and forced expiratory volume in one second (% pred.), and the strongest independent contributors to AQLQ(S) score were R20 and forced vital
capacity (% pred.). A history of one or more exacerbations within the previous year was independently associated with R20.

Conclusions and Clinical Relevance
Previously reported markers of small airway obstruction do not appear to be independently associated with asthma disease expression. In contrast, the IOS parameter R20, a marker of mean airway resistance of both large and small airways, appears to have independent clinical significance. These observations require confirmation in prospective longitudinal studies.
Introduction

Asthma is a common inflammatory airway disease that is estimated to affect 300 million people worldwide. Inhaled corticosteroids (ICS) comprise the mainstay of asthma therapy, but deposition of most standard topical therapies is limited to the large conducting airways. Persistent asthma, in which symptoms and/or airway inflammation are inadequately controlled despite topical therapy, imposes a disproportionate burden on individual patients and society, and is relatively common. Indeed, a large randomised controlled trial comparing ICS with combination ICS and long-acting β-agonist (LABA) only achieved total control of asthma at one year in 28% and 41% of patients respectively. The causes of persistence are not completely understood but include poor patient adherence, inadequate inhaler technique and corticosteroid resistance. A further possibility is that patients with persistent asthma have pathology in the small airways (usually defined as those with an internal diameter < 2mm) that standard topical therapy cannot reach.

The forced expiratory volume in one second (FEV₁) is often used to monitor asthma control, and as an outcome measure in clinical trials. However, FEV₁ is thought to be insensitive to small airway obstruction, and therefore a number of putative markers of small airway obstruction have been suggested, as summarised in Table 3.8. Measures of expiratory air trapping such as the forced vital capacity (FVC) and the ratio of residual volume (RV) to total lung capacity (TLC) are often assumed to represent small airway obstruction, although conclusive evidence in support of this assertion is lacking. Impulse oscillometry (IOS), a variant of the forced oscillation technique (FOT), allows the non-invasive assessment of lung mechanics, and may provide novel insights into small airway obstruction. The structural interpretation of IOS/FOT parameters is not fully understood, and has been the subject of much research, often based around computational models of lung impedance. An additional complication is that values of resistance and reactance measured using different equipment may not be directly comparable. Resistance at 20Hz (R20) may be a marker of the general level of airway resistance throughout the airway tree, while the difference between R5 and R20 (R5-R20) may represent the heterogeneity of airway resistance. Measures of low-frequency reactance such as the reactance at 5Hz and reactance area (AX) are likely to reflect airway closure. Multiple breath inert gas washout (MBW) is a test of gas...
mixing efficiency and ventilation heterogeneity within the airway tree, with lung clearance index (LCI) being a general marker of ventilation heterogeneity\textsuperscript{130,233}. The specific ventilation inequality and respiratory dead space components of LCI may be extracted using the novel parameters $\text{LCI}_{\text{vent}}$ and $\text{LCI}_{\text{ds}}$, respectively. The indices $S_{\text{cond}}$ and $S_{\text{acin}}$ derived from MBW are thought to represent ventilation heterogeneity arising due to convective and diffusive-convective mechanisms, respectively\textsuperscript{131}, with convective mechanisms occurring in both the large and small conducting airways and diffusive-convective mechanisms occurring more distally in the airway tree, in the region of the acinar entrance. To summarise, while a number of physiological markers have been proposed that may provide greater insight into small airway obstruction than spirometry, these are unlikely to be completely specific to the small airways, as defined above. Moreover, it is known that the large and small airways are not independent of each other, but that they instead form a complex interacting network that may exhibit emergent properties such as catastrophic closure and re-opening\textsuperscript{124}. Therefore, the distinction between markers of large and small airway obstruction may be to a certain extent artificial.
### Table 3.8: Physiological interpretations of airway obstruction markers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Interpretation of abnormal result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forced expiratory volume in one second (FEV₁)</td>
<td>Reduced ventilatory function</td>
</tr>
<tr>
<td>Forced vital capacity (FVC)</td>
<td>Air trapping</td>
</tr>
<tr>
<td>Ratio of forced expiratory volume in one second to forced vital capacity (FEV₁/FVC)</td>
<td>Expiratory flow limitation</td>
</tr>
<tr>
<td>Ratio of residual volume to total lung capacity (RV/TLC)</td>
<td>Air trapping</td>
</tr>
<tr>
<td>Ratio of alveolar volume by single breath helium dilution to total lung capacity (VA)</td>
<td>Abnormal convective ventilation distribution</td>
</tr>
<tr>
<td>Lung clearance index (LCI)</td>
<td>Ventilation heterogeneity</td>
</tr>
<tr>
<td>LCI&lt;sub&gt;vent&lt;/sub&gt;</td>
<td>Specific ventilation inequality component of lung clearance index</td>
</tr>
<tr>
<td>LCI&lt;sub&gt;ds&lt;/sub&gt;</td>
<td>Increased dead space component of lung clearance index</td>
</tr>
<tr>
<td>S&lt;sub&gt;cond&lt;/sub&gt;</td>
<td>Conductive ventilation heterogeneity</td>
</tr>
<tr>
<td>S&lt;sub&gt;acin&lt;/sub&gt;</td>
<td>Acinar ventilation heterogeneity</td>
</tr>
<tr>
<td>Resistance at 5Hz (R5)</td>
<td>General marker of airway resistance</td>
</tr>
<tr>
<td>Resistance at 20Hz (R20)</td>
<td>General marker of airway resistance</td>
</tr>
<tr>
<td>Resistance at 5Hz minus resistance at 20Hz (R5-R20)</td>
<td>Heterogeneity of airway resistance</td>
</tr>
<tr>
<td>Reactance at 5Hz (X5)</td>
<td>Airway closure</td>
</tr>
<tr>
<td>Reactance area (AX)</td>
<td>Airway closure</td>
</tr>
</tbody>
</table>
Nevertheless, there is some evidence that the putative markers of small airway obstruction described above may relate to disease expression in patients with asthma. Bourdin et al found that single breath nitrogen phase III slope correlated with Asthma Control Questionnaire (ACQ) score \(^{156}\), although these authors did not control for possible associations between nitrogen phase III slope and FEV\(_1\). Farah et al found that changes in S\(_{\text{cond}}\) and S\(_{\text{acin}}\) correlated significantly with changes in five-point ACQ scores following treatment with high-dose ICS \(^{157}\). The same group showed that MBW parameters could predict the symptomatic response to ICS up- or down-titration \(^{159}\). Moreover, Thompson et al found that S\(_{\text{acin}}\) was raised during asthma exacerbations and fell markedly as the exacerbation resolved \(^{158}\). Shi et al reported that AX and R5-R20 were significantly higher in children with uncontrolled asthma compared to those with controlled asthma, and that this effect was more apparent with pre-bronchodilator than post-bronchodilator measurements \(^{126}\). Similarly, Takeda et al found that R20, R5-R20 and X5 were independent predictors of patient-reported outcome measures such as ACQ score and Asthma Quality of Life Questionnaire (AQLQ) score in adults with asthma \(^{127}\). Sorkness et al found that at any given level of airflow obstruction, as measured with the FEV\(_1\)/FVC (% pred.), patients with severe asthma had more severe air trapping, as measured by reduced FVC (% pred.) and increased RV/TLC (% pred.) \(^{92}\). However, there has as yet been no study examining the contributions of each of these putative small airway obstruction markers to clinical outcomes in a single well-characterised group of adults with asthma. Moreover, with the exception of the study by Bourdin et al \(^{156}\), physiological measurements in the above studies were performed having withheld bronchodilator medications, and it is therefore possible that variations in large airway bronchial tone may have contributed to the associations that were observed. We therefore wished to specifically investigate post-bronchodilator measurements, in order to eliminate these variations and focus on the effects of long-term remodelling changes in the smaller airways.

In this study, we aimed to test the hypotheses that small airway obstruction markers are associated with (i) increased asthma severity, as evidenced by higher treatment requirements, (ii) impaired asthma control and quality of life, and (iii) frequent exacerbations.
Materials and methods

Subjects
Seventy-four patients with asthma and 18 healthy subjects with no history of respiratory disease were recruited. All participants were over 18 years of age. Current smokers (defined as people who have smoked within the previous year) and ex-smokers with a greater than 10 pack-year smoking history were excluded from the study. Asthma was diagnosed by a specialist asthma physician in a secondary care setting according to current British Thoracic Society guidelines. Asthma severity was classified according to the current Global Initiative for Asthma (GINA) treatment steps. The study protocol was approved by the National Research Ethics Committee – East Midlands Leicester (approval number 08/H0406/189) and all subjects gave their written informed consent.

Study protocol
All participants attended a single visit in the stable state, not less than six weeks following any asthma exacerbation. Exacerbations were defined as worsening asthma symptoms for ≥ 3 days requiring additional therapy (short burst oral prednisolone and/or antibiotics) following either primary or secondary care consultation. Clinical and physiological assessment was performed in the following sequence:

i) Collection of demographic and clinical details
ii) Completion of six-point ACQ (ACQ-6) and standardised AQLQ (AQLQ(S))
iii) Administration of salbutamol 400 micrograms via metered dose inhaler and spacer
iv) Impulse oscillometry, using Jaeger MasterScreen Impulse Oscillometry System (Viasys Healthcare GmbH, Hoechberg, Germany)
vi) Lung volumes using body plethysmography
vii) Single-breath determination of carbon monoxide uptake
viii) Spirometry

All physiological tests were performed in the seated position by individuals with appropriate training and accreditation. Physiological tests were performed 10 minutes
after administration of a short-acting bronchodilator (salbutamol 400 micrograms). This was administered via a metered dose inhaler and spacer, with each 100 microgram actuation being inhaled in a separate inhalation to TLC, followed by a 5-10 second breath-hold.

**Statistical analysis**
Statistical analyses were performed using SPSS 20 (IBM Corporation, Somers, New York, USA) and Prism 6 (GraphPad Software Inc., La Jolla, California, USA). A p value of < 0.05 was taken as the threshold for statistical significance. Comparisons between or across groups were performed using Student’s T test or one-way analysis of variance for parametric data, the Mann-Whitney U test or Kruskal-Wallis test for non-parametric data, and Fisher’s exact test or the Chi-squared test for proportions. Bonferroni/Dunn corrections for multiple comparisons were used as appropriate. Correlations between continuous variables were calculated using Pearson’s correlation coefficient (R). Log-normally distributed variables were log-transformed as appropriate. Linear regression models were constructed (SPSS 20, stepwise algorithm, with probability for variable entry < 0.05 and probability for variable removal > 0.10) to determine the physiological determinants of ACQ and AQLQ scores. A logistic regression model (SPSS 20, forward conditional algorithm, with probability for variable entry < 0.05 and probability for variable removal > 0.10) was constructed to determine the physiological predictors of the exacerbation-prone phenotype, defined as having had at least one asthma exacerbation during the preceding year. The pool of variables that could potentially be entered into the linear or logistic regression models comprised FEV\(_1\) (% pred.), FVC (% pred.), FEV\(_1\)/FVC, TLC (% pred.), RV/TLC (% pred.), alveolar volume using single breath helium dilution (VA), VA/TLC, LCI, LCI\(_{vent}\), LCI\(_{ds}\), S\(_{cond}\), S\(_{acin}\), R5, R20, log-transformed R5-R20, X5 and log-transformed AX. Predicted values and standardised residuals for FEV\(_1\), FVC, TLC, RV/TLC, KCO and DL\(_{CO}\) were calculated using published regression equations\(^{216}\). The lower limit of normal for FEV\(_1\) was defined as 1.645 standardised residuals below predicted, and the upper limit of normal for RV/TLC was defined as 1.645 standardised residuals above predicted. Upper limits of normal for R20, R5-R20, AX, LCI and S\(_{acin}\) were defined as the mean + 1.645 standard deviations in the healthy control group.
Results

Demographic, clinical and physiological data across groups are shown in Tables 3.9 and 3.10. The groups did not differ significantly with respect to age, sex or body mass index. The small airway obstruction markers $S_{\text{acin}}$, R5-R20 and AX did not differ significantly between the asthma severity groups. However, markers of total (R5) and mean large and small airway resistance (R20) were significantly higher in the GINA 4-5 group compared to the GINA 1-3 group (0.47 vs 0.37, $p < 0.05$ for R5; 0.39 vs 0.31, $p < 0.01$ for R20).

Table 3.11 shows correlations between physiological variables and both ACQ-6 and AQLQ(S) scores, as well as FEV$_1$ (% pred.). ACQ-6 score correlated significantly with FEV$_1$ (% pred.), RV/TLC (% pred.), TLC, VA, VA/TLC, diffusing capacity of the lung for carbon monoxide (DLCO) (% pred.), functional residual capacity from multiple breath washout (FRC$_{\text{mbw}}$), R5 and R20, but not with $S_{\text{acin}}$, log-transformed R5-R20 or log-transformed AX. The strongest correlation was with R20 ($R = 0.369$, $p < 0.01$). AQLQ(S) score correlated significantly with FEV$_1$ (% pred.), FVC (% pred.), RV/TLC (% pred.), TLC, VA, VA/TLC, DLCO (% pred.), FRC$_{\text{mbw}}$, R5, R20 and log-transformed R5-R20, but not with $S_{\text{acin}}$ or log-transformed AX. The strongest correlation was again with R20 ($R = -0.430$, $p < 0.01$). Linear regression models, shown in Table 3.12, revealed that the strongest independent contributors to ACQ-6 score were R20 and FEV$_1$ (% pred.), and the strongest independent contributors to AQLQ(S) score were R20 and FVC (% pred.).

Table 3.13 shows physiological variables between patients with and without the exacerbation-prone phenotype, defined as having had at least one asthma exacerbation within the previous year. $S_{\text{acin}}$, R5-R20 and AX did not differ significantly between the groups. R5 and R20 were significantly higher in the exacerbation-prone group compared to the non-exacerbation-prone group, while FVC (% pred.), TLC, VA and DLCO (% pred.) were significantly lower. A logistic regression model revealed that the only independent predictor of the exacerbation-prone phenotype was R20. In particular, a 0.1 kPa·L$^{-1}$·s increase in R20 was associated with an odds ratio of 1.86 for having the exacerbation-prone phenotype (confidence interval of odds ratio 1.12 to 3.10, $p < 0.05$).
Figure 3.8 shows scatterplots of FEV\textsubscript{1} standardised residuals versus selected small airway obstruction markers. Patients were divided into four quadrants depending upon whether their FEV\textsubscript{1} was normal or low (fixed airflow obstruction negative or positive [FAO\textsuperscript{-} or FAO\textsuperscript{+}], respectively), and whether the given small airway obstruction marker was normal or high (small airway obstruction negative or positive [SAO\textsuperscript{-} or SAO\textsuperscript{+}], respectively). In each case, the majority of patients were concordant (FAO\textsuperscript{-}/SAO\textsuperscript{-} or FAO\textsuperscript{+}/SAO\textsuperscript{+}). Figure 3.9 shows pie charts indicating the proportion of patients that were concordant (FAO\textsuperscript{-}/SAO\textsuperscript{-} or FAO\textsuperscript{+}/SAO\textsuperscript{+}) or discordant (FAO\textsuperscript{-}/SAO\textsuperscript{+} or FAO\textsuperscript{+}/SAO\textsuperscript{-}) for each small airway obstruction marker.
Table 3.9: Demographic and clinical data across asthma severity groups

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 18)</th>
<th>Mild-moderate asthma# (n = 43)</th>
<th>Severe asthma# (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.3 (3.9)</td>
<td>57.2 (2.0)</td>
<td>53.8 (2.5)</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>50</td>
<td>51</td>
<td>39</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.8 (1.2)</td>
<td>27.0 (0.8)</td>
<td>30.0 (1.4)</td>
</tr>
<tr>
<td>Atopy (% atopic)</td>
<td>-</td>
<td>79</td>
<td>71</td>
</tr>
<tr>
<td>Duration of asthma (years)*</td>
<td>-</td>
<td>18.7 (2.9)</td>
<td>25.3 (3.2)</td>
</tr>
<tr>
<td>Age of onset of asthma (years)*</td>
<td>-</td>
<td>38.4 (3.3)</td>
<td>28.3 (3.8)</td>
</tr>
<tr>
<td>ICS dose (BDP equivalent [mcg])****</td>
<td>-</td>
<td>547 (66)</td>
<td>1726 (118)</td>
</tr>
<tr>
<td>Oral prednisolone use (%)****</td>
<td>-</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>Leukotriene receptor antagonist use (%)**</td>
<td>-</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>Oral theophylline use (%)**</td>
<td>-</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Exacerbations in past year****</td>
<td>-</td>
<td>1.0 (0.28)</td>
<td>3.5 (0.56)</td>
</tr>
<tr>
<td>ACQ-6 score***</td>
<td>-</td>
<td>0.99 (0.12)</td>
<td>1.82 (0.22)</td>
</tr>
<tr>
<td>AQLQ(S) score*</td>
<td>-</td>
<td>5.65 (0.16)</td>
<td>5.00 (0.23)</td>
</tr>
</tbody>
</table>

BMI = body mass index; ICS = inhaled corticosteroid; BDP = Beclometasone dipropionate; ACQ-6 = Six-point Asthma Control Questionnaire; AQLQ(S) = standardised Asthma Quality of Life Questionnaire. #Mild-moderate asthma refers to Global Initiative for Asthma (GINA) treatment steps 1-3; Severe asthma refers to GINA treatment steps 4-5. Data expressed as mean (standard error), †median (interquartile range) or proportions. Groups compared using Student’s T test or one-way analysis of variance for parametric data, †Kruskal-Wallis test for non-parametric data, and Fisher’s exact test or the Chi-squared test for proportions. Bonferroni/Dunn corrections for multiple comparisons used as appropriate. Significant differences across groups denoted *(p < 0.05), ***(p < 0.01), ****(p < 0.001) or *****(p < 0.0001). Significant differences between GINA 1-3 and GINA 4-5 groups denoted ¥(p < 0.05) or ¥¥(p < 0.01).
Table 3.10: Physiological data across asthma severity groups

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 18)</th>
<th>Mild-moderate asthma (n = 43)</th>
<th>Severe asthma (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; (% pred.)***</td>
<td>113.3 (4.8)</td>
<td>90.8 (3.4)**</td>
<td>86.9 (4.6)***</td>
</tr>
<tr>
<td>FVC (% pred.)*</td>
<td>117.2 (5.6)</td>
<td>101.1 (3.0)*</td>
<td>105.0 (4.2)</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC (%)***</td>
<td>80.7 (1.0)</td>
<td>73.1 (1.5)*</td>
<td>68.4 (2.2)***</td>
</tr>
<tr>
<td>TLC (L)</td>
<td>6.39 (0.37)</td>
<td>6.14 (0.21)</td>
<td>6.00 (0.30)</td>
</tr>
<tr>
<td>TLC (% pred.)</td>
<td>104.3 (3.9)</td>
<td>104.9 (2.5)</td>
<td>107.8 (3.2)</td>
</tr>
<tr>
<td>RV/TLC (% pred.)***</td>
<td>89.2 (3.2)</td>
<td>110.4 (3.1)***</td>
<td>108.7 (2.7)***</td>
</tr>
<tr>
<td>VA (L)</td>
<td>5.58 (0.34)</td>
<td>5.07 (0.22)</td>
<td>4.88 (0.27)</td>
</tr>
<tr>
<td>VA/TLC (%)**</td>
<td>90.3 (1.7)</td>
<td>82.0 (1.6)**</td>
<td>80.8 (1.4)**</td>
</tr>
<tr>
<td>DLCO (% pred.)</td>
<td>89.3 (2.9)</td>
<td>91.2 (2.5)</td>
<td>89.5 (2.9)</td>
</tr>
<tr>
<td>KCO (% pred.)</td>
<td>97.4 (2.9)</td>
<td>107.4 (2.7)</td>
<td>104.6 (3.0)</td>
</tr>
<tr>
<td>FRC&lt;sub&gt;mbw&lt;/sub&gt; (L)</td>
<td>2.52 (0.19)</td>
<td>2.45 (0.11)</td>
<td>2.39 (0.17)</td>
</tr>
<tr>
<td>LCI</td>
<td>7.28 (0.27)</td>
<td>7.79 (0.20)</td>
<td>7.94 (0.22)</td>
</tr>
<tr>
<td>LCI&lt;sub&gt;vent&lt;/sub&gt;</td>
<td>1.20 (0.02)</td>
<td>1.23 (0.02)</td>
<td>1.25 (0.02)</td>
</tr>
<tr>
<td>LCI&lt;sub&gt;ds&lt;/sub&gt;</td>
<td>1.13 (0.01)</td>
<td>1.17 (0.01)</td>
<td>1.18 (0.02)</td>
</tr>
<tr>
<td>S&lt;sub&gt;cond&lt;/sub&gt; (L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.033 (0.007)</td>
<td>0.051 (0.006)</td>
<td>0.038 (0.005)</td>
</tr>
<tr>
<td>S&lt;sub&gt;acuc&lt;/sub&gt; (L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.118 (0.014)</td>
<td>0.175 (0.019)</td>
<td>0.184 (0.020)</td>
</tr>
<tr>
<td>R5 (kPa·L&lt;sup&gt;-1&lt;/sup&gt;·s)***</td>
<td>0.32 (0.03)</td>
<td>0.37 (0.02)</td>
<td>0.47 (0.03)**, V</td>
</tr>
<tr>
<td>R20 (kPa·L&lt;sup&gt;-1&lt;/sup&gt;·s)***</td>
<td>0.29 (0.02)</td>
<td>0.31 (0.01)</td>
<td>0.39 (0.02)**, V</td>
</tr>
<tr>
<td>R5-R20 (kPa·L&lt;sup&gt;-1&lt;/sup&gt;·s)</td>
<td>0.03 (0.01 – 0.06)</td>
<td>0.05 (0.03 – 0.11)</td>
<td>0.05 (0.01 – 0.15)</td>
</tr>
<tr>
<td>X5 (kPa·L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>-0.10 (0.01)</td>
<td>-0.13 (0.01)</td>
<td>-0.15 (0.02)</td>
</tr>
<tr>
<td>AX (kPa·L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.23 (0.16 – 0.54)</td>
<td>0.41 (0.24 – 0.66)</td>
<td>0.47 (0.23 – 1.27)**</td>
</tr>
</tbody>
</table>
**Legend for Table 3.10**

FEV$_1$ = forced expiratory volume in one second; FVC = forced vital capacity; TLC = total lung capacity; RV = residual volume; VA = alveolar volume (single-breath helium dilution); DLCO = diffusing capacity of the lung for carbon monoxide; KCO = carbon monoxide transfer coefficient; FRC$\text{mbw}$ = functional residual capacity from multiple breath washout; LCI = lung clearance index; LCI$\text{vent}$ = specific ventilation inequality component of lung clearance index; LCI$\text{ds}$ = dead space component of lung clearance index; R5/R20 = resistance at 5Hz/20Hz; R5-R20 = resistance at 5Hz minus resistance at 20Hz; X5 = reactance at 5Hz; AX = reactance area.

*Mild-moderate asthma refers to Global Initiative for Asthma (GINA) treatment steps 1-3; Severe asthma refers to GINA treatment steps 4-5. Data expressed as mean (standard error), †median (interquartile range) or proportions. Groups compared using Student’s T test or one-way analysis of variance for parametric data, †Kruskal-Wallis test for non-parametric data, and Fisher’s exact test or the Chi-squared test for proportions. Bonferroni/Dunn corrections for multiple comparisons used as appropriate. Significant differences across groups denoted *(p < 0.05), **(p < 0.01), ****(p < 0.001) or *****(p < 0.0001). Significant differences compared to healthy control group denoted *(p < 0.05), ***(p < 0.01) or ***(p < 0.001). Significant differences between GINA 1-3 and GINA 4-5 groups denoted ¥*(p < 0.05) or ¥¥*(p < 0.01).
Table 3.11: Correlations between clinical outcome measures and physiological variables

<table>
<thead>
<tr>
<th></th>
<th>ACQ-6 score</th>
<th>AQLQ(S) score</th>
<th>FEV(_1) (% pred.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV(_1) (% pred.)</td>
<td>-.285*</td>
<td>.289*</td>
<td>-</td>
</tr>
<tr>
<td>FVC (% pred.)</td>
<td>-.230</td>
<td>.299*</td>
<td>.835**</td>
</tr>
<tr>
<td>FEV(_1)/FVC</td>
<td>-.166</td>
<td>.100</td>
<td>.657**</td>
</tr>
<tr>
<td>TLC</td>
<td>-.252*</td>
<td>.254*</td>
<td>.100</td>
</tr>
<tr>
<td>TLC (% pred.)</td>
<td>-.118</td>
<td>.154</td>
<td>.416**</td>
</tr>
<tr>
<td>RV/TLC (% pred.)</td>
<td>.268*</td>
<td>-.322**</td>
<td>-.573**</td>
</tr>
<tr>
<td>VA</td>
<td>-.300*</td>
<td>.322**</td>
<td>.214</td>
</tr>
<tr>
<td>VA/TLC</td>
<td>-.248*</td>
<td>.274*</td>
<td>.358**</td>
</tr>
<tr>
<td>DLCO (% pred.)</td>
<td>-.286*</td>
<td>.313**</td>
<td>.348**</td>
</tr>
<tr>
<td>KCO (% pred.)</td>
<td>-.084</td>
<td>.019</td>
<td>-.216</td>
</tr>
<tr>
<td>FRC(_{mbw})</td>
<td>-.240*</td>
<td>.243*</td>
<td>.106</td>
</tr>
<tr>
<td>LCI</td>
<td>.151</td>
<td>-.202</td>
<td>-.406**</td>
</tr>
<tr>
<td>LCI(_{vent})</td>
<td>.051</td>
<td>-.052</td>
<td>-.383**</td>
</tr>
<tr>
<td>LCI(_{ds})</td>
<td>.104</td>
<td>-.142</td>
<td>-.352**</td>
</tr>
<tr>
<td>S(_{cond})</td>
<td>.139</td>
<td>.025</td>
<td>-.196</td>
</tr>
<tr>
<td>S(_{acin})</td>
<td>.160</td>
<td>-.168</td>
<td>-.200</td>
</tr>
<tr>
<td>R5</td>
<td>.341**</td>
<td>-.405**</td>
<td>-.340**</td>
</tr>
<tr>
<td>R20</td>
<td>.369**</td>
<td>-.430**</td>
<td>-.169</td>
</tr>
<tr>
<td>log R5-R20</td>
<td>.221</td>
<td>-.250*</td>
<td>-.379**</td>
</tr>
<tr>
<td>X5</td>
<td>-.070</td>
<td>.091</td>
<td>.402**</td>
</tr>
<tr>
<td>log AX</td>
<td>.173</td>
<td>-.202</td>
<td>-.492**</td>
</tr>
</tbody>
</table>
Legend for Table 3.11

ACQ-6 = Six-point Asthma Control Questionnaire; AQLQ(S) = standardised Asthma Quality of Life Questionnaire; FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; TLC = total lung capacity; RV = residual volume; VA = alveolar volume (single-breath helium dilution); DLCO = diffusing capacity of the lung for carbon monoxide; KCO = carbon monoxide transfer coefficient; FRC_{mbw} = functional residual capacity from multiple breath washout; LCI = lung clearance index; LCI_{vent} = specific ventilation inequality component of lung clearance index; LCI_{dead} = dead space component of lung clearance index; R5/R20 = resistance at 5Hz/20Hz; R5-R20 = resistance at 5Hz minus resistance at 20Hz; X5 = reactance at 5Hz; AX = reactance area.

Pearson’s correlation coefficients are shown. Significant correlations are indicated *(p < 0.05)* or ***(p < 0.01)***.
Table 3.12: Linear regression models assessing the contributions of physiological variables to ACQ-6 and AQLQ(S) scores

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Constant term</th>
<th>Independent variables</th>
<th>Unstandardised coefficient (B)</th>
<th>Standardised coefficient (β)</th>
<th>p value</th>
<th>Model R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACQ-6 score</td>
<td>1.129</td>
<td>R20 (kPa·L⁻¹·s)</td>
<td>3.276</td>
<td>0.330</td>
<td>0.005</td>
<td>0.187</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FEV₁ (% pred.)</td>
<td>-0.010</td>
<td>-0.229</td>
<td>0.046</td>
<td></td>
</tr>
<tr>
<td>AQLQ(S) score</td>
<td>5.459</td>
<td>R20 (kPa·L⁻¹·s)</td>
<td>-4.355</td>
<td>-0.398</td>
<td>&lt; 0.0005</td>
<td>0.229</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FVC (% pred.)</td>
<td>0.014</td>
<td>0.248</td>
<td>0.026</td>
<td></td>
</tr>
</tbody>
</table>

ACQ-6 = Six-point Asthma Control Questionnaire; AQLQ(S) = standardised Asthma Quality of Life Questionnaire; R20 = resistance at 20Hz; FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity.
Table 3.13: Demographic and physiological variables in exacerbation-prone and non-exacerbation-prone patients with asthma

<table>
<thead>
<tr>
<th></th>
<th>Non-exacerbation-prone*</th>
<th>Exacerbation-prone*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 34)</td>
<td>(n = 40)</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>59.9 (1.5)</td>
<td>52.2 (2.5)</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>59</td>
<td>35</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3 (0.9)</td>
<td>29.0 (1.2)</td>
</tr>
<tr>
<td>FEV₁ (% pred.)</td>
<td>94.0 (4.5)</td>
<td>85.1 (3.3)</td>
</tr>
<tr>
<td>FVC (% pred.)*</td>
<td>108.2 (3.7)</td>
<td>97.9 (3.1)</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>70.1 (2.0)</td>
<td>72.1 (1.7)</td>
</tr>
<tr>
<td>TLC (L)**</td>
<td>6.59 (0.23)</td>
<td>5.66 (0.24)</td>
</tr>
<tr>
<td>TLC (% pred.)</td>
<td>109.8 (3.3)</td>
<td>103.1 (2.3)</td>
</tr>
<tr>
<td>RV/TLC (% pred.)</td>
<td>106.0 (3.4)</td>
<td>115.6 (3.9)</td>
</tr>
<tr>
<td>VA (L)**</td>
<td>5.48 (0.23)</td>
<td>4.58 (0.23)</td>
</tr>
<tr>
<td>VA/TLC (%)</td>
<td>82.8 (1.6)</td>
<td>80.3 (1.4)</td>
</tr>
<tr>
<td>DLCO (% pred.)**</td>
<td>96.1 (2.9)</td>
<td>85.8 (2.2)</td>
</tr>
<tr>
<td>KCO (% pred.)</td>
<td>108.4 (2.9)</td>
<td>104.4 (2.7)</td>
</tr>
<tr>
<td>FRC_mbw (L)</td>
<td>2.58 (0.13)</td>
<td>2.30 (0.13)</td>
</tr>
<tr>
<td>LCI</td>
<td>8.01 (0.24)</td>
<td>7.71 (0.19)</td>
</tr>
<tr>
<td>LCI_vent</td>
<td>1.25 (0.02)</td>
<td>1.23 (0.02)</td>
</tr>
<tr>
<td>LCI_ds</td>
<td>1.18 (0.02)</td>
<td>1.16 (0.01)</td>
</tr>
<tr>
<td>S_cond (L⁻¹)</td>
<td>0.052 (0.007)</td>
<td>0.052 (0.009)</td>
</tr>
<tr>
<td>S_acio (L⁻¹)</td>
<td>0.197 (0.023)</td>
<td>0.170 (0.018)</td>
</tr>
<tr>
<td>R5 (kPa·L⁻¹·s)*</td>
<td>0.37 (0.03)</td>
<td>0.45 (0.02)</td>
</tr>
<tr>
<td>R20 (kPa·L⁻¹·s)**</td>
<td>0.31 (0.02)</td>
<td>0.37 (0.02)</td>
</tr>
<tr>
<td>R5-R20 (kPa·L⁻¹·s) †</td>
<td>0.04 (0.02 – 0.09)</td>
<td>0.06 (0.03 – 0.13)</td>
</tr>
<tr>
<td>X5 (kPa·L⁻¹·s)</td>
<td>-0.14 (0.02)</td>
<td>-0.13 (0.01)</td>
</tr>
<tr>
<td>AX (kPa·L⁻¹) †</td>
<td>0.33 (0.20 – 0.71)</td>
<td>0.51 (0.29 – 1.24)</td>
</tr>
</tbody>
</table>
Legend for Table 3.13
FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; TLC = total lung
capacity; RV = residual volume; VA = alveolar volume (single-breath helium dilution); DLCO =
diffusing capacity of the lung for carbon monoxide; KCO = carbon monoxide transfer
coefficient; FRC_mbw = functional residual capacity from multiple breath washout; LCI = lung
clearance index; LCI_vent = specific ventilation inequality component of lung clearance index;
LCI_ds = dead space component of lung clearance index; R5/R20 = resistance at 5Hz/20Hz; R5-
R20 = resistance at 5Hz minus resistance at 20Hz; X5 = reactance at 5Hz; AX = reactance area.
*Exacerbation-prone defined as having had at least one asthma exacerbation in the previous
year.
Data expressed as mean (standard error), †median (interquartile range) or proportions. Groups
compared using Student’s T test for parametric data, †Mann-Whitney U test for non-parametric
data or Fischer’s exact test for proportions. Significant differences between groups denoted *(p
< 0.05), **(p < 0.01), ****(p < 0.001) or *****(p < 0.0001).
Figure 3.8: Scatterplots of forced expiratory volume in one second standardised residuals against small airway obstruction markers in patients with asthma

Scatterplots are shown of forced expiratory volume in one second (FEV₁) standardised residuals (SR) against residual volume to total lung capacity ratio SR (Panel A), resistance at 20Hz (Panel B), resistance at 5Hz minus resistance at 20Hz (Panel C), reactance area (Panel D), lung clearance index (Panel E) and S_{acin} (Panel F). Dotted lines indicate lower limits of normal for FEV₁ and upper limits of normal for small airway obstruction markers. Patients with normal FEV₁ and abnormal small airway obstruction markers are indicated in red, and patients with low FEV₁ but normal small airway obstruction markers are indicated in blue.

Panel A

Panel B
Figure 3.9: Pie charts showing proportions of patients with concordance or discordance between forced expiratory volume in one second and small airway obstruction markers

Pie charts are displayed showing the proportions of patients with (i) concordance between FEV$_1$ and small airway obstruction markers (ie. both normal or both abnormal), (ii) elevated small airway obstruction markers but normal FEV$_1$ (FAO$^-$ SAO$^+$), or (iii) low FEV$_1$ but normal small airway obstruction markers (FAO$^+$ SAO$^-$). The small airway obstruction markers analysed are residual volume to total lung capacity ratio (Panel A), resistance at 20Hz (Panel B), resistance at 5Hz minus resistance at 20Hz (Panel C), reactance area (Panel D), lung clearance index (Panel E) and $S_{acin}$ (Panel F).

Panel A (RV/TLC)

Panel B (R20)
Discussion

In this study we investigated the relationship between clinical outcomes and a comprehensive panel of physiological measurements in a group of patients with asthma being managed in a secondary care setting, particularly focussing on measurements that are often considered to reflect small airway obstruction. We found that $S_{acin}$, $R_{5-R20}$ and $AX$ did not differ significantly between patients with mild-moderate asthma and those with severe asthma, nor did these parameters correlate significantly with asthma clinical outcomes. Our results on the surface appear to be discordant with those of previous studies\textsuperscript{126,127,157-159}, in which positive associations between these markers and clinical outcomes were reported. However, in these studies bronchodilators were withheld prior to the study visits, whereas we performed all physiological tests after the administration of a bronchodilator, in order to eliminate the effect of large airway smooth muscle tone, and thus accentuate as much as possible the effects of the distal small airways. It is therefore possible that the associations that were observed in previous studies were at least partly related to the effect of proximal airway tone impacting upon the heterogeneity of distal airway calibre. Indeed, it has previously been observed that pre-bronchodilator physiological measurements correlate more strongly with asthma outcomes than post-bronchodilator measurements\textsuperscript{126}.

An unexpected finding of our study was the striking relationship between the IOS parameter $R_{20}$ and a number of clinically important outcome measures such as asthma control, quality of life and exacerbations. Moreover, in multivariate linear regression analyses, the contribution of $R_{20}$ was additive to that of traditional spirometric outcome measures such as FEV$_1$ and FVC, suggesting that it may represent a distinct facet of asthma disease expression. Although the structural correlates of IOS parameters have not yet been defined, $R_{20}$ is thought to reflect the mean level of airway resistance, including both the large and small airways, whereas $R_{5-R20}$ represents the heterogeneity of airway resistance. Computational models and ex vivo prototypic models of lung impedance based upon realistic airway geometry may shed further light on this area in the near future\textsuperscript{212}. Interestingly, the ACQ was significantly associated with FEV$_1$, whereas the AQLQ associated more with FVC. This suggests that asthma quality of life may be particularly affected by air trapping.
The association observed in this study between absolute lung volumes and asthma exacerbations is intriguing and, to our knowledge, has not been previously reported. In particular, we found that patients with a history of one or more exacerbations within the previous year had significantly lower TLC and VA than those without a history of recent exacerbations. This was contributed to partly by a lower TLC (% pred.) in the exacerbation-prone group, although this did not reach statistical significance, and partly by a greater proportion of females in the exacerbation-prone group, although this also did not reach statistical significance. The reduced DLCO (% pred.) seen in the exacerbation-prone group was driven primarily by the lower absolute lung volumes, since neither Kco (% pred.) nor the VA/TLC ratio differed significantly between the two groups. These observations suggest that a preserved TLC may have a protective effect against exacerbations of asthma, a possibility that merits further investigation. However, a limitation of this analysis is that exacerbations were recorded retrospectively, and it is therefore possible that the physiological associations observed were due to the after-effects of previous exacerbations, rather than being predictive of future exacerbations.

We found that physiological variables explained approximately one fifth of the variance of ACQ-6 and AQLQ(S) scores. The lack of a strong association between physiological variables and patient-reported outcome measures in patients with asthma has been previously documented\textsuperscript{259,260}, although the causes of this are poorly understood. One possible explanation is that patients with asthma become accustomed to a certain level of disease control, thus regarding this as their normal baseline state. Therefore, patient-reported measures of asthma control or quality of life may be related more strongly to changes in physiological variables, rather than to their absolute values. Moreover, it is likely that subjective measures of asthma control are influenced by a large number of factors other than the function of the lower airways, including obesity\textsuperscript{261}, deconditioning\textsuperscript{262}, dysfunctional breathing\textsuperscript{263,264}, vocal cord dysfunction\textsuperscript{264}, and psychological factors\textsuperscript{265}.

Our study was limited by its cross-sectional design, and a number of important questions remain about the role of small airway obstruction in the clinical expression of asthma. Most notably, it is not known whether small airway obstruction affects the long-term outcome of patients with asthma, and longitudinal studies are required to
address this issue. With this limitation in mind, we performed an exploratory analysis on our cross-sectional data to assess which small airway obstruction markers might hold promise as indicators of incipient airflow obstruction in patients with asthma and normal spirometry. We found that for R5-R20 and AX, a significant proportion of patients (15% and 16% respectively) displayed abnormally high values in the presence of normal FEV$_1$, whereas there were fewer patients who had low FEV$_1$ and normal R5-R20 and AX. This suggests that these indices may be sensitive early markers of airflow obstruction, but longitudinal follow-up would be required to determine whether this group of patients is at increased risk of developing spirometric airflow obstruction in the future. Interestingly, the MBW parameter LCI did not appear to be particularly sensitive in detecting asthma patients with airway obstruction, with only 5% of patients having a high LCI and normal FEV$_1$, compared to 20% of patients who had a low FEV$_1$ and normal LCI. These results contrast with observations made in cystic fibrosis (CF) and non-CF bronchiectasis (Section 3.2 of this thesis), in which LCI appeared to be a significantly more sensitive marker of airway obstruction than FEV$_1$. This suggests that ventilation heterogeneity is a relatively subtle phenomenon in patients with asthma, particularly when compared to suppurative lung diseases such as CF and non-CF bronchiectasis. Nevertheless, the alternative MBW parameter S$_{acim}$ was abnormally raised in the presence of a normal FEV$_1$ in 12% of patients with asthma, and therefore merits investigation as an early marker of airway disease alongside the IOS parameters R20, R5-R20 and AX.

In conclusion, we have investigated the clinical correlates of a broad panel of physiological parameters in a well-characterised group of patients with asthma. Measurements that have traditionally been considered to represent small airway obstruction, such as S$_{acim}$, R5-R20 and AX, do not appear to be associated with impaired asthma control or quality of life cross-sectionally, although the long-term significance of these parameters requires further investigation. In contrast, the IOS parameter R20 is strongly and independently associated with adverse outcome. Further studies are required to confirm this novel finding and to investigate its clinical significance.
3.5 Characterisation of acinar airspace involvement in patients with asthma using hyperpolarised $^3$He magnetic resonance and quantitative computed tomography

Abstract

Background
The multiple breath inert gas washout (MBW) parameter $S_{acin}$ is thought to be a specific marker of acinar airway involvement, but has not been validated using quantitative imaging techniques in asthma. We aimed to utilise $^3$He diffusion magnetic resonance ($^3$He-MR) and quantitative computed tomography (CT) densitometry to determine the nature of acinar airway involvement in patients with asthma.

Methods
Thirty-seven patients with asthma and seventeen age-matched healthy controls underwent spirometry, body plethysmography, MBW and $^3$He-MR. A subset of patients with asthma (n = 27) underwent quantitative CT densitometry.

Results
The apparent diffusion coefficient (ADC) at 1s was significantly higher in patients with asthma and a high $S_{acin}$ compared to healthy controls (0.024 vs 0.017, $p < 0.05$), but ADC at 13ms did not differ significantly between the groups. $S_{acin}$ correlated strongly with ADC at 1s ($R = 0.65$, $p < 0.001$), but weakly with ADC at 13ms ($R = 0.38$, $p < 0.05$). ADC at both 13ms and 1s correlated strongly with the mean lung density expiratory / inspiratory ratio, a CT marker of expiratory air trapping ($R = 0.77$, $p < 0.0001$ for ADC at 13ms; $R = 0.72$, $p < 0.001$ for ADC at 1s). CT markers of emphysema did not differ significantly between control and asthma groups.

Conclusion
The MBW parameter $S_{acin}$ is associated with subtle alterations in diffusion within the acinar airways in patients with asthma. The precise nature and clinical significance of the underlying structural abnormality requires further investigation.
**Introduction**

Asthma is a chronic inflammatory airway disease that is characterised by variable airflow obstruction, airway hyperresponsiveness and structural remodelling in both the large and small airways\(^\text{14}\). Inhaled corticosteroids (ICS) are the mainstay of asthma therapy, but optimal treatment requires that the drug is delivered adequately to the site of inflammation within the airway tree. In particular, the deposition of traditional large particle inhalers has often been limited to the larger conducting airways, and there has been increasing interest in targeting the small airways with extra-fine inhaled therapies\(^\text{36}\).

While it is known that inflammatory and structural changes in asthma occur in the smaller conducting airways\(^\text{47-56}\), it is not known whether the lesion extends to the more distal intra-acinar airways. The acinar airways of the lung constitute the majority of the airway surface area and comprise respiratory bronchioles, alveolar ducts and alveoli\(^\text{161}\). Understanding the role and contribution of the acinar airways to asthma is important because currently available inhaled therapies are not designed to provide penetration to this compartment\(^\text{266}\). A number of tools are available to non-invasively probe the structure of the acinar airways in patients with asthma. These include the physiological assessment of gas mixing using multiple breath inert gas washout (MBW)\(^\text{128}\), measurement of gas diffusion using hyperpolarised noble gas magnetic resonance techniques\(^\text{178}\), and computed tomography (CT) densitometry to evaluate expiratory air trapping\(^\text{167}\). However to date there has not been a comprehensive assessment of the acinar airways in asthma using these approaches.

There are thought to be two independent mechanisms of gas mixing inefficiency in the lungs, namely convection-dependent inhomogeneity (CDI) and diffusion-convection-dependent inhomogeneity (DCDI)\(^\text{140,142}\). CDI arises due to unequal convective ventilation between relatively large lung units subtended by conducting airways. DCDI is a more complex mechanism that occurs due to an interaction between convective and diffusive gas flows at the convection-diffusion front, the region of the airway tree at which these flows are of approximately equal magnitude. The MBW parameters \(S_{\text{cond}}\) and \(S_{\text{acin}}\) were proposed by Verbanck *et al.* as measures of CDI and DCDI, respectively\(^\text{131}\). Since in health, the convection-diffusion front is thought to be located...
within the pulmonary acinus, $S_{\text{acin}}$ was proposed as a putative physiological marker of acinar airspace disease. Elevations in $S_{\text{acin}}$ have been observed in patients with asthma, leading to the suggestion that this condition is characterised by a specific structural abnormality in the pulmonary acinus. However, the precise nature of this structural abnormality has not been elucidated.

Hyperpolarised $^3$helium diffusion magnetic resonance ($^3$He-MR) is a technique that allows microstructural changes at the level of alveoli and acinar airways to be examined non-invasively, under resting physiological conditions. The apparent diffusion coefficient (ADC) of $^3$He within the pulmonary acinus may be measured across a wide range of timescales, from 1ms to 10s. Short timescales correspond to diffusion within a single alveolus or alveolar duct, while long timescales correspond to diffusion within the acinar airways, and possibly along collateral ventilation pathways. Air trapping may be assessed using physiological measurements of lung volumes, or with imaging techniques such as quantitative CT densitometry.

We aimed to utilise $^3$He-MR at multiple diffusion timescales and quantitative CT densitometry to determine the structural correlates of the multiple breath washout marker $S_{\text{acin}}$ in asthma. We hypothesised that asthma patients with an elevated $S_{\text{acin}}$ would manifest altered long range diffusion suggestive of intra-acinar airway disease. We also hypothesised that the degree of acinar involvement in asthma would be independent of lung hyperinflation. We sought to test these hypotheses in a cohort of carefully phenotyped adults with asthma.

**Methods**

Thirty-seven patients with asthma and seventeen age-matched healthy control subjects were recruited. All participants were never smokers or ex-smokers with less than 5 pack years’ smoking history. Asthma was diagnosed in a secondary care setting according to British Thoracic Society guidelines. The study was approved by the National Research and Ethics Committee – East Midlands, Leicester, and all participants gave their written informed consent.
Patients with asthma completed the six-point Asthma Control Questionnaire (ACQ)\textsuperscript{250} and the standardised Asthma Quality of Life Questionnaire (AQLQ(S))\textsuperscript{258}. Participants were administered 200 micrograms of salbutamol via a metered-dose inhaler and spacer, to minimise the confounding effects of airway smooth muscle tone on physiological and imaging assessments. Spirometry, body plethysmography and measurement of carbon monoxide diffusing capacity were performed according to American Thoracic Society / European Respiratory Society guidelines\textsuperscript{90,215,217}. Induced sputum inflammatory cell counts were obtained in patients with asthma using a previously published method\textsuperscript{267}. MBW was performed according to current guidelines\textsuperscript{130} using the sulphur hexafluoride (SF\textsubscript{6}) wash-in method\textsuperscript{132}, as described in Section 2.5.

\textsuperscript{3}He-MR was performed using a 0.15 T permanent magnet system (Intermagnetics General Corporation, New York, NY) and a Surrey Medical Imaging Systems console (Surrey, UK). Participants were scanned in the supine position, and inhaled 600ml of a \textsuperscript{3}He/\textsuperscript{4}He mixture from functional residual capacity (FRC), followed by a breath-hold lasting between 2 and 10 seconds, depending upon the pulse sequence being performed. Short-timescale ADC (13ms) was measured using a diffusion-weighted Carr-Purcell-Meiboom-Gill technique\textsuperscript{224}, and long-timescale ADC (1s) was measured using a stimulated echo sequence\textsuperscript{225}. The first seven patients with asthma and the first two healthy controls to enter the study took part in a pilot phase in which only short timescale ADC measurements were made.

The effect of lung volume changes on short-timescale ADC have been previously reported, with a strong positive correlation observed between the degree of lung inflation and short-timescale ADC\textsuperscript{196}. In order to aid the interpretation of our results, we also investigated the relationship between lung volume and long-timescale ADC, in three healthy control subjects and three patients with asthma. Long-timescale ADC measurements were performed at specified lung volumes above either residual volume (RV) or FRC. The absolute values of RV and FRC were determined using body plethysmography.

A subset of patients with asthma (n = 27) were further characterised using quantitative computed tomography (CT) densitometry, as described in Section 2.8. Scans were
obtained at full inspiration and full expiration. VIDA Apollo image analysis software (VIDA Diagnostics, Coralville, Iowa) was used for quantitative analysis of lung densitometry. The main parameters extracted were the ratio of mean lung density on expiration to inspiration (MLD E/I), a marker of expiratory air trapping\textsuperscript{171}, and the fifteenth lower percentile of the inspiratory lung attenuation curve (P\textsubscript{15}), a marker of emphysema\textsuperscript{222}.

Statistical analyses were performed using SPSS 20 (IBM Corporation, Somers, New York, USA) and Prism 6 (GraphPad Software Inc., La Jolla, California, USA). Group comparisons were performed using the Student’s \( t \) test, one-way analysis of variance with Tukey test for multiple comparisons, or the Mann-Whitney \( U \) test for continuous variables, and Fisher’s exact test or the Chi-squared test for proportions. Relationships between continuous variables were investigated using Pearson’s correlation coefficient. Previous data on the group standard deviation of ADC at 1s was not available for use in a sample size calculation. However, Wang \textit{et al}\textsuperscript{184} reported a 0.0051 cm\(^2\)s\(^{-1}\) difference in mean ADC at 1.5s between healthy and asthma groups, with a group standard deviation of 0.0026 cm\(^2\)s\(^{-1}\) in the healthy group and 0.0055 cm\(^2\)s\(^{-1}\) in the asthma group, using similar methodology to our own. We calculated that to detect this difference between healthy and asthma groups at 90% power, using a \( t \) test with a 5% significance level, we would require 15 patients in each group.

\section*{Results}

\textbf{Asthma patient-reported and clinical outcomes in patients with an elevated S\textsubscript{acin}}

Table 3.1 shows the demographic and clinical characteristics of the participant groups. Patients with asthma were divided into S\textsubscript{acin}-normal and S\textsubscript{acin}-high groups, with the upper limit of normal for S\textsubscript{acin} being defined as the mean + 1.64 standard deviations in the age-matched control group (0.204 L\(^{-1}\)). The three groups were well-matched for age and sex. The S\textsubscript{acin}-high group had evidence of suboptimal asthma control, quality of life, and greater treatment utilisation compared to patients with a normal S\textsubscript{acin}. These observations were present despite similar levels of eosinophilic airway inflammation in both groups.
Physiological phenotyping of asthmatics with an elevated S<sub>ac</sub>

Table 3.1 shows physiological parameters in the participant groups. The S<sub>ac</sub>-high group exhibited significantly worse expiratory flow limitation and expiratory air trapping than the S<sub>ac</sub>-normal group. FEV<sub>1</sub> (% pred.) was significantly lower in the S<sub>ac</sub>-high group compared to the S<sub>ac</sub>-normal group (69.3 vs 90.9, p < 0.01), and the ratio of residual volume to total lung capacity (RV/TLC) was significantly higher (48.3% vs 38.2%, p < 0.01), as was the FRC (% pred.) (131.5% vs 103.7%, p < 0.01). Carbon monoxide transfer coefficient (K<sub>CO</sub>) did not differ significantly between the groups.

Imaging-based phenotyping of asthmatics with an elevated S<sub>ac</sub>

Figure 3.10 shows the CT densitometry data in the two asthma groups. There was evidence of expiratory air trapping in the S<sub>ac</sub>-high group, with a significantly raised MLD E/I compared to the S<sub>ac</sub>-normal group (0.89 vs 0.83, p < 0.05). However, the inspiratory P<sub>15</sub> did not differ between the groups, suggesting that a raised S<sub>ac</sub> is not associated with emphysema in patients with asthma. Figure 3.11 shows the short and long timescale ADC measurements across the three groups. ADC at 1s was significantly higher in the S<sub>ac</sub>-high group compared to the healthy control group (0.024 vs 0.017, p < 0.05), with a trend towards a significant difference between the S<sub>ac</sub>-high and S<sub>ac</sub>-normal asthma groups (0.024 vs 0.019, p = 0.09).

Evaluation of the contribution of lung volume to apparent diffusion coefficients

Figure 3.12 shows correlations between ADCs and S<sub>ac</sub> (Panels A and B), FRC (% pred.) (Panels C and D) and MLD E/I (Panels E and F) in patients with asthma. S<sub>ac</sub> correlated weakly with ADC at 13ms (R = 0.38, p < 0.05), but strongly with ADC at 1s (R = 0.65, p < 0.001). ADC at both 13ms and 1s correlated strongly with the functional residual capacity percent predicted (R = 0.73, p < 0.0001 for ADC at 13ms; R = 0.68, p < 0.0001 for ADC at 1s) and with the mean lung density expiratory / inspiratory ratio, a CT marker of expiratory air trapping (R = 0.77, p < 0.0001 for ADC at 13ms; R = 0.72, p < 0.0001 for ADC at 1s). However, in healthy subjects there were no significant correlations between ADC at 13ms / 1s and either S<sub>ac</sub> or FRC (% pred.).

Figure 3.13 shows the relationship between lung inflation and ADC at 1s in three healthy volunteers (Panel A) and three patients with asthma (Panel B). The correlation
was positive but weak in both cases, only reaching statistical significance in the patients with asthma \( (p < 0.05) \). The slope of the lines was shallow, with a 50% increase in lung inflation resulting in a 3.7% increase in ADC in healthy volunteers, and a 4.5% increase in patients with asthma.
Table 3.14: Demographic and clinical characteristics of participant groups in magnetic resonance study

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 17)</th>
<th>Asthma $S_{acin}$ normal (n = 20)</th>
<th>Asthma $S_{acin}$ high (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.4 (3.3)</td>
<td>54.2 (3.1)</td>
<td>61.2 (1.9)</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>47</td>
<td>40</td>
<td>65</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.6 (2.6)</td>
<td>164.8 (2.5)</td>
<td>169.7 (1.9)</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>75.0 (2.7)</td>
<td>78.1 (3.3)</td>
<td>90.4 (5.0) *</td>
</tr>
<tr>
<td>Body mass index (kg/m^2)*</td>
<td>25.8 (0.8)</td>
<td>28.9 (1.3)</td>
<td>31.2 (1.4) **</td>
</tr>
<tr>
<td>Age of onset of asthma symptoms (years)</td>
<td>-</td>
<td>23.4 (5.0)</td>
<td>27.5 (5.3)</td>
</tr>
<tr>
<td>Duration of asthma (years)</td>
<td>-</td>
<td>30.9 (3.8)</td>
<td>33.7 (5.1)</td>
</tr>
<tr>
<td>Atopic status (% positive)</td>
<td>-</td>
<td>85</td>
<td>82</td>
</tr>
<tr>
<td>ACQ-6 score*</td>
<td>-</td>
<td>1.43 (0.26)</td>
<td>2.14 (0.22)</td>
</tr>
<tr>
<td>AQLQ(S) score¥</td>
<td>-</td>
<td>5.61 (0.23)</td>
<td>4.95 (0.31)</td>
</tr>
<tr>
<td>Sputum neutrophil count (%)</td>
<td>-</td>
<td>57.2 (6.0)</td>
<td>61.8 (7.1)</td>
</tr>
<tr>
<td>Sputum eosinophil count (%)*</td>
<td>-</td>
<td>2.69 (1.23–5.89)</td>
<td>1.76 (0.76–4.04)</td>
</tr>
<tr>
<td>Blood eosinophil count ($\times 10^9$/L)</td>
<td>-</td>
<td>0.33 (0.04)</td>
<td>0.34 (0.07)</td>
</tr>
<tr>
<td>Daily dose of inhaled corticosteroid (beclometasone dipropionate equivalent [μg]) Median Range</td>
<td>-</td>
<td>1000 0–2000</td>
<td>1600 200–2000</td>
</tr>
<tr>
<td>Use of long-acting beta-agonists (% of subjects)</td>
<td>-</td>
<td>75</td>
<td>94</td>
</tr>
<tr>
<td>Regular use of oral prednisolone (% of subjects)</td>
<td>-</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>Use of leukotriene receptor antagonist (% of subjects)</td>
<td>-</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>Use of a methylxanthine (% of subjects)¥</td>
<td>-</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td>Asthma treatment step‡,¥</td>
<td>-</td>
<td>1 : 6 : 9 : 4</td>
<td>1 : 0 : 9 : 7</td>
</tr>
<tr>
<td>Refractory asthma (% positive)¶,¥</td>
<td>-</td>
<td>45</td>
<td>76</td>
</tr>
</tbody>
</table>
Legend for Table 3.14

ACQ-6 = six-point Asthma Control Questionnaire; AQLQ(S) = standardised Asthma Quality of Life Questionnaire.

† Expressed as geometric mean (95% confidence interval). Log-transformed data compared between groups using Student’s t test.

‡ As defined by the Global Initiative for Asthma2. Expressed as number of patients receiving treatment at step 2: step 3: step 4: step 5.

¶ Refractory asthma defined according to the American Thoracic Society Workshop definition268.

Data expressed as mean (standard error) or proportions, unless stated otherwise. Groups compared using one-way analysis of variance with Tukey test for multiple comparisons or Student’s t test for parametric data, Mann-Whitney U test for non-parametric data, and Chi-squared test or Fisher’s exact test for proportions. Significant differences across or between groups denoted *(p < 0.05) with trends towards significance denoted †*(p < 0.1). Significant differences compared to healthy control group denoted #*(p < 0.05) or ##*(p < 0.01).
Table 3.15: Physiological data across participant groups in magnetic resonance study

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 17)</th>
<th>Asthma S\textsubscript{acin} normal (n = 20)</th>
<th>Asthma S\textsubscript{acin} high (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV\textsubscript{1} (% pred.)****</td>
<td>104.5 (3.5)</td>
<td>90.9 (4.3)</td>
<td>69.3 (5.0) #¥#¥¥</td>
</tr>
<tr>
<td>FVC (% pred.)**</td>
<td>119.9 (3.8)</td>
<td>106.0 (3.5) #</td>
<td>100.1 (3.5) **#</td>
</tr>
<tr>
<td>FEV\textsubscript{1}/FVC (%)****</td>
<td>72.1 (1.7)</td>
<td>70.6 (2.5)</td>
<td>55.6 (3.3) #¥¥¥</td>
</tr>
<tr>
<td>FRC (L)**</td>
<td>3.67 (0.26)</td>
<td>3.08 (0.23)</td>
<td>4.28 (0.26) #¥</td>
</tr>
<tr>
<td>FRC (% pred.)*</td>
<td>114.4 (6.2)</td>
<td>103.7 (6.7)</td>
<td>131.5 (6.2) #¥</td>
</tr>
<tr>
<td>TLC (L)</td>
<td>6.92 (0.48)</td>
<td>5.70 (0.34)</td>
<td>6.77 (0.39)</td>
</tr>
<tr>
<td>TLC (% pred.)</td>
<td>109.8 (3.7)</td>
<td>103.3 (3.8)</td>
<td>109.5 (3.4)</td>
</tr>
<tr>
<td>RV/TLC (%)****</td>
<td>31.9 (2.2)</td>
<td>38.2 (1.9)</td>
<td>48.3 (2.3) #¥¥¥</td>
</tr>
<tr>
<td>Va/TLC (%)****</td>
<td>88.2 (1.8)</td>
<td>82.0 (1.9)</td>
<td>74.3 (1.9) #¥</td>
</tr>
<tr>
<td>KCO (mmol\textperiodcentered min\textsuperscript{-1}\textperiodcentered kPa\textsuperscript{-1}\textperiodcentered L\textsuperscript{-1})</td>
<td>1.55 (0.06)</td>
<td>1.66 (0.06)</td>
<td>1.58 (0.07)</td>
</tr>
<tr>
<td>LCI****</td>
<td>7.34 (0.26)</td>
<td>7.43 (0.25)</td>
<td>9.59 (0.31) #¥¥¥¥</td>
</tr>
<tr>
<td>LCI\textsubscript{vent}****</td>
<td>1.20 (0.02)</td>
<td>1.22 (0.03)</td>
<td>1.41 (0.03) #¥¥¥¥</td>
</tr>
<tr>
<td>LCI\textsubscript{at}****</td>
<td>1.14 (0.01)</td>
<td>1.14 (0.01)</td>
<td>1.25 (0.01) #¥¥¥¥</td>
</tr>
<tr>
<td>S\textsubscript{cond} (L\textsuperscript{-1})</td>
<td>0.029 (0.004)</td>
<td>0.054 (0.015)</td>
<td>0.068 (0.012)</td>
</tr>
<tr>
<td>S\textsubscript{acin} (L\textsuperscript{-1})****</td>
<td>0.120 (0.012)</td>
<td>0.115 (0.011)</td>
<td>0.319 (0.026) #¥¥¥¥¥</td>
</tr>
</tbody>
</table>
Legend for Table 3.15

FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; FRC = functional residual capacity from body plethysmography; TLC = total lung capacity; RV = residual volume; VA = alveolar volume from single breath helium dilution; KCO = carbon monoxide transfer coefficient; LCI = lung clearance index; LCI_{vent} = specific ventilation inequality component of lung clearance index; LCI_{ds} = dead space component of lung clearance index.

Data expressed as mean (standard error). Groups compared using one-way analysis of variance with Tukey test for multiple comparisons or Student’s t test. Significant differences across groups denoted *(p < 0.05), ***(p < 0.001) or ****(p < 0.0001). Significant differences compared to healthy control group denoted *(p < 0.05), ***(p < 0.001) or *****(p < 0.0001). Significant differences between asthma S_{acin}-low and S_{acin}-high groups denoted ¥(p < 0.05), ¥¥(p < 0.01), ¥¥¥(p < 0.001) or ¥¥¥¥(p < 0.0001).
Figure 3.10: Quantitative computed tomography densitometry between groups

*Error bars indicate means ± standard errors of the mean.*
Figure 3.11: Apparent diffusion coefficients (ADC) across groups

*Error bars indicate means ± standard errors of the mean.*

A

B

$p < 0.05$
Figure 3.12: Correlations between $^3$He-MR, CT and physiological variables in patients with asthma

Best-fit linear regression lines and Pearson correlation coefficients are shown.
Figure 3.13: Change in apparent diffusion coefficient (%) against change in volume of gas in the lungs (%) in healthy subjects and patients with asthma

Correlations are shown between percentage change in ADC and percentage change in volume of gas in the lungs in three healthy subjects (Panel A) and three patients with asthma (Panel B). The three participants in each case are denoted with different symbols. \( V \) = volume of gas in lungs; \( V_{FRC} \) = volume of gas in lungs at functional residual capacity; \( ADC_V \) = ADC at lung volume \( V \); \( ADC_{FRC} \) = ADC at functional residual capacity (extrapolated).

Panel A

![Graph A](image)

Panel B

![Graph B](image)
Discussion

The main finding of this study is that in patients with asthma, the MBW parameter $S_{\text{acin}}$ is strongly associated with elevations in long-timescale ADC. However, this association is not observed in healthy subjects. Moreover, elevations in long timescale ADC cannot be reproduced purely by lung inflation, suggesting that such elevations result from a specific structural abnormality in the pulmonary acinus in patients with asthma.

A number of previous studies have investigated the clinical significance of the acinar lesion in asthma. Farah et al found that improvements in $S_{\text{acin}}$ were independently associated with improvements in five-point ACQ score following the initiation of ICS treatment\(^{157}\), and that markers of ventilation heterogeneity could predict the response to inhaled corticosteroid dose titration\(^{159}\). Thompson et al found that $S_{\text{acin}}$ correlated with asthma severity, as measured using the Global Initiative for Asthma treatment steps, and that asthma exacerbations were associated with increases in $S_{\text{acin}}$\(^{158}\). However, in the above studies MBW was performed using nitrogen as the inert tracer gas, which may probe less distal structures than SF\(_6\)\(^{130}\), and additionally MBW was performed without the administration of a bronchodilator, so that the influence of large airway bronchial tone was not eliminated. Indeed it has been shown that bronchodilator administration results in complete or partial normalisation of a high baseline $S_{\text{acin}}$ in both children and adults with asthma\(^{150,152,153}\), suggesting that large airway bronchodilator tone may impact upon measurements of distal airway function. The study reported in Section 3.4 of this thesis found that $S_{\text{acin}}$, performed post-bronchodilator using the SF\(_6\) wash-in method, was not independently associated with ACQ scores, AQLQ scores or asthma exacerbations, suggesting that a raised $S_{\text{acin}}$ in asthma may be a marker of pre-symptomatic early remodelling in the distal airways. Longitudinal studies are required to assess whether this abnormality progresses to fixed airflow obstruction in asthma, as may be the case in other obstructive airway diseases such as cystic fibrosis\(^{132}\) and bronchiolitis obliterans post-allogeneic haematopoietic stem cell transplant\(^{269}\).

There is some evidence that treating the small airways with extra-fine inhaled therapies can improve asthma control. Barnes et al found in a retrospective cohort study that asthma control was more likely to be achieved in the year following the initiation or
step-up of an extra-fine ICS compared to a large particle formulation\textsuperscript{270}. Verbanck \textit{et al} found that $S_{\text{acin}}$ improved following the replacement of standard corticosteroid inhalers with an extra-fine inhaler, in those patients who had a raised $S_{\text{acin}}$ at baseline\textsuperscript{160}. However, while it is known that extra-fine ICS provide improved penetration into the smaller conducting airways\textsuperscript{271-275}, it is not clear whether or not this extends into the pulmonary acinus. In particular, there are few studies that have specifically measured drug deposition within this compartment, and theoretical models suggest that very small particles ($< 0.5 \ \mu m$ in diameter) may simply remain suspended in the air and be exhaled without depositing in the airways\textsuperscript{266}.

The acinar airways form an asymmetrically dichotomous branching network in three-dimensional space that may be described in terms of its mean airway radius, branch length and branch angle. Short timescale ADC is mainly sensitive to changes in acinar airway radius, since an increase in this parameter reduces the restriction to transverse displacement of helium. Long timescale ADC is a measure of the network properties of the acinar airways, with higher values being associated with greater interconnectedness and reduced tortuosity of the acinus. Long timescale ADC is relatively insensitive to acinar airway radius, since at long diffusion times transverse displacement is negligible compared to longitudinal displacement along the airway axis. An increase in branch length would theoretically cause elevation of long-timescale ADC because a given helium atom would then encounter less branch points, each of which entails a change in direction in three-dimensional space, and would thus travel a greater distance in any given direction. Long timescale ADC may also be affected by the width of the alveolar sleeve surrounding the acinar airways, as well as the size of the alveolar mouth opening. Verbanck and Paiva simulated axial diffusion within an alveolar duct model, with the two main parameters of their model being (i) the ratio of luminal diameter to total diameter ($s/S$), where the total diameter includes the alveolar sleeve surrounding the duct, and (ii) the ratio of the alveolar mouth opening diameter to the mean alveolar width in the axial direction (AM/AW)\textsuperscript{276}. The authors found that a low $s/S$ ratio (ie. a relatively large alveolar sleeve width) was associated with reduced axial diffusion, due to the retarding effect of radial diffusion into the alveolar sleeve. The AM/AW ratio had a far more modest effect on axial diffusion, with a low ratio (ie. relatively narrow alveolar mouth openings) associated with a small reduction in axial diffusion. This may be explained by the fact that narrow alveolar mouth openings result in gas molecules
becoming trapped in the alveoli, thus retarding axial diffusion. However, narrow alveolar mouth openings also result in less gas molecules entering the alveoli in the first place, and it is likely that these two opposing effects accounted for the relatively modest dependence of axial diffusion on the AM/AW ratio. A further factor that may influence long timescale ADC is the presence of collateral channels. Simulations of long timescale ADC within an anatomically realistic asymmetrically dichotomous model of the acinus yielded values that were of the same order as those observed experimentally in healthy subjects. The addition of intra-acinar collateral channels to the model produced significantly increased values of simulated long timescale ADC.

An important question to address is whether the correlation between $S_{\text{acina}}$ and long timescale ADC represents a true structural change in the pulmonary acinus, or whether the relationship is driven by the presence of expiratory air trapping and hyperinflation in patients with raised $S_{\text{acina}}$. Hajari *et al* utilised $^3$He MR lung morphometry to assess the changes that occur in the acinar airways during lung inflation in healthy subjects. They concluded that lung inflation occurs primarily by alveolar recruitment, and to a lesser extent by the expansion of alveolar ducts. The alveolar sleeve width actually decreased with increasing lung inflation. The expansion of alveolar ducts would be expected to increase short timescale ADC, and indeed it is known that short timescale ADC has a strong linear relationship with lung inflation in healthy subjects. It might be expected that a reduction in alveolar sleeve width with increasing lung inflation would result in an increase in long timescale ADC, on the basis of the alveolar duct model alluded to above. However, we observed only minor effects of lung inflation on long timescale ADC, suggesting that hyperinflation alone cannot account for the strong association between $S_{\text{acina}}$ and long timescale ADC. Nevertheless, ADC did appear to fall somewhat when measurements were taken near residual volume, suggesting that airway closure could result in more restricted diffusion.

We observed strong correlations between the CT marker of expiratory air trapping MLD E/I and both short and long-timescale ADC, suggesting that there may be common structural abnormalities at the level of the acinar airways that result in both expiratory air trapping and altered diffusion in the distal airspaces. A possible method of elucidating these abnormalities in future studies may be micro-CT of surgical lung biopsies or resected lung specimens, as has been performed in patients with COPD.
We found no evidence of emphysema in patients with asthma and a raised $S_{\text{acin}}$, with neither $P_{15}$ nor $K_{CO}$ differing between the $S_{\text{acin}}$-normal and $S_{\text{acin}}$-high groups. Previous studies have also found no evidence of histological emphysema in patients with asthma, although a subtle loss of alveolar-parenchymal attachments has been observed, which could theoretically lead to loss of lung elastic recoil and dynamic airway collapse\textsuperscript{17}.

We conclude that the MBW parameter $S_{\text{acin}}$ appears to be associated with a structural abnormality in the pulmonary acinus in patients with asthma, causing subtle alterations in diffusion within the acinar airways. However, a number of questions remain to be answered. In particular, it is not known whether currently available small-particle inhalers provide significant deposition into the acinar airways, or what the benefits if any of acinar drug deposition would be. Longitudinal studies are required to determine whether acinar airway disease is a precursor to the development of fixed airflow obstruction in patients with asthma, and whether treatment with extra-fine ICS or systemic therapies could prevent this adverse outcome.
3.6 Randomised controlled trial of the prostaglandin D2 receptor antagonist QAW039 in persistent eosinophilic asthma

Abstract

Background
Asthma is a chronic inflammatory airway disease that imposes a substantial burden of morbidity and healthcare costs worldwide. There is evidence that eosinophils are a primary driver of asthma, and that control of eosinophilic airway inflammation reduces the frequency of asthma exacerbations.

Methods
We performed a single-centre, randomised, double-blind, placebo-controlled, parallel-group clinical trial of the prostaglandin D2 receptor antagonist QAW039 in 61 subjects with persistent eosinophilic asthma. The treatment phase lasted for three months, during which subjects received either QAW039 225mg twice per day orally, or placebo. The primary outcome was the change in sputum eosinophil percentage from baseline to post-treatment. Secondary and exploratory outcomes included changes in Asthma Control Questionnaire score (ACQ), Standardised Asthma Quality of Life Score (AQLQ(S)) and forced expiratory volume in one second (FEV₁).

Results
QAW039 was associated with a 5.2-fold reduction in geometric mean sputum eosinophil percentage, from 4.88 at baseline to 0.91 post-treatment, versus a 1.3-fold reduction in the placebo group (p = 0.005). Mean AQLQ(S) scores fell by 0.17 in the placebo group and increased by 0.27 in the QAW039 group (p < 0.05), but changes in ACQ score did not differ between the groups. Mean post-bronchodilator FEV₁ fell by 100 ml in the placebo group and increased by 110 ml in the QAW039 group (p < 0.05). QAW039 displayed a favourable side-effect profile, with no serious adverse events reported.

Conclusions
QAW039 is effective at attenuating eosinophilic airway inflammation in patients with persistent eosinophilic asthma, and has a favourable safety profile. There is evidence that QAW039 improves lung function and asthma-related quality of life.
Introduction

Asthma is a chronic inflammatory airway disease that is characterised by heterogeneity with respect to clinical phenotype and response to therapy. Eosinophilic airway inflammation, mediated by the T helper 2 (Th2) axis, is a feature of the two most commonly recognised phenotypes of asthma, namely early-onset atopic and late-onset non-atopic asthma. Treatment strategies that specifically target eosinophilic airway inflammation have been shown to reduce exacerbations of asthma. Moreover, treatments such as anti-IL-5 and anti-IL-13 that modify the Th2 axis are particularly effective in patients with uncontrolled eosinophilic airway inflammation, but may be less effective in an unselected population.

There is increasing evidence that the actions of prostaglandin D2 (PGD2) upon the chemoattractant receptor homologous molecule expressed on Th2 cells (CRTH2) may play an important role in mediating eosinophilic airway inflammation in asthma and allergic diseases. The CRTH2 receptor mediates the migration of Th2 cells, delays their apoptosis and stimulates them to produce the cytokines IL-4, IL-5 and IL-13. Furthermore, CRTH2 is also expressed by eosinophils, and directly mediates their chemotaxis and degranulation. CRTH2 is therefore considered to be a highly promising novel drug target in the treatment of asthma, and a number of small molecule antagonists to this receptor are currently in clinical development. QAW039 is an orally active highly selective and potent antagonist of PGD2 that binds to the CRTH2 receptor, but not to the more general homeostatic PGD2 receptor DP1. QAW039 would therefore be expected to bind to CRTH2 receptors on Th2 cells and eosinophils, and to inhibit their migration into airway tissues.

We tested the hypothesis that, in patients with a sputum eosinophilia (≥ 2%) and persistent, moderate-to-severe asthma, 12-weeks treatment with QAW039 at a dose of 225mg twice per day (compared to placebo), on top of conventional treatment, reduces the levels of eosinophils in induced sputum. Secondary objectives were to determine the effects of QAW039 on asthma symptoms, as measured by the Asthma Control Questionnaire (ACQ), and to assess safety and tolerability of QAW039 as compared to placebo. Exploratory objectives included assessment of the effect of QAW039 (compared to placebo) on the forced expiratory volume in one second (FEV1) and...
health-related quality of life as measured by the standardised Asthma Quality of Life Questionnaire (AQLQ(S)). A number of novel physiological and imaging outcome measures were incorporated into the study design in order to provide additional mechanistic information, and to assess their responsiveness in the context of an intervention.

**Methods**

**Subjects**
Participants were older than 18 years of age and had a clinical diagnosis of asthma that was supported by one or more of the following criteria: an increase in FEV<sub>1</sub> of ≥ 12% and ≥ 200ml from its pre-bronchodilator value following the inhalation of 400μg salbutamol, a provoked fall in FEV<sub>1</sub> of 20% by methacholine at ≤ 16mg/ml while on inhaled corticosteroids (ICS), or a change in FEV<sub>1</sub> of > 12% over two measurements during the previous year. Participants were recruited from a refractory asthma clinic providing tertiary care for a mixed urban and rural population of 4 million people, as well as from secondary care asthma and general respiratory clinics in the region. Suitable participants were also identified through the screening of local primary care databases. Inclusion criteria were current treatment with ICS, a sputum eosinophil count of ≥ 2% at screening, and either an ACQ score ≥ 1.5 at randomisation or ≥ 1 exacerbations (requiring higher than the patient’s normal dose of systemic corticosteroids for ≥ 3 days) in the past 12 months. Exclusion criteria included serious coexisting illness, pregnancy or lactation, the possibility of conception, a history of malignancy within the previous five years, recent (within 6 weeks of screening) lower respiratory tract infection or exacerbation of asthma requiring > 10mg of oral prednisolone per day, the use of omalizumab within 6 months before randomisation into the study, and the use of immunosuppressive medication (except low-dose [≤ 10mg prednisolone per day] oral corticosteroids) within 30 days before randomisation. All subjects provided written informed consent. The study protocol was approved by the National Research Ethics Committee (East Midlands) and the United Kingdom Medicines and Healthcare Products Regulatory Agency.
Figure 3.14: QAW039 study protocol

Screening: 1 week
Run-in: 2 weeks
Treatment: 12 weeks
Wash-out: 6 weeks

QAW039 225 mg orally twice daily

Placebo

Visit 1  Visit 2  Visit 3  Visit 4  Visit 5  Visit 6
Design of the study
The study was a single-centre, randomised, double-blind, placebo-controlled, parallel-group clinical trial conducted from February 2012 through June 2013. The protocol of the study is summarised in Figure 3.1

At a screening visit (Visit 1, Day -21), demographic and clinical details were collected, and inclusion and exclusion criteria were reviewed. An induced sputum sample was collected to assess eligibility based upon a sputum eosinophil count of $\geq 2\%$. Regular treatment was kept constant from this time until the end of the study. One week later, a two-week placebo run-in period was commenced (Visit 2, Day -14). Following this, patients attended a baseline visit (Visit 3, Day 0), at which they completed the ACQ, and eligibility based upon the inclusion and exclusion criteria was again assessed, taking into account the ACQ score. If patients fulfilled the criteria, they proceeded to undertake the remainder of the study visit tests, and were then randomised in a 50:50 ratio to receive either QAW039 at a dose of 225mg twice per day, or an identical placebo. Randomisation was performed by the trial pharmacist using previously generated treatment allocation cards, and was stratified by whether or not participants were receiving treatment with regular oral corticosteroids. Patients completed the ACQ and AQLQ(S). The fractional exhaled nitric oxide at 50ml/s ($\text{FeNO}_{50}$) was measured using a NIOX MINO device (Aerocrine AB, Solna, Sweden). Patients undertook impulse oscillometry using the Jaeger MasterScreen Impulse Oscillometry System (Viasys Healthcare GmbH, Hoechberg, Germany). Multiple breath inert gas washout was performed using the sulphur hexafluoride wash-in method$^{132}$, followed by body plethysmography, measurement of carbon monoxide diffusing capacity and pre-bronchodilator spirometry. An induced sputum sample was then collected. Salbutamol (400μg via a metered-dose inhaler and spacer) was administered, followed by the measurement of post-bronchodilator spirometry. A blood sample was drawn for the measurement of blood eosinophil count. Inspiratory and expiratory computed tomography (CT) was then performed. Six weeks following randomisation, patients attended a mid-treatment visit (Visit 4, Day 42), at which they completed the ACQ and AQLQ(S) questionnaires, pre- and post-bronchodilator spirometry was performed, an induced sputum sample was obtained, and a blood sample was drawn for the measurement of blood eosinophil count. Twelve weeks following randomisation, patients attended an end-of-treatment visit (Visit 5, Day 84), which incorporated the
same assessment schedule as Visit 3. Patients then began a six-week placebo washout period, in which all participants received placebo. Following this, patients attended an end-of-study visit (Visit 6, Day 126) and undertook the same assessments as at Visits 3 and 5, except that CT scans were not performed.

Criteria for withdrawal from the study were defined *a priori*, and included withdrawal of informed consent, asthma exacerbation, pregnancy and adverse events for which continued exposure to the study drug would be detrimental. Safety was assessed at each study visit on the basis of history and physical examination, vital signs, haematology, blood chemistry, urinalysis and an electrocardiogram. Because of the expected anti-eosinophilic effects of QAW039, results of sputum and blood eosinophil counts obtained during Visits 5 and 6 were not disclosed to the investigators during the study.

**Statistical analysis**

The primary outcome of the study was the change in sputum eosinophil percentage between the baseline visit (Visit 3) and the post-treatment visit (Visit 5). As sputum eosinophil percentage is known to follow a log-normal distribution, the analysis was based on a log_{10}-transformed scale. Secondary outcomes included the change from baseline to post-treatment with respect to ACQ score. Exploratory outcomes included the change from baseline to post-treatment with respect to AQLQ(S) score and FEV_1.

All participants who were randomised were included in the intention-to-treat population, and missing data due to withdrawals or otherwise were imputed using the last observation carried forward. Participants who completed the study up to the post-treatment visit (Visit 5) without major protocol deviations were included in the per-protocol population. The study was aimed to power for a 50% reduction in sputum eosinophil percentage, equivalent to an absolute reduction in log_{10} sputum eosinophil percentage of 0.301^{286}. In order to detect this difference between groups with 80% power, we required 21 patients in each group. With 30 patients per arm to be randomised, we expected 24 patients to complete the post-treatment assessments, assuming a 20% dropout rate during the course of the treatment phase. Statistical analyses were performed using SPSS 20 (IBM Corporation, Somers, New York, USA) and Prism 6 (GraphPad Software Inc., La Jolla, California, USA). Between-group comparisons at baseline were performed using unpaired t-tests for parametric data, the Mann-Whitney U test for non-parametric data and Fisher’s exact test for proportions.
Between- and within-group comparisons of the change from baseline to post-treatment with respect to primary, secondary and exploratory outcomes were performed using unpaired and paired t-tests respectively.

**Results**

**Enrolment and baseline characteristics**

Figure 3.15 shows the numbers of subjects who attended a screening visit, were randomly assigned to a study group, and who completed the study up to the post-treatment visit. A total of 117 patients attended a screening visit, of which 61 fulfilled the inclusion and exclusion criteria and were randomised. Thirty-two patients were assigned to receive placebo and 29 to receive QAW039. Three patients withdrew in the placebo group and three patients in the QAW039 group. In each case, the reason for withdrawal was an exacerbation of asthma. One patient was assigned to QAW039 but was incorrectly dispensed placebo at the mid-treatment visit. This patient was excluded from the per-protocol analysis but included in the intention-to-treat analysis. The groups were well-matched for baseline characteristics (Table 3.16), with the only statistically significant difference between the groups being a higher proportion of patients using a methylxanthine in the QAW039 group.
Figure 3.15: Number of patients who were screened, randomised and completed the study up to the post-treatment visit

- Screened for eligibility: 117
- Did not meet selection criteria: 52 (Run-in: 65, Did not meet selection criteria: 4)
- Randomised: 61
- Assigned to QAW039: 29 (4 withdrew during treatment - 3 asthma exacerbations, - 1 protocol violation)
- Assigned to placebo: 32 (3 withdrew during treatment due to asthma exacerbations)
- Intention-to-treat population: 29
  - Per-protocol population: 25
- Intention-to-treat population: 32
  - Per-protocol population: 29
### Table 3.16a Baseline Characteristics of Subjects in the Intention-to-Treat Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>QAW039 (n = 29)</th>
<th>Placebo (n = 32)</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (no. of subjects)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18</td>
<td>13</td>
<td>0.13</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>50</td>
<td>50</td>
<td>0.98</td>
</tr>
<tr>
<td>Range</td>
<td>20 – 80</td>
<td>19 – 69</td>
<td></td>
</tr>
<tr>
<td>Age at onset of symptoms (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>18</td>
<td>21</td>
<td>0.46</td>
</tr>
<tr>
<td>Range</td>
<td>2 – 46</td>
<td>2 – 57</td>
<td></td>
</tr>
<tr>
<td>Body-mass index (kg/m²)</td>
<td>31.1 ± 5.9</td>
<td>29.5 ± 5.9</td>
<td>0.30</td>
</tr>
<tr>
<td>Positive atopic status (% of subjects)‡</td>
<td>93</td>
<td>88</td>
<td>0.67</td>
</tr>
<tr>
<td>Total IgE (U/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>167.3</td>
<td>163.4</td>
<td>0.88</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>81.9 – 450.7</td>
<td>78.9 – 489.1</td>
<td></td>
</tr>
<tr>
<td>Presence of nasal polyps (% of subjects)</td>
<td>14</td>
<td>16</td>
<td>1.00</td>
</tr>
<tr>
<td>Severe exacerbations per subject in previous year (no.)</td>
<td>2</td>
<td>2</td>
<td>0.74</td>
</tr>
<tr>
<td>Previous admission to the intensive care unit for asthma (% of subjects)</td>
<td>21</td>
<td>16</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Plus-minus values are means ± standard deviation (SD) unless otherwise stated.

† P values were calculated with the use of a two-sided unpaired t-test for parametrically distributed variables, Fisher’s exact test for comparison of proportions, and the Mann-Whitney U test for comparison of non-parametric variables.

‡ Positive atopic status was defined as a positive skin test or radioallergosorbent test for any of a panel of specified aeroallergens.
Table 3.16b Baseline Characteristics of Subjects in the Intention-to-Treat Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>QAW039 (n = 29)</th>
<th>Placebo (n = 32)</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁ after bronchodilator use (% of predicted value)</td>
<td>79.6 ± 24.3</td>
<td>86.4 ± 26.1</td>
<td>0.30</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>66.1 ± 14.7</td>
<td>69.3 ± 11.5</td>
<td>0.35</td>
</tr>
<tr>
<td>Improvement in FEV₁ after bronchodilator use (%)</td>
<td></td>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td>Median</td>
<td>8.9</td>
<td>11.4</td>
<td></td>
</tr>
<tr>
<td>Interquartile range</td>
<td>5.2 – 14.0</td>
<td>5.8 – 28.5</td>
<td></td>
</tr>
<tr>
<td>Eosinophil count in sputum (%) ¶</td>
<td>4.88 ± 1.27</td>
<td>5.11 ± 1.24</td>
<td>0.82</td>
</tr>
<tr>
<td>Eosinophil count in blood (×10⁹/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FENO₅₀ (ppb)</td>
<td>30.5 ± 1.4</td>
<td>36.3 ± 1.6</td>
<td>0.41</td>
</tr>
<tr>
<td>Score on Juniper Asthma Control Questionnaire</td>
<td>1.90 ± 0.83</td>
<td>2.23 ± 0.90</td>
<td>0.14</td>
</tr>
<tr>
<td>Score on Asthma Quality of Life Questionnaire</td>
<td>5.42 ± 1.04</td>
<td>5.02 ± 0.95</td>
<td>0.13</td>
</tr>
<tr>
<td>Daily dose of inhaled corticosteroid – beclometasone dipropionate equivalent (µg)</td>
<td>1600 – 3000</td>
<td>1600 – 2000</td>
<td>0.57</td>
</tr>
<tr>
<td>Median</td>
<td>1600</td>
<td>1600</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>400 – 3000</td>
<td>200 – 2000</td>
<td></td>
</tr>
<tr>
<td>Use of long-acting beta-agonists (% of subjects)</td>
<td>89.7</td>
<td>84.4</td>
<td>0.71</td>
</tr>
<tr>
<td>Use of oral prednisolone Regular use (% of subjects)</td>
<td>24.1</td>
<td>21.9</td>
<td>1.00</td>
</tr>
<tr>
<td>Mean</td>
<td>9</td>
<td>7</td>
<td>0.59</td>
</tr>
<tr>
<td>Range</td>
<td>5 – 10</td>
<td>5 – 10</td>
<td></td>
</tr>
<tr>
<td>Use of montelukast (% of subjects)</td>
<td>34.5</td>
<td>12.5</td>
<td>0.07</td>
</tr>
<tr>
<td>Use of a methylxanthine (% of subjects)</td>
<td>34.5</td>
<td>6.3</td>
<td>0.009</td>
</tr>
</tbody>
</table>
Legend for Table 3.16b

Plus-minus values are means ± standard deviation (SD) unless otherwise stated. FENO_{50} denotes the fraction of exhaled nitric oxide in exhaled air at a flow rate of 50 ml/s, FEV_{1} forced expiratory volume in one second, and FVC forced vital capacity.

† P values were calculated with the use of a two-sided unpaired t-test for parametrically distributed variables, Fisher’s exact test for comparison of proportions, and the Mann-Whitney U test for comparison of non-parametric variables.

¶ Values are geometric means ± log_{10} SD.
Efficacy

Figures 3.16 and 3.17 show the changes from baseline to the mid-treatment, end-of-treatment and end-of-study visits with respect to the primary outcome and the main secondary and exploratory outcomes, in the per-protocol population. Table 3.17 shows all efficacy outcome measures at baseline and post-treatment in the per-protocol population. Table 3.18 shows the equivalent data in the intention-to-treat population. All statistically significant differences between groups in the per-protocol analysis were also present in the intention-to-treat analysis.

Primary outcome

The geometric mean sputum eosinophil percentage fell from 4.88 at baseline to 0.91 post-treatment in the QAW039 group (p < 0.0001), and from 5.11 at baseline to 3.58 post-treatment in the placebo group (p = 0.43). The geometric mean fold change in sputum eosinophil percentage from baseline to post-treatment was 0.74 (1.3-fold reduction) in the placebo group and 0.19 (5.2-fold reduction) in the QAW039 group, with a statistically significant difference between the groups (p = 0.005).

Secondary and exploratory outcomes

The mean six-point ACQ score (ACQ-6), which included questions about asthma symptoms and bronchodilator use but excluded the pre-bronchodilator FEV₁ component, increased by 0.11 in the placebo group and fell by 0.25 in the QAW039 group, with the changes not reaching statistical significance within either group or between groups. The mean AQLQ(S) score fell by 0.17 in the placebo group and increased by 0.27 in the QAW039 group, with a statistically significant difference between groups (p = 0.036). The mean post-bronchodilator FEV₁ fell by 100 ml in the placebo group and increased by 110 ml in the QAW039 group, a statistically significant difference between groups (p = 0.004), and a statistically significant improvement from baseline within the QAW039 group (p = 0.001). The mean pre-bronchodilator FEV₁ also increased by 110 ml in the QAW039 group, a statistically significant change from baseline (p = 0.022), but the between-group comparison did not reach statistical significance. The mean ratio of residual volume (RV) to total lung capacity (TLC) increased by 0.3% in the placebo group and fell by 3.2% in the QAW039 group, a statistically significant change within the QAW039 group (p = 0.019) and between the groups (p = 0.036). The MBW parameters LCI, LCI_{vent} and LCI_{ds} fell significantly
following treatment in the QAW039 group, but the between-group comparisons did not reach statistical significance.

**Safety**

QAW039 had an acceptable side-effect profile throughout the study period. There were no serious adverse events reported, and no patient withdrawals due to side-effects of QAW039.
Figure 3.16: Changes from baseline to mid-treatment, post-treatment and post-washout visits with respect to main outcomes in the per-protocol population

*Orange lines represent the QAW039 group, blue lines represent the placebo group. Error bars denote 95% confidence intervals. P values are for between-group comparisons of the change from baseline.*
Figure 3.17: Changes from baseline to post-treatment and post-washout visits with respect to lung clearance index and resistance at 5Hz in the per protocol population

Orange lines represent the QAW039 group, blue lines represent the placebo group. Error bars denote 95% confidence intervals. P values are for between-group comparisons of the change from baseline.
Table 3.17a: Outcome Measures at Baseline and Post-Treatment in the Per-Protocol Population

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Baseline values</th>
<th>Post-treatment values</th>
<th>Change from baseline to post-treatment values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QAW039 (n = 25)</td>
<td>Placebo (n = 29)</td>
<td>QAW039 (n = 25)</td>
</tr>
<tr>
<td>Eosinophil count in sputum (%) ‡</td>
<td>4.69 ± 1.21</td>
<td>4.97 ± 1.24</td>
<td>0.91 ± 0.68</td>
</tr>
<tr>
<td>Eosinophil count in blood (×10⁹/L)</td>
<td>0.36 ± 0.26</td>
<td>0.35 ± 0.24</td>
<td>0.35 ± 0.28</td>
</tr>
<tr>
<td>FENO50 (ppb)</td>
<td>38.0 ± 25.5</td>
<td>43.7 ± 35.5</td>
<td>31.5 ± 20.2</td>
</tr>
<tr>
<td>ACQ-6 score</td>
<td>1.71 ± 0.95</td>
<td>2.07 ± 0.93</td>
<td>1.47 ± 0.96</td>
</tr>
<tr>
<td>AQLQ score</td>
<td>5.44 ± 0.99</td>
<td>5.05 ± 0.99</td>
<td>5.71 ± 0.90</td>
</tr>
<tr>
<td>Pre-bronchodilator FEV₁ (L)</td>
<td>2.33 ± 0.87</td>
<td>2.26 ± 0.97</td>
<td>2.44 ± 0.82</td>
</tr>
<tr>
<td>Pre-bronchodilator FVC (L)</td>
<td>3.54 ± 0.89</td>
<td>3.42 ± 1.18</td>
<td>3.57 ± 0.91</td>
</tr>
<tr>
<td>Pre-bronchodilator FEV₁/FVC (%)</td>
<td>64.9 ± 14.4</td>
<td>64.0 ± 11.3</td>
<td>67.4 ± 11.7</td>
</tr>
<tr>
<td>Post-bronchodilator FEV₁ (L)</td>
<td>2.55 ± 0.89</td>
<td>2.62 ± 1.04</td>
<td>2.66 ± 0.83</td>
</tr>
<tr>
<td>Post-bronchodilator FVC (L)</td>
<td>3.68 ± 0.87</td>
<td>3.71 ± 1.19</td>
<td>3.73 ± 0.89</td>
</tr>
<tr>
<td>Post-bronchodilator FEV₁/FVC (%)</td>
<td>68.1 ± 14.1</td>
<td>68.7 ± 11.6</td>
<td>70.8 ± 12.8</td>
</tr>
</tbody>
</table>
Table 3.17b: Outcome Measures at Baseline and Post-Treatment in the Per-Protocol Population

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Baseline values</th>
<th>Post-treatment values</th>
<th>Change from baseline to post-treatment values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QAW039 (n = 25)</td>
<td>Placebo (n = 29)</td>
<td>QAW039 (n = 25)</td>
</tr>
<tr>
<td>RV (L)</td>
<td>2.74 ± 1.18</td>
<td>2.91 ± 1.27</td>
<td>2.52 ± 1.18</td>
</tr>
<tr>
<td>TLC (L)</td>
<td>6.43 ± 1.46</td>
<td>6.48 ± 1.53</td>
<td>6.37 ± 1.33</td>
</tr>
<tr>
<td>RV/TLC (%)</td>
<td>42.0 ± 12.4</td>
<td>44.5 ± 13.9</td>
<td>38.8 ± 12.7</td>
</tr>
<tr>
<td>VA (L)</td>
<td>5.06 ± 1.18</td>
<td>4.81 ± 1.34</td>
<td>5.10 ± 1.12</td>
</tr>
<tr>
<td>VA/TLC (%)</td>
<td>79.2 ± 11.3</td>
<td>74.9 ± 13.9</td>
<td>80.7 ± 11.0</td>
</tr>
<tr>
<td>Kco (mmol•min⁻¹•kPa⁻¹•L⁻¹)</td>
<td>1.67 ± 0.38</td>
<td>1.64 ± 0.27</td>
<td>1.63 ± 0.27</td>
</tr>
<tr>
<td>DLco (mmol•min⁻¹•kPa⁻¹)</td>
<td>8.30 ± 2.17</td>
<td>7.82 ± 2.42</td>
<td>8.25 ± 2.00</td>
</tr>
<tr>
<td>LCI</td>
<td>8.94 ± 1.66</td>
<td>9.18 ± 1.90</td>
<td>8.40 ± 1.46</td>
</tr>
<tr>
<td>LCIvent</td>
<td>1.34 ± 0.11</td>
<td>1.39 ± 0.17</td>
<td>1.30 ± 0.11</td>
</tr>
<tr>
<td>LCIds</td>
<td>1.23 ± 0.09</td>
<td>1.24 ± 0.08</td>
<td>1.19 ± 0.07</td>
</tr>
<tr>
<td>Scond (L⁻¹)</td>
<td>0.068 ± 0.034</td>
<td>0.074 ± 0.038</td>
<td>0.061 ± 0.031</td>
</tr>
<tr>
<td>Sact (L⁻¹)</td>
<td>0.205 ± 0.107</td>
<td>0.219 ± 0.112</td>
<td>0.211 ± 0.095</td>
</tr>
</tbody>
</table>
Table 3.17c: Outcome Measures at Baseline and Post-Treatment in the Per-Protocol Population

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Baseline values</th>
<th>Post-treatment values</th>
<th>Change from baseline to post-treatment values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QAW039 (n = 25)</td>
<td>Placebo (n = 29)</td>
<td>QAW039 (n = 25)</td>
</tr>
<tr>
<td>R5 (kPa\cdot L^{-1}\cdot s)</td>
<td>0.55 ± 0.26</td>
<td>0.61 ± 0.20</td>
<td>0.52 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>0.37 ± 0.13</td>
<td>0.42 ± 0.10</td>
<td>0.36 ± 0.10</td>
</tr>
<tr>
<td>R5-R20 (kPa\cdot L^{-1}\cdot s)</td>
<td>0.18 ± 0.15</td>
<td>0.19 ± 0.16</td>
<td>0.16 ± 0.12</td>
</tr>
<tr>
<td>AX (kPa\cdot L^{-1})</td>
<td>2.44 ± 2.24</td>
<td>2.74 ± 2.69</td>
<td>1.96 ± 1.87</td>
</tr>
<tr>
<td>Inspiratory MLD (HU)</td>
<td>-826 ± 37</td>
<td>-838 ± 35</td>
<td>-839 ± 29</td>
</tr>
<tr>
<td>Expiratory MLD (HU)</td>
<td>-701 ± 65</td>
<td>-719 ± 49</td>
<td>-704 ± 69</td>
</tr>
<tr>
<td>MLD expiratory / inspiratory ratio</td>
<td>0.85 ± 0.07</td>
<td>0.86 ± 0.06</td>
<td>0.84 ± 0.07</td>
</tr>
<tr>
<td>RB1 wall area (mm²)</td>
<td>41.6 ± 10.0</td>
<td>37.2 ± 10.6</td>
<td>41.0 ± 13.5</td>
</tr>
<tr>
<td>RB1 wall percentage</td>
<td>62.7 ± 5.8</td>
<td>63.9 ± 2.8</td>
<td>63.9 ± 5.3</td>
</tr>
<tr>
<td>RB1 luminal area (mm²)</td>
<td>26.3 ± 13.5</td>
<td>21.5 ± 7.9</td>
<td>24.7 ± 14.4</td>
</tr>
<tr>
<td>Outcome</td>
<td>Baseline values</td>
<td>Post-treatment values</td>
<td>Change from baseline to post-treatment values</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------</td>
<td>-----------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>QAW039 (n = 29)</td>
<td>Placebo (n = 32)</td>
<td>QAW039 (n = 29)</td>
</tr>
<tr>
<td>Eosinophil count in sputum (%) ‡</td>
<td>4.88 ± 1.27</td>
<td>5.11 ± 1.24</td>
<td>1.15 ± 1.11</td>
</tr>
<tr>
<td>Eosinophil count in blood (×10⁹/L)</td>
<td>0.38 ± 0.27</td>
<td>0.36 ± 0.23</td>
<td>0.37 ± 0.28</td>
</tr>
<tr>
<td>FENO₅₀ (ppb)</td>
<td>30.5 ± 1.4</td>
<td>36.3 ± 1.6</td>
<td>32.2 ± 19.8</td>
</tr>
<tr>
<td>ACQ-6 score</td>
<td>1.68 ± 0.95</td>
<td>2.13 ± 0.91</td>
<td>1.45 ± 0.97</td>
</tr>
<tr>
<td>AQLQ score</td>
<td>5.42 ± 1.04</td>
<td>5.02 ± 0.95</td>
<td>5.65 ± 0.98</td>
</tr>
<tr>
<td>Pre-bronchodilator FEV₁ (L)</td>
<td>2.25 ± 0.87</td>
<td>2.21 ± 0.95</td>
<td>2.35 ± 0.82</td>
</tr>
<tr>
<td>Pre-bronchodilator FVC (L)</td>
<td>3.52 ± 0.88</td>
<td>3.34 ± 1.16</td>
<td>3.55 ± 0.90</td>
</tr>
<tr>
<td>Pre-bronchodilator FEV₁/FVC (%)</td>
<td>62.9 ± 14.8</td>
<td>64.5 ± 11.6</td>
<td>65.3 ± 12.7</td>
</tr>
<tr>
<td>Post-bronchodilator FEV₁ (L)</td>
<td>2.46 ± 0.89</td>
<td>2.58 ± 1.00</td>
<td>2.55 ± 0.85</td>
</tr>
<tr>
<td>Post-bronchodilator FVC (L)</td>
<td>3.67 ± 0.86</td>
<td>3.64 ± 1.15</td>
<td>3.70 ± 0.88</td>
</tr>
<tr>
<td>Post-bronchodilator FEV₁/FVC (%)</td>
<td>66.1 ± 14.7</td>
<td>69.3 ± 11.5</td>
<td>68.5 ± 13.9</td>
</tr>
</tbody>
</table>
Table 3.18b: Outcome Measures at Baseline and Post-Treatment in the Intention-to-Treat Population

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Baseline values</th>
<th>Post-treatment values</th>
<th>Change from baseline to post-treatment values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QAW039 (n = 29)</td>
<td>Placebo (n = 32)</td>
<td>QAW039 (n = 29) Placebo (n = 32)</td>
</tr>
<tr>
<td>RV (L)</td>
<td>2.81 ± 1.18</td>
<td>2.86 ± 1.24</td>
<td>2.61 ± 1.20 2.87 ± 1.28 -0.19 (-0.45 – 0.07) 0.00 (-0.15 – 0.15) 0.17</td>
</tr>
<tr>
<td>TLC (L)</td>
<td>6.52 ± 1.45</td>
<td>6.38 ± 1.53</td>
<td>6.46 ± 1.35 6.35 ± 1.45 -0.05 (-0.34 – 0.24) -0.03 (-0.17 – 0.12) 0.87</td>
</tr>
<tr>
<td>RV/TLC (%)</td>
<td>42.5 ± 12.7</td>
<td>44.5 ± 13.2</td>
<td>39.8 ± 13.1 44.7 ± 14.3 -2.8 (-5.1 – -0.5) § 0.2 (-1.6 – 2.1) 0.041</td>
</tr>
<tr>
<td>VA (L)</td>
<td>5.08 ± 1.18</td>
<td>4.74 ± 1.31</td>
<td>5.11 ± 1.12 4.67 ± 1.38 0.03 (-0.16 – 0.22) -0.08 (-0.27 – 0.12) 0.42</td>
</tr>
<tr>
<td>VA/TLC (%)</td>
<td>78.5 ± 11.3</td>
<td>75.1 ± 13.3</td>
<td>79.8 ± 11.1 74.1 ± 15.3 1.3 (-2.1 – 4.7) -1.0 (-4.4 – 2.3) 0.33</td>
</tr>
<tr>
<td>KCO (mmol•min⁻¹•kPa⁻¹•L⁻¹)</td>
<td>1.66 ± 0.36</td>
<td>1.64 ± 0.26</td>
<td>1.63 ± 0.26 1.68 ± 0.25 -0.04 (-0.10 – 0.03) 0.04 (-0.01 – 0.09) 0.06</td>
</tr>
<tr>
<td>DLCO (mmol•min⁻¹•kPa⁻¹)</td>
<td>8.29 ± 2.02</td>
<td>7.74 ± 2.35</td>
<td>8.24 ± 1.87 7.81 ± 2.46 -0.05 (-0.25 – 0.15) 0.07 (-0.21 – 0.35) 0.51</td>
</tr>
<tr>
<td>LCI</td>
<td>9.05 ± 1.57</td>
<td>9.27 ± 2.24</td>
<td>8.50 ± 1.50 9.01 ± 2.33 -0.55 (-0.88 – -0.21) § -0.26 (-0.73 – 0.20) 0.33</td>
</tr>
<tr>
<td>LCIvent</td>
<td>1.36 ± 0.12</td>
<td>1.40 ± 0.20</td>
<td>1.32 ± 0.12 1.39 ± 0.22 -0.04 (-0.07 – 0.00) § 0.00 (-0.04 – 0.04) 0.19</td>
</tr>
<tr>
<td>LCIds</td>
<td>1.23 ± 0.09</td>
<td>1.24 ± 0.09</td>
<td>1.20 ± 0.07 1.23 ± 0.10 -0.03 (-0.05 – -0.01) § 0.00 (-0.03 – 0.02) 0.06</td>
</tr>
<tr>
<td>Scond (L⁻¹)</td>
<td>0.068 ± 0.032</td>
<td>0.076 ± 0.039</td>
<td>0.061 ± 0.030 0.074 ± 0.039 -0.007 (-0.023 – 0.009) -0.002 (-0.014 – 0.010) 0.62</td>
</tr>
<tr>
<td>Sacic (L⁻¹)</td>
<td>0.215 ± 0.114</td>
<td>0.213 ± 0.112</td>
<td>0.210 ± 0.104 0.222 ± 0.153 -0.005 (-0.028 – 0.018) 0.008 (-0.017 – 0.033) 0.43</td>
</tr>
</tbody>
</table>
Table 3.1c: Outcome Measures at Baseline and Post-Treatment in the Intention-to-Treat Population

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Baseline values</th>
<th>Post-treatment values</th>
<th>Change from baseline to post-treatment values</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QAW039 (n = 29)</td>
<td>Placebo (n = 32)</td>
<td>QAW039 (n = 29)</td>
<td>Placebo (n = 32)</td>
</tr>
<tr>
<td>R5 (kPa•L⁻¹•s)</td>
<td>0.55 ± 0.25</td>
<td>0.60 ± 0.19</td>
<td>0.52 ± 0.20</td>
<td>0.63 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>0.36 ± 0.12</td>
<td>0.42 ± 0.10</td>
<td>0.36 ± 0.10</td>
<td>0.43 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>0.19 ± 0.15</td>
<td>0.18 ± 0.15</td>
<td>0.16 ± 0.12</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td>AX (kPa•L⁻¹)</td>
<td>2.53 ± 2.19</td>
<td>2.64 ± 2.60</td>
<td>2.12 ± 1.90</td>
<td>2.83 ± 3.32</td>
</tr>
<tr>
<td>Inspiratory MLD (HU)</td>
<td>-831 ± 37</td>
<td>-837 ± 35</td>
<td>-840 ± 28</td>
<td>-845 ± 26</td>
</tr>
<tr>
<td>Expiratory MLD (HU)</td>
<td>-715 ± 70</td>
<td>-720 ± 48</td>
<td>-716 ± 71</td>
<td>-731 ± 46</td>
</tr>
<tr>
<td>MLD expiratory / inspiratory</td>
<td>0.86 ± 0.07</td>
<td>0.86 ± 0.06</td>
<td>0.85 ± 0.07</td>
<td>0.87 ± 0.05</td>
</tr>
<tr>
<td>RB1 wall area (mm²)</td>
<td>40.2 ± 10.1</td>
<td>36.5 ± 10.5</td>
<td>39.9 ± 12.5</td>
<td>35.5 ± 10.8</td>
</tr>
<tr>
<td>RB1 wall percentage</td>
<td>63.0 ± 6.1</td>
<td>63.7 ± 3.1</td>
<td>64.0 ± 5.5</td>
<td>63.2 ± 3.5</td>
</tr>
<tr>
<td>RB1 luminal area (mm²)</td>
<td>25.2 ± 13.2</td>
<td>21.3 ± 7.9</td>
<td>24.0 ± 13.4</td>
<td>21.2 ± 8.2</td>
</tr>
</tbody>
</table>
Legend for Tables 3.17 and 3.18

FENO_{50} denotes the fraction of exhaled nitric oxide in exhaled air at a flow rate of 50 ml/s, ACQ-6 six-point Asthma Control Questionnaire score, AQLQ Asthma Quality of Life Questionnaire score, FEV₁ forced expiratory volume in one second, FVC forced vital capacity, RV residual volume, TLC total lung capacity, VA alveolar volume by single breath helium dilution, KCO carbon monoxide transfer coefficient, DLCO carbon monoxide diffusing capacity, LCI lung clearance index, LCI_{vent} = specific ventilation inequality component of lung clearance index, LCI_{ds} = dead space component of lung clearance index, R5/R20 = resistance at 5Hz/20Hz, AX reactance area, MLD mean lung density, HU Hounsfield Units, and RB1 right upper lobe apical segmental bronchus.

* Plus-minus values are mean ± standard deviation (SD), and changes from baseline to post-treatment are mean (95% confidence interval), unless otherwise stated.  
† P values were calculated with the use of a two-sided unpaired t-test comparing the baseline-to-post-treatment changes between the QAW039 and placebo groups, unless otherwise stated.  
‡ Baseline and post-treatment values are geometric means ± log_{10} SD. Change from baseline to post-treatment is the geometric mean (95% confidence interval) fold-change in sputum eosinophil percentage.  
¶ P value was calculated using a two-sided unpaired t-test comparing the baseline-to-post-treatment changes between the QAW039 and placebo groups with respect to log-transformed sputum eosinophil percentage.  
§ Denotes a significant (p < 0.05) change from baseline to post-treatment values within a treatment group. P values were calculated using a two-sided paired t-test comparing baseline and post-treatment values in each treatment group. Sputum eosinophil percentage was log-transformed prior to analysis.
Discussion

We found that QAW039 significantly reduced sputum eosinophil percentage compared to placebo in patients with persistent, moderate-to-severe asthma and a sputum eosinophilia. The 5.2-fold reduction seen was comparable to that observed with mepolizumab\textsuperscript{29,30}, and appreciably greater than that observed with montelukast\textsuperscript{287,288}. Unlike mepolizumab\textsuperscript{29,30}, QAW039 did not have any significant effect on the blood eosinophil count. This suggests that CRTH2 receptor blockade attenuates the migration of eosinophils into the airway tissues, but may not affect the release of eosinophils from the bone marrow in humans. We did not observe a significant reduction in FeNO with QAW039. Interestingly, reductions in FeNO have been observed with lebrikizumab (anti-IL-13)\textsuperscript{31} but not mepolizumab (anti-IL-5)\textsuperscript{29}, suggesting that the production of FeNO may be partly dependent upon the precise T\textsubscript{h}2 cytokine profile.

In this study, QAW039 significantly improved AQLQ(S) scores compared to placebo, with non-significant improvements in ACQ-6 scores. In addition, QAW039 significantly improved expiratory flow limitation (post-bronchodilator FEV\textsubscript{1}) and expiratory air trapping (RV/TLC ratio) compared to placebo. Previous interventional studies have shown that anti-eosinophilic treatments or strategies exert their major therapeutic effect through the reduction in asthma exacerbations\textsuperscript{29,30,281}, although effects on FEV\textsubscript{1} have also been observed\textsuperscript{30,31}. The treatment period in this study was not long enough to observe a significant effect on exacerbations, and future studies should examine the hypothesis that QAW039 reduces the frequency of exacerbations in patients with eosinophilic asthma. Following a six-week placebo wash-out period, we noted a prompt rebound effect in the QAW039 group with respect to sputum eosinophil percentage, ACQ-6 and AQLQ(S) scores, and FEV\textsubscript{1}. This suggests that the short-term improvements in asthma quality of life and FEV\textsubscript{1} seen with QAW039 were driven by reversible processes such as reductions in airway wall oedema or mucus production. Future studies are required to examine the longer-term effects of QAW039 on airway remodelling.

Two previous clinical trials of CRTH2 receptor antagonists in asthma have yielded mixed results. In a randomized controlled study of OC000459 in patients with asthma not currently receiving ICS, significant improvements were observed in FEV\textsubscript{1} and
asthma quality of life. However, this compound has not yet been tested in patients with moderate-to-severe asthma who have higher treatment requirements, and it is therefore unknown whether OC000459 would have additional clinical benefit in this important group. The alternative compound AMG853, a dual CRTH2 and D-prostanoid receptor antagonist, was not effective in improving asthma symptoms or FEV1 in patients with moderate-to-severe asthma. In these two studies, patient selection was not based upon evidence of eosinophilic airway inflammation. Previous experience has shown that targeting anti-eosinophilic therapies to patients with evidence of uncontrolled Th2-driven inflammation results in improved efficacy. The positive results obtained in our study should therefore not be extrapolated to an unselected group of patients with moderate-to-severe asthma.

We utilised a number of novel physiological and imaging outcome measures in this study such as multiple breath inert gas washout (MBW), impulse oscillometry and quantitative CT. These modalities did not appear to be as responsive as standard outcome measures such as FEV1. However, a number of the MBW outcomes, namely LCI, LCI_{vent} and LCI_{ds}, did show statistically significant improvements within the QAW039 group, and the change in LCI_{ds} from baseline to post-treatment was close to showing a statistically significant difference between the QAW039 and placebo groups (p = 0.06). Moreover, a number of these techniques are still at a relatively early stage of development, and improvements may be anticipated in the future. In particular, we assessed changes in airway wall geometry with quantitative CT using the dimensions of a single lobar bronchus. Newer techniques such as computational fluid dynamics, which take into account the morphology of the first six generations of the airway tree, may provide novel insights into the effect of an intervention on airway resistance.

We conclude that QAW039 is effective at attenuating eosinophilic airway inflammation in patients with persistent eosinophilic asthma, and has a favourable safety profile. There is evidence that QAW039 improves lung function and asthma-related quality of life. Longer-term studies are required to confirm these findings and to investigate the effect of QAW039 on preventing asthma exacerbations.
4 Conclusions

4.1 Summary of findings

The aim of the work presented in this thesis was to assess the clinical utility and structural correlates of putative non-invasive measurements of small airway obstruction in patients with asthma. The rationale underpinning this aim was the widely-stated hypothesis that small airway disease may represent a cause of disease persistence in asthma, since conventional inhaled therapies do not penetrate to the very distal airways. The accurate measurement of small airway obstruction would potentially enable treatments that targeted the small airway compartment, such as small particle inhalers or systemic therapies, to be selectively administered to the patient group most likely to benefit from them. Moreover, small airway biomarkers could be used to assess the response to these treatments in clinical practice, or within the context of interventional trials. A further possibility, albeit speculative, is that small airway markers may provide an early warning for the incipient development of incompletely reversible airflow obstruction in patients with asthma.

We first undertook a period of methodological validation, particularly focusing on the multiple breath inert gas washout (MBW) technique, since this method is currently less well standardised than spirometry, body plethysmography and impulse oscillometry (IOS). Utilising an acrylic glass lung model, we determined that measurements of functional residual capacity performed using our MBW method are accurate and repeatable in vitro, and we also demonstrated good within-visit repeatability in vivo. This provided increased confidence in the results presented in the remainder of the thesis using this technique.

The MBW test yields a large amount of data, but only one data point is utilised to derive the lung clearance index (LCI), the most commonly used MBW parameter. Phase III slope analysis had been adequately explored by other investigators, but it did not appear that the information content of the standard washout curve, comprising breath-by-breath exhaled inert gas concentration, had been fully assessed. We therefore derived two novel parameters, LCI_{vent} and LCI_{ds}, based upon a simple two-compartment lung model, which estimated the contribution towards LCI of specific ventilation
inequality and increased respiratory dead space, respectively. These parameters were found to be repeatable in patients with asthma, cystic fibrosis (CF) and non-cystic fibrosis bronchiectasis. Moreover, there was evidence that they could discriminate between different phenotypes of cystic fibrosis, a condition that is characterised by severe ventilation heterogeneity. A further important observation was that in patients with both non-CF bronchiectasis and CF, LCI_{vent} and LCI_{ds} were strongly correlated, suggesting that specific ventilation inequality and increased respiratory dead space are not completely independent. Since these mechanisms are expected to occur mainly in the proximal and the distal airways respectively, it may be surmised that the calibre of the large and small airways are also related to a certain extent. The association between large and small airway obstruction may be purely statistical, in that the two processes may tend to coexist in the same patients due to a common underlying pathological process, or alternatively, the calibre of the large and small airways may interact due to the network properties of the airway tree. In either case, it is clear that attempting to draw a sharp distinction between large and small airway obstruction is somewhat artificial. Nevertheless, this does not preclude the possibility that some patients may have disproportionate small airway disease, and that this characteristic could be measured and used to stratify therapy.

The next study dealt with the repeatability of putative small airway biomarkers, both within-visit and between-visit. We found that IOS parameters exhibited good within-visit repeatability, and were stable over time. With the notable exception of S_{cond}, MBW parameters exhibited good within-visit repeatability, and were moderately stable over time. We then investigated the relationship between putative small airway markers and the clinical expression of asthma. We found that the main physiological predictors of asthma symptoms, quality of life and exacerbations were spirometric measures of expiratory flow limitation and measures of airway resistance using IOS. The most predictive IOS parameter was the resistance at 20Hz (R20), which is often considered to be a marker of large airway disease, although in reality the structural correlates of IOS parameters are not fully understood. Nevertheless, the fact that FEV\textsubscript{1} and R20 are independent predictors of asthma control suggests that they probe two different aspects of asthma pathophysiology or disease expression.
The MBW parameter $S_{acin}$ was introduced as a specific marker of acinar airspace disease, but the structural correlates of this index had not previously been validated using either imaging or histological techniques. We utilised hyperpolarised $^3$He magnetic resonance to probe diffusion within the acinar airspaces, in two polar groups of patients with asthma, one of which comprised patients with a normal $S_{acin}$, and the other of which comprised patients with a raised $S_{acin}$. We found that long-timescale diffusion of $^3$He was less restricted in patients with a high $S_{acin}$. Moreover, the correlation between $S_{acin}$ and long-timescale ADC could not be accounted for purely by expiratory air trapping and lung hyperinflation. We therefore concluded that there was evidence for a structural abnormality in the pulmonary acinus in patients with asthma causing subtle alterations in diffusion within this compartment.

The final study presented in this thesis was a randomised controlled trial of a novel anti-eosinophilic agent, the CRTH2 receptor antagonist QAW039. The primary outcome of the study was met, in that the drug very effectively reduced sputum eosinophil counts. In addition, there was evidence that QAW039 improves asthma quality of life and FEV$_1$. It therefore appears that QAW039 is a promising agent for the treatment of persistent eosinophilic asthma. The main relevance of this study to the present thesis was the inclusion of a number of novel exploratory outcome measures in the study design. These included MBW, IOS and quantitative CT parameters. Statistically significant improvements were observed within the QAW039 group for a number of MBW parameters, suggesting their possible utility as outcome measures in future interventional trials.

4.2 Future work

The work presented in this thesis may be built upon in a number of ways in the future. The validation study we performed using the photoacoustic gas analyser-based MBW system suggests that it may be suitable for development into a commercial product for clinical or research use, an undertaking that would benefit from academic input. We utilised a one-compartment lung model to validate our Innocor system, but in future this may be refined to include two parallel compartments subtended by airways with different resistances, in order to simulate ventilation heterogeneity.
Further studies are also required to understand the structural basis of IOS and MBW parameters, including the novel parameters $L_{CI_{vent}}$ and $L_{CI_{ds}}$. This work is likely to involve a combination of computational modelling, and experiments using physical rapid prototype models of the human airways. With respect to IOS, the prevailing paradigm that $R_{20}$ reflects large airways and $R_{5-R20}$ reflects small airways is likely to be overly simplistic. It is now possible to produce patient-specific physical models of the upper airways down to the segmental branches of the bronchial tree, from CT images. The impedance of these models can be measured using IOS or other variants of the forced oscillation technique. Patient-specific computational models of the airway tree utilise CT images to derive the geometry of the first six generations, and the remainder of the bronchial tree, which is beyond the resolution of CT, is ‘grown out’ using an automated algorithm. The impedance of these models may be simulated, and the effects of altering the model, for instance by imposing homogeneous or heterogeneous airway constriction, can then be investigated. These techniques are likely to provide for the first time a true understanding of the structural significance of IOS parameters. A similar approach may be utilised to model gas mixing in the airways, although simulating the interaction between convection and diffusion within the whole airway tree is likely to be computationally intractable. However, it is likely that useful insights can be gained using simplified forward models that incorporate both diffusive and convective effects. Altering the structure of the model and then entering the output of the simulation into the inverse model described in Section 2.7 may yield further insights into the structural correlates of $L_{CI_{vent}}$ and $L_{CI_{ds}}$.

In this thesis, the diffusive component of ventilation heterogeneity was investigated using hyperpolarised $^3$He diffusion MR. Magnetic resonance techniques may also be utilised to quantify the regional fractional ventilation throughout the lungs, and the coupling of this technique with standard MBW is a further potentially fruitful area of research. In particular, this would allow the convective component of ventilation heterogeneity to be better understood.

We examined the clinical significance of small airway biomarkers cross-sectionally, and found that the IOS parameter $R_{20}$ appeared to independently predict asthma control, quality of life and exacerbations. Longitudinal studies are required to
investigate the possibility that one or more of these markers may predict the future development of spirometric fixed airflow obstruction, which is known to be associated with worse clinical outcomes in patients with asthma\textsuperscript{4}. Early intervention at this stage may potentially alter the natural history of the disease. Future work following up our asthma cohort is planned in order to determine the baseline predictors of accelerated lung function decline in this patient group.
References


3) Braman SS. The global burden of asthma. Chest. 2006; 130(Suppl. 1): 4S-12S.


7) Barnes PJ. Against the Dutch hypothesis: asthma and chronic obstructive pulmonary disease are distinct diseases. Am J Respir Crit Care Med. 2006; 174(3): 240-3


17) Mauad T, Silva LF, Santos MA, Grinberg L, Bernardi FD, Martins MA, Saldiva PH, Dolhnikoff M. Abnormal alveolar attachments with decreased elastic fiber


42) Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJ, Pauwels RA, Pedersen SE; GOAL Investigators Group. Can guideline-defined asthma control


200) Samee S, Altes T, Powers P, de Lange EE, Knight-Scott J, Rakes G, Mugler JP 3rd, Ciambotti JM, Alford BA, Brookeman JR, Platts-Mills TA. Imaging the lungs in asthmatic patients by using hyperpolarized helium-3 magnetic resonance:


