MULTIPLE-BREATH-INERT GAS WASHOUT
TO ASSESS VENTILATION INHOMOGENEITY IN
CHRONIC OBSTRUCTIVE PULMONARY DISEASE

A THESIS
SUBMITTED TO THE UNIVERSITY OF MANCHESTER
FOR THE DEGREE OF
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FACULTY OF BIOLOGY MEDICINE AND HEALTH

ALAN STEPHEN BELL

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SCHOOL OF BIOLOGICAL SCIENCES
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ABSTRACT

A Thesis submitted by Alan Stephen Bell for the degree of Master of Philosophy in the Faculty of Medical and Human Sciences (University of Manchester)

Multiple-Breath Inert Gas Washout to Assess Ventilation Inhomogeneity in Chronic Obstructive Pulmonary Disease (Submitted: June 2016)

Background: COPD is a heterogeneous condition with increased morbidity and mortality. Clinical hallmarks include dyspnoea, cough, sputum production, and non-reversible airflow obstruction. It is also associated with exacerbations that become more prevalent as severity increases, leading to further deterioration in health status and accelerated disease progression. Exacerbations are poorly understood and subjective measures are relied upon for diagnosis and monitoring recovery. There is a large amount of current evidence to suggest that increased resistance and VI manifest in mild disease prior to symptoms developing. Recently MBW has been shown to effectively measure VI using LCI in cystic fibrosis.

Aims: The aim of this thesis was to evaluate the sensitivity of LCI as a clinical measure of airways disease in COPD. The following were investigated: 1) LCI comparison between COPD and HC’s. 2) COPD LCI\textsubscript{N2} comparison to other physiological tests. 3) LCI\textsubscript{N2} pre and post salbutamol. 4) The intra-test and inter-test variability of LCI. 5) Comparison of LCI measured on 2 different systems. 6) Practicalities of MBW in COPD. 7) Impact of exacerbations on MBW parameters.

Methods: Fifty-four COPD and 12 HC’s were recruited and performed LCI\textsubscript{N2}, LCI\textsubscript{SF6} and spirometry, with a repeatability visit separated by ≥24 hrs. The COPD subjects (n = 54) also completed IOS, DL\textsubscript{CO}, plethysmography, with LCI\textsubscript{N2} reversibility collected in a sub-set (n = 25). Subjects who exacerbated and performed LCI\textsubscript{N2} (n = 9) were tested on day of onset, 2 weeks post treatment and after 6 weeks.

Results and Conclusions: LCI\textsubscript{N2} was able to differentiate between COPD and HC group. Twenty % of COPD subjects with an FEV\textsubscript{1} within normal range were found to have abnormal VI. LCI\textsubscript{N2} did not differ after treatment with salbutamol (p> 0.05) but did correlate with R\textsubscript{5}-R\textsubscript{20}, X\textsubscript{s}, RV/TLC, R\textsubscript{aw} and G\textsubscript{aw}. Both MBW\textsubscript{N2} and MBW\textsubscript{SF6} had low intra and inter test variability but MBW\textsubscript{N2} produced significantly greater LCI and FRC% values. The difference between the two FRC% methods became disproportionally greater with disease severity. FRC\textsubscript{N2} % produced values greater than FRC\textsubscript{pleth} % with mean 156% (45) and 137% (36) respectively. Test time was greatly extended for both methods in moderate to severe subjects which may limit testing in clinical practice. In the exacerbation cohort LCI\textsubscript{N2} was not found to significantly change at the onset of an exacerbation nor throughout recovery. Data suggestes that LCI is best used in mild COPD patients and the N\textsubscript{2} system may be erroneous and requires more research in a COPD population.
DECLARATION

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Dedication

This thesis is dedicated to my daughter Amelia Jean Bell, born on the 17th May 2016 at 6:54am, 6 weeks before submission of this thesis. The completion of this work has been challenging yet illuminating and tiring yet intriguing with moments of accomplishment as well as despair. From the moment I held my daughter for the first time I was inspired to ensure I complete this work to the highest level possible. I will carry this ethos into everything I do in the future so that I may provide the lifestyle and opportunities that my new family deserves.

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I undertook 90% of all testing discussed in this thesis however a special thank you goes to Mr. Paul Hitchen and Mr. Philip Lawrence for providing their time and skills in helping data collection, performing LCI and lung function testing on occasions when needed.
My Fiancé, Ann-Marie Duffy, for her support and understanding throughout the completion of this work, especially during her pregnancy.

My parents, Mr. Alan Bell Snr and Mrs. Renee Bell, who have always promoted a work ethic to succeed and have provided financial support when required. I only hope that completion of this thesis is one of the many things in life that they can be proud of and justify the sacrifices that they have made on my behalf.
The Author

Alan Stephen Bell

2006 BSc. Sport and Exercise Science, Lancaster University

2010 Association of Respiratory Technology and Physiology (ARTP) Part I Qualification

2012 BSc. Clinical Physiology, Manchester Metropolitan University

2012 Association of Respiratory Technology and Physiology (ARTP) Part II Qualification

Abstracts & Publications

Past and current abstracts and publication that are under review can be found in the appendix.

Abstracts

ERS, Munich 2014 (Poster Discussion)

http://erj.ersjournals.com/content/44/Suppl_58/P1989

ERS, Munich 2014 (Poster)

http://erj.ersjournals.com/content/44/Suppl_58/P2130

ERS, London 2016 (Poster)

Bell, A., Singh, D., Horsley, A. (2016) Over-reading of FRC using N₂ MBW compared to plethysmography and SF₆ MBW in COPD. *European Respiratory Journal*, 48 (Suppl 60). Available at:
http://erj.ersjournals.com/content/48/suppl_60/PA2256
## List of Abbreviations

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<tr>
<td>$\chi^2$</td>
<td>Coherence</td>
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<tr>
<td>AECOPD</td>
<td>Acute exacerbations in COPD</td>
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<td>ACQ</td>
<td>Asthma Control Questionnaire</td>
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<td>ATS</td>
<td>American Thoracic Society</td>
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<td>BDP</td>
<td>Beclomethasone Dipropionate</td>
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<td>BLS</td>
<td>Basic Life Support</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CAT™</td>
<td>COPD Assessment Test</td>
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<td>CCQ</td>
<td>Clinical COPD Questionnaire</td>
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<tr>
<td>Cet</td>
<td>Alveolar concentration</td>
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<td>CEV</td>
<td>Cumulative expired volume</td>
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<td>CF</td>
<td>Cystic Fibrosis</td>
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<td>CH$_4$</td>
<td>Methane</td>
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<tr>
<td>CLE</td>
<td>Centrilobular emphysema</td>
</tr>
<tr>
<td>CO</td>
<td>Carbon Monoxide</td>
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<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
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<td>COPD</td>
<td>Chronic Obstructive Pulmonary Disease</td>
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<td>CT</td>
<td>Computerised Tomography</td>
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<td>DL$_{CO}$</td>
<td>Diffusing capacity</td>
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<tr>
<td>E0</td>
<td>Clinic visit at onset of COPD exacerbation</td>
</tr>
<tr>
<td>E2</td>
<td>Clinic visit after 2 weeks of COPD exacerbation onset</td>
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<tr>
<td>E6</td>
<td>Clinic visit after 6 weeks of COPD exacerbation onset</td>
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<tr>
<td>ERS</td>
<td>European Respiratory Society</td>
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<td>EVC</td>
<td>Expired vital capacity</td>
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<td>FEV$_1$</td>
<td>Forced expired volume in 1 second</td>
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<tr>
<td>FEV$_1$/FVC</td>
<td>Ratio of FEV$_1$ divided by FVC</td>
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<td>FEF$_{25-75%}$</td>
<td>Mean forced expiratory flow between 25% and 75% of the forced vital capacity</td>
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<td>FRC</td>
<td>Functional residual capacity</td>
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<tr>
<td>FRC$_{pleth}$</td>
<td>Functional residual capacity measured by whole body plethysmography</td>
</tr>
<tr>
<td>F$_{res}$</td>
<td>Resonance frequency</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>GOLD</td>
<td>Global Initiative for Chronic Obstructive Lung Disease</td>
</tr>
<tr>
<td>G$_{aw}$</td>
<td>Airway conductance</td>
</tr>
<tr>
<td>HC</td>
<td>Healthy Controls</td>
</tr>
<tr>
<td>HRCT</td>
<td>High resolution computerised tomography</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IC</td>
<td>Inspiratory capacity</td>
</tr>
<tr>
<td>ICS</td>
<td>Inhaled corticoid steroid</td>
</tr>
<tr>
<td>IVC</td>
<td>Inspired vital capacity</td>
</tr>
<tr>
<td>ISOLDE</td>
<td>Inhaled Steroids in Obstructive Lung Disease in Europe</td>
</tr>
<tr>
<td>K$_{CO}$</td>
<td>Carbon monoxide transfer coefficient</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>LCI</td>
<td>Lung clearance index</td>
</tr>
<tr>
<td>L/sec</td>
<td>Litre per second</td>
</tr>
<tr>
<td>m</td>
<td>Metre</td>
</tr>
<tr>
<td>m$^2$</td>
<td>Metre squared</td>
</tr>
<tr>
<td>MBW</td>
<td>Multiple-Breath Washout</td>
</tr>
<tr>
<td>MBW$_{N2}$</td>
<td>Multiple-Breath Nitrogen Washout</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MBW&lt;sub&gt;SF6&lt;/sub&gt;</td>
<td>Multiple-Breath Sulphur Hexafluoride Washout</td>
</tr>
<tr>
<td>MCID</td>
<td>Minimum Clinical Important Difference</td>
</tr>
<tr>
<td>mins</td>
<td>Minutes</td>
</tr>
<tr>
<td>mls</td>
<td>Millilitre</td>
</tr>
<tr>
<td>MM</td>
<td>Molecular Mass</td>
</tr>
<tr>
<td>mm</td>
<td>Millimetre</td>
</tr>
<tr>
<td>mMRC</td>
<td>Modified British Medical Research Council Questionnaire</td>
</tr>
<tr>
<td>N&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Oxygen</td>
</tr>
<tr>
<td>PLE</td>
<td>Panlobular emphysema</td>
</tr>
<tr>
<td>PRO</td>
<td>Patient Reported Outcomes</td>
</tr>
<tr>
<td>QC</td>
<td>Quality control</td>
</tr>
<tr>
<td>R&lt;sub&gt;t&lt;/sub&gt;</td>
<td>Total respiratory system resistance</td>
</tr>
<tr>
<td>R&lt;sub&gt;20&lt;/sub&gt;</td>
<td>Proximal respiratory system resistance</td>
</tr>
<tr>
<td>R&lt;sub&gt;5&lt;/sub&gt;-R&lt;sub&gt;20&lt;/sub&gt;</td>
<td>Peripheral respiratory system resistance</td>
</tr>
<tr>
<td>R&lt;sub&gt;rs&lt;/sub&gt;</td>
<td>Respiratory system resistance</td>
</tr>
<tr>
<td>R&lt;sub&gt;aw&lt;/sub&gt;</td>
<td>Airways resistance</td>
</tr>
<tr>
<td>RV</td>
<td>Residual volume</td>
</tr>
<tr>
<td>S&lt;sub&gt;acin&lt;/sub&gt;</td>
<td>Ventilation inhomogeneity in acinar lung zones</td>
</tr>
<tr>
<td>S&lt;sub&gt;cond&lt;/sub&gt;</td>
<td>Ventilation inhomogeneity in conductive lung zones</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SF&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Sulphur Hexafluoride</td>
</tr>
<tr>
<td>SpO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Peripheral capillary oxygen saturation</td>
</tr>
<tr>
<td>sG&lt;sub&gt;aw&lt;/sub&gt;</td>
<td>Specific airway conductance</td>
</tr>
<tr>
<td>sR&lt;sub&gt;aw&lt;/sub&gt;</td>
<td>Specific airways resistance</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lung capacity</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<td>---------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>$V_A$</td>
<td>Alveolar volume</td>
</tr>
<tr>
<td>$VA/Q$</td>
<td>Ventilation-Perfusion ratio</td>
</tr>
<tr>
<td>$VI$</td>
<td>Ventilation inhomogeneity</td>
</tr>
<tr>
<td>$V_T$</td>
<td>Tidal volume</td>
</tr>
<tr>
<td>$X_5$</td>
<td>Respiratory system reactance at 5 Hz</td>
</tr>
<tr>
<td>$X_{rs}$</td>
<td>Respiratory system reactance</td>
</tr>
<tr>
<td>$Z_{rs}$</td>
<td>Respiratory system impedance</td>
</tr>
</tbody>
</table>
CHAPTER 1

1.0 General Introduction

Chronic obstructive pulmonary disease (COPD) is one of the leading chronic inflammatory diseases of the airways and a growing global problem. In 2002 COPD was reported to be the fourth most frequent cause of death in the world, which is set to rise the third by 2020 (Barnes, 2004; Lopez and Murray, 1998). It is directly linked to increased mortality, with current prevalence being 4-10% of all adults in Europe (Halbert, et al., 2003). In addition to the impact on public health COPD has an ever-burdening economic impact upon society with current treatments unable to slow the progression of the disease, leading to increased hospital admissions as severity increases. This prevalence, combined with research that shows small airway changes due to lung injury early in the disease, emphasises the need for techniques that aid in earlier detection and that are sensitive enough to detect changes in response to new medications (Maleki-Yazdi et al, 2007). The disease is heterogeneous in nature and is defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) as a;

‘…common preventable and treatable disease characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients.’ (GOLD, 2011)

It is characterised by persistent and progressive airflow obstruction with pathological manifestations of chronic inflammation of both the central and peripheral airways (bronchitis), and airway wall thickening and destruction of alveoli walls (emphysema). The parenchymal lesions lead to small airway abnormalities with increased resistance and decreased elastic recoil, with an increase in mucus secretions blocking the lumen of the small airways (Contoli, et al., 2010). Clinical hallmarks of COPD
include dyspnoea, cough and sputum production, as well as non-reversible airflow obstruction quantified as a ratio of the volume of air measured after 1 second of a forced expiration as a function of the total volume forcibly expired (FEV₁/FVC), which will be < 0.7 in the presence of obstruction along with a faster than age related decrease in forced expired volume in 1 second (FEV₁) over time (GOLD, 2011). As the disease progresses exacerbation frequency increases, the degree of hyperinflation increases, and exercise capacity is decreased resulting in a reduction in quality of life. This multi-faceted definition, due to the heterogeneous pathophysiology of the disease resulted in the GOLD 2006 guidelines being updated in 2011. In addition to the conventional staging system using FEV₁, associated risk factors were included with an aim to personalise the impact of COPD on an individual basis to determine severity and monitor progression (figure 1.1).
Severity is now classified as A, B, C or D and incorporates FEV₁ percent predicted, assessment of symptoms, and breathlessness as measured by patient reported outcomes using the COPD Assessment Test/COPD Control Questionnaire (CAT/CCQ) and Modified British Medical Research Council Questionnaire (mMRC); along with the risk of exacerbation measured by medical history. This approach has been adopted to reflect the heterogeneous characteristics of COPD (GOLD, 2011).

This updated staging system included the use of FEV₁ and exacerbation history along with either a measure of dyspnoea (using the modified Medical Research Council (mMRC) or a health status, such as the COPD Assessment Test (CAT) score. Even with this new improved multi-dimensional approach to COPD classification and management the limitations of using a marker of the large conducting airways (FEV₁) could still be further enhanced by the addition of more sensitive pulmonary functions tests. There is a large amount of evidence to suggest that increased resistance and heterogeneity of the small airways have a large impact on airflow limitation, with abnormalities manifesting prior to symptoms of breathlessness and measurable effects on the larger airways (Konstantinos, Kostikas and Kontakiotes, 2013).
1.1 Exacerbations

The recent GOLD guidelines (2011) also highlight the impact of exacerbations upon the severity of COPD. Exacerbations occur as a result of a rise in inflammation in the lung. The primary cause of this is through an infection via bacteria, a virus, or a combination of both; with a lesser number of exacerbations being attributed to an elevated exposure to air pollution (Celli and Barnes, 2007) (figure 1.2).

A single definition of COPD exacerbations does not exist but current accepted literature all include a worsening of respiratory symptoms, with increased sputum volume and purulence (Celli and Barnes, 2007; Mackay, et al., 2012; Mallia and Johnston, 2005). COPD exacerbations have both a large economic and individual effect. Patients that exacerbate, especially on a frequent basis (>2 per year), express deterioration in health status, accelerated disease progression, and increased mortality with a greater financial cost of treatment and risk of hospital admission (Celli and Barnes, 2007; Soler-Cataluna, et al., 2005). This makes the methods used to prevent, detect and monitor exacerbations of paramount importance. There are a limited amount of physiological tests employed in exacerbation assessments. Spirometry is the predominant physiological test but FEV$_1$ has not only been found to be a poor predictor of exacerbation in the case of the inhaled steroids in obstructive lung disease in Europe (ISOLDE) clinical trial but it has also been found to weakly correlate with exacerbation frequency (Burge, et al., 2000). The increasing evidence of the involvement of the small airways can explain one reason for the limited sensitivity of FEV$_1$ in exacerbations. From a pathophysiological perspective sputum is known to be produced and reside in the small airways and during exacerbations the elevated sputum volume and purulence as a result of increased inflammation, as well as bacterial and/or viral load indicates that the small airways are the main site that warrant investigation. It has been shown that pathogenic exposure that causes an abnormal adaptive immune response can be linked by the number of lymphoid follicles in
the small airway and the severity of COPD (Hogg, et al., 2004). When this is applied in terms of physiology, FEV$_1$ is known to represent airflow resistance of the large airways and has been shown to provide variable changes in results prior to and during exacerbations in which patient distress can be evident without any detectable change in lung function (Seemungal, et al., 1998). This highlights the need for a change in approach of physiological outcomes in the assessment of exacerbations that are able to include the small airways and that are sensitive enough to detect disease effects.

Figure 1.2 The main mechanisms related to a rise in inflammation, leading to an exacerbation in COPD. Bacterial and/or viral infections account for 78% of all exacerbations in patients with COPD (Celli and Barnes, 2007).
1.2 Current Therapy

Current COPD therapeutic interventions consist of a combination of bronchodilators (β₂-agonists and anti-cholinergic) and inhaled corticosteroids (ICS). Bronchodilators aid in the reduction of air trapping and improve both exercise capacity and patient reported outcomes. The underlying inflammation is controlled through the use of ICS, however to date no medication has been shown to have a positive long-term effect on lung function decline (GOLD, 2011).

This has led researchers to not only investigate new medicines but also to evaluate the efficiency of delivery and the level of deposition current medications are able achieve. Focus is increasingly being directed towards the accessibility of the small airways both in terms of pharmacology and the available physiological tools to make accurate measurements. In recent years it has been proven that due to the relative size of the small airways they are a deposit site for pathogens, as well as an attraction site for inflammatory mediators which result in bronchoconstriction, which can be reversed through pharmacological intervention (McNulty and Usmani, 2014; Persson, 2008). Indeed extra-fine pharmacological formulations of bronchodilators, individually or in combination with a corticosteroid, have been shown to result in significant improvements in exercise capacity and patient reported outcomes (PRO’s) due to reductions in residual volume (RV) when no effect was observed on FEV₁. One study reported a reduction in RV of 13% when subjects were administered extra-fine Beclomethasone Dipropionate (BDP) (John, et al., 2005). This evidence highlights that the distal lung is a site of inflammation and airway abnormality that when targeted by selective intervention can contribute to an improvement in clinical outcomes, which may be achieved along with a reduced dose exposure of corticosteroids. In addition to reducing the amount of the delivered dose required, the frequency of medication administration could be simplified to aid in COPD management and increase compliance of medication use. These findings reinforce the
need for accurate measurement techniques, which are sensitive to their treatment effects.

**1.3 Requirements for Clinical Endpoints**

Clinical endpoints are the tools used in both clinical practice and clinical trials to evaluate patients' clinical presentation, the different pathophysiology of diseases; as well as an outcome measure to determine disease severity. They also serve to monitor progression and to provide reflective evidence of a treatment effect. This importance requires each and every endpoint to be both sensitive and repeatable (Cazzola, et al., 2008). The sensitivity of such a test in COPD should be descriptive of the pathophysiology of the disease, able to provide evidence of physiological change in relation to disease severity, exacerbation and treatment. An endpoint should also be repeatable with the minimal amount of variation both within test occurring during the same session and repeated measures on multiple days or weeks. The multifaceted approach employed by the GOLD committee reflects the fact that multiple endpoints are required to improve the identification and management of COPD and its heterogeneous manifestations. The current guidelines acknowledge the importance the small airways play as an early site of inflammation and airway abnormality but are not able to suggest a robust physiological clinical outcome measure that reflects both the early onset of the disease in the small airways, changes during exacerbations and evaluation of pharmacological interventions. It would be of great benefit to researchers, clinicians as well as patients to identify a measure that provides further information regarding the management of COPD.
1.4 Airway Physiology

In terms of airway resistance ($R_{aw}$) the Hagen-Poiseuille’s law of fluid dynamics can be amended from a fluid to a gas and applied to lung physiology to measure the pressure changes of airflow through the bronchial tree (Sutera, 1993). This denotes that a small change in diameter of an airway can have large effects upon $R_{aw}$, as the radius is inversely proportional to the $4^{th}$ power. This highlights that $R_{aw}$ is not constant throughout the bronchial tree and is increased in airways with smaller diameters but also that the level of $R_{aw}$ throughout the respiratory tract can be affected by airway changes as a result of disease.

The respiratory tree comprises of 23 generations of dichotomous branching tubes (figure 1.3). The first 16 generations are the conducting airways, known as anatomical dead space, since no gas exchange take place and gas transport is by convection with high relative flow velocity (Robinson, Goldman and Gustafsson, 2009). The terminal bronchioles from generation 16 onwards divide into the respiratory bronchioles and subsequently alveoli (alveolar ducts and sacs; generations 17-23). They are termed the acinar airways where diffusive gas exchange takes place. The small airways are defined as those with a diameter <2 millimetres (mm) that correspond to generations 8-23. The small airways contain the acinar airways as well as a selection of the conducting airways, which differ from large airways as they are lined with surfactant; and lack cartilage support and mucous glands (McNulty and Usmani, 2014). As the airways divide the flow velocity decreases so that the peripheral airway gas velocity and airway resistance ($R_{aw}$) have a low impact upon airflow limitation accounting for only 10% of total $R_{aw}$, despite accounting for 95% of the total lung volume (TLC) (Macklem and Mead, 1967). Inflammation of these small airways may therefore progress prior to the manifestation of clinical symptoms, which typically do not arise until disease is advanced.
Figure 1.3 The defined generations of the bronchial tree. Small airways are defined as those < 2mm in diameter and include the terminal bronchioles, respiratory bronchioles, alveolar ducts and alveolar sacs (McNulty and Usmani, 2014).
1.5 Role of the Small Airways

Structural and inflammatory damage of the small airways is apparent in all obstructive lung diseases and occurs before the onset of symptoms. In COPD the small airways present with smooth muscle hypertrophy and an increased level of lymphocytic and neutrophilic inflammation in comparison not only to healthy controls (HC) but also to asymptomatic smokers (Saetta, et al., 1998; Turato, et al., 2002). These processes induce small airway injury, which can be associated to disease severity. There have been studies utilizing multi-detector computerised tomography (CT) and histological analysis to investigate the small airways in healthy subjects and 2 sub-groups of emphysema: Centrilobular (CLE) and Panlobular (PLE) (Hogg, et al., 2004; McDonough, et al., 2011). A marked reduction was found in the number of terminal bronchioles and total airway cross-sectional area in both emphysema sub-groups. The loss of terminal bronchioles preceded the emphysematous destruction evident in the CLE sub-group (figure 1.4).

Figure 1.4. Multi-detector CT results of the number of airways per lung generation, between emphysema patients and healthy controls. The bars represent airways size (dark blue 2mm, green 2.5mm, grey 3mm, light blue 4mm) and lines represent the lungs of healthy controls (blue), CLE (yellow) and PLE (red) (McDonough, et al., 2011).
The changes in the small airways were associated with increased smooth muscle mass, mucus hyper-secretion, (which increases the surface tension), and persistent inflammation. Decreasing number of small airways correlated with the severity of COPD (figure 1.5) and served as a prediction of mortality in patients with advanced disease (Hogg, et al., 2007).

Such findings demonstrate that the small airways of COPD patients are an important site for expression of the disease, and highlights structural changes such as airway narrowing and reduction in number, which can precede inflammatory adaptation in the acinar part of the lung. The small airways are thus subject to an abnormal adaptive immune response that is variable and poorly understood.

![Figure 1.5](image)

**Figure 1.5.** The number of small airways (2-2.5 mm) per lung pair captured by computed tomography (CT) in patients with varying degrees of COPD. Compared to control group the number of small airways was inversely proportional to the severity of COPD (McDonough, et al., 2011).
1.6 Physiological Markers of Small Airways

Physiologically small airway damage results in abnormal gas distribution and mixing termed ventilation inhomogeneity (VI), as well as gas trapping, increased airways resistance ($R_{aw}$) and airflow obstruction. The anatomical position of the small airways ensures that they remain a difficult compartment of the lung area to measure. Physiological testing offers a non-invasive method of assessing the impact of disease on the airways. A summary of techniques to assess the small airways can be found in Table 1. Repeated CT examinations are useful in assessing the small airways although this method is limited not only by radiation exposure but also due to the difficulty in discriminating between airway and vascular abnormalities (Konstantinos, Kostikas and Kontakiotis, 2013). Pulmonary function tests are routinely used to determine small airway damage and therapeutic change with varying degrees of success.

Table 3.1. Different methods of small airway evaluation

<table>
<thead>
<tr>
<th>Methods</th>
<th>Parameters Measured</th>
</tr>
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<tbody>
<tr>
<td>Spirometry</td>
<td>FEF$_{25-75%}$, FVC</td>
</tr>
<tr>
<td>Whole-Body Plethysmography</td>
<td>RV, RV/TLC, $R_{aw}$, $sG_{aw}$, $G_{aw}$</td>
</tr>
<tr>
<td>Impulse Oscillometry</td>
<td>$R_5$, $R_{20}$, $R_5$-$R_{20}$, $X_5$</td>
</tr>
<tr>
<td>Multiple-Breath Washout</td>
<td>$S_{cond}$, $S_{acin}$</td>
</tr>
<tr>
<td>Imaging</td>
<td>HRCT</td>
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</tbody>
</table>

FEF$_{25-75\%}$, mean forced expiratory flow between 25% and 75% of the forced vital capacity (FVC); FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; $R_{aw}$, airways resistance; $sR_{aw}$, specific airways resistance; $G_{aw}$, airways conductance; $sG_{aw}$, specific airways conductance; $R_5$, total respiratory system resistance; $R_{20}$, central respiratory system resistance; $R_5$-$R_{20}$, peripheral respiratory system resistance; $X_5$, lung capacitance; $S_{cond}$, ventilation inhomogeneity in conductive lung zones; $S_{acin}$, ventilation inhomogeneity in acinar lung zones; HRCT, high resolution computed tomography.
1.6.1 Spirometry

Spirometry is the most widely used test in pulmonary disease detection, classification, progression and prognosis, as well as the most common outcome marker in clinical trials. Testing involves the patient in a seated position, wearing a nose-peg, inhaling to TLC before instantaneously exhaling with maximal effort until the lungs reach RV. Spirometry indices (figure 1.6) are reported as flow in litres per second (L/sec) and volume in litres (L) and reflects $R_{aw}$ which is heavily influenced by the distal portions of the lung (Robinson, Goldman and Gustafsson, 2009). FEV$_1$ is a globally accepted endpoint in clinical practice. It is the main physiological marker used to define, classify and track the progression of COPD, as well as the primary endpoint employed in clinical trials to evaluate new COPD treatments (GOLD, 2011; Jones and Agusti, 2006). The measurement is highly repeatable (within 150 millilitres), not time consuming and when smoking cessation has occurred it demonstrates a slower rate of decline (Anthonisen, et al., 1994).

**Flow Volume Loop**

![Flow Volume Loop](image)

**Figure 1.6. Flow volume loop.** The expiratory portion of a flow volume loop illustrating PEF, peak expiratory flow; FEF$_{25}$%, forced expiratory flow at 25% of the forced vital capacity (FVC); FEF$_{75}$%, forced expiratory flow at 75% of the forced vital capacity (FVC); FEF$_{25-75}$%, mean forced expiratory flow between 25% and 75% of the forced vital capacity (FVC); FEV$_1$, forced expired volume in 1 second; FVC, forced vital capacity.
Included as part of the GOLD multifaceted approach to COPD management FEV\textsubscript{1} has been shown to be poorly correlated with patient reported outcomes (PRO’s) and exercise performance (Wolkove, et al., 1989). This is because FEV\textsubscript{1} is reflective of large airway dysfunction, where the proximal airway generations are predominantly affected by turbulent airflow limitation, which only becomes a significantly detectable factor after sufficient progression of the disease (Polak, 2008). This characteristic makes FEV\textsubscript{1} a relatively insensitive tool for assessing distal airway abnormalities where the underlying mechanisms resulting in lung injury manifest early in COPD.

The FEF\textsubscript{25-75}, average flow rate across the middle portion between 25% and 75% of a forced vital capacity (FVC), has higher sensitivity for measuring airway dysfunction compared to FEV\textsubscript{1} and is proposed as a marker of distal airway abnormalities and air trapping (Kraemer, et al., 2005). The use of this measurement in terms of distal lung evaluation is limited due to being highly dependent upon lung volume (FVC). This means that any change in FVC will directly affect the FEF\textsubscript{25-75}. This direct link to lung volume results in both poor intra-test reproducibility and between test variability (Boggs, et al., 1982; Quanjer, et al., 2014).

Overall spirometry is a valuable endpoint in COPD but it does have limitations. It is a poor descriptor of the small airways. The measurement technique is highly dependent upon patient effort and the ability of the operator to coach the correct technique and identify sub-maximal efforts. It can also lead to the patient experiencing symptoms such as headache, dizziness and even syncope during or after testing.
1.6.2 Whole-Body Plethysmography

Plethysmographic assessment allows for the determination of lung volumes and airways resistance. These include total lung capacity (TLC), inspiratory capacity (IC), functional residual capacity (FRC) and residual volume (RV) in addition to characteristics of airway resistance ($R_{aw}$) and its reciprocals termed airway conductance ($G_{aw}$; $1/R_{aw}$), which is achieved by relating air-flow and driving pressure, as well as specific airways conductance ($sG_{aw}$; $G_{aw}/TLC$) which is independent of lung volume.

![Figure 1.7. Whole-body plethysmography.](image)

The airtight compartment allows for the measurement of $R_{aw}$, before calculating FRC as the pressure change in the box due to chest expansion during breathing being equal and opposite to intra-thoracic volume. From this an ERV manoeuvre followed by an inspired vital capacity is performed to calculate the remaining lung volumes.

The measurement was developed in 1956 (DuBois, Botelho and Comroe, 1956) based on Boyle's law, which modelled the lung as a closed compartment where for a fixed amount of gas, the relative changes in volume are equal and opposite to the changes in pressure. The test involves the patient being seated in an airtight chamber (figure 1.7), breathing at tidal volume ($V_T$) through a pneumotach or mass flow...
sensor, whilst wearing a nose clip and cheeks supported by the palms of their hands to improve the measurement of pressure change at the mouth. $R_{aw}$ is measured once stable breathing is achieved (representing FRC), as the patient performs small panting breaths of at least 0.5 L at a rate of 1 per second, which equates to 1 hertz (Hz). The breathing circuit is then occluded, whilst the patient continues the inspiratory and expiratory efforts, which constitutes increases in the chest wall and decreases in intra-thoracic pressure. This increase in chest wall volume increases the chamber pressure, and this pressure change is equal and opposite to intra-thoracic volume and allows for the determination of FRC (Konstantinos, Kostikas and Kontakiotis, 2013). Once the occlusion is removed the patient slowly exhales from FRC to RV before performing an inspired vital capacity (IVC), which allows for the calculations of RV, VC, IC and TLC (figure 1.8).

![Figure 1.8. The assessment of $R_{aw}$ and lung volumes, using whole-body plethysmography. $R_{aw}$ is measured during panting manoeuvres performed after a relaxed tidal breathing baseline has been established. Pressure changes at the mouth are then measured during shallow breaths against an occlusion to determine TGV before an ERV and IVC is performed to calculate RV, IC and TLC. (Wanger, et al., 2005).](image-url)
This test is well validated with standardised guidelines jointly issued by the American Thoracic Society (ATS) and the European Respiratory Society (ERS). Repeatability, as measured by coefficients of variation (CV), has been reported to be 3.5-6.7% for FRC, 9.5-12.4% for RV and 4.5-10% for IC (Cazzola, et al., 2008; Pellegrino, Rodarte and Brusasco, 1998). RV is the amount of air remaining in the lungs after an expired vital capacity (EVC) has been performed. This volume of trapped gas increases with age but is increased further in COPD as airways prematurely close due to occlusion and increased lung compliance. As a result TLC can often be raised (> 120%) signifying hyperinflation, therefore the RV/TLC ratio is a descriptor of gas trapping and estimates the observed increase in RV due to the nature of disease (O'Donnell and Laveneziana, 2006).

When assuming that TLC remains constant FRC and IC have been shown to correlate with PRO’s such as dyspnoea more than FEV₁. Bronchodilator treatment effects have also been demonstrated by a reduction in lung volumes and an increase in exercise capacity. An increase in IC has shown that a single dose of 300 mg of indacaterol, which is one example of a long-acting β₂ agonist, was statistically superior (P<0.05) to 12 mg of formoterol (long-acting β₂ agonist) twice daily, thus decreasing the frequency of medication use for patients (Beier, et al., 2009). Indacaterol 300 mg was also proven to result in a statistically significant reduction in resting hyperinflation (increase in IC and decrease in FRC) after 14 days of treatment in comparison to placebo (Khindri, et al., 2009). In addition to this indacaterol was found to increase exercise capacity both by ergometer endurance time during a constant load exercise challenge as well as dynamic hyperinflation during ergometry at peak exercise. These types of findings are due to an improvement in lung compliance that decreases the work of breathing and reduces the oxygen debt. This combined with an increase of functional strength of the inspiratory muscles aides in improving the respiratory cycle of COPD patients.
In summary plethysmography is an invaluable tool for both clinical practice and clinical trials. The test itself can be time consuming, and is highly dependent on patient effort and technique, which becomes increasingly difficult as obstruction becomes more severe. The equipment has larger cost implications than spirometry but remains an accessible test in most lung function clinics and has standardised guidelines authored jointly by the ATS and ERS. The evaluation of $R_{aw}$ is not specific for small airways and is not captured during tidal breathing ($V_T$) which limits its application in relation to patient’s symptoms and health status in comparison to more sensitive testing of total respiratory resistance such as impulse oscillometry (IOS) (McNulty and Usmani, 2014).
1.6.3 IOS

Tests that are based on $V_T$ analysis require minimal instruction and are physically less demanding in comparison to dynamic and static lung volume measurements. This type of testing provides physiological evidence of disease when the patient is in a relaxed and rested state, thus more closely reflecting lung mechanics in daily life. One such method of analysing the state of the respiratory system during $V_T$ is impulse oscillometry (IOS). The test is based upon an adaptation of Ohm’s law where current through a conductor between two points is directly proportional to the potential difference across the two points. When this is adapted for airflow, IOS superimposes frequency waves to the respiratory tract to investigate the resistance to airflow through different portions of the lung (Paredi, et al., 2010). The test involves the subject being in a seated position, whilst wearing a nose-clip, and placing their hands on their cheeks for support. The subject breathes through a pneumotachograph in a relaxed manor whilst their $V_T$ is recorded for 30-90 seconds. During this time a loudspeaker produces multiple oscillating frequency waves of around 5 to 30 Hz to the respiratory tract (figure 1.9). Lower frequencies have a slower cycle time and a larger wavelength that enables them to penetrate the periphery of the lung, providing information on the entire respiratory tract. The higher frequency signals have a faster cycle time and a shorter wavelength and thus cannot penetrate beyond the large airways.
Figure 1.9. A simplified lung model demonstrating the impact of various frequency waveforms on different portions of the lung. High frequency sound waves only penetrate the large airways and so represent the resistance in the central airways. The low frequencies are able to penetrate both the large and small airways and represent the total resistance of the airways. (Horsley and Siddiqui, 2015).

The instantaneous pressure-flow relationship, measured at the mouth at varying frequencies, are analysed in a Fourier transformation to determine impedance ($Z_{rs}$), which reflects the mechanical properties of the respiratory tract (McNulty and Usmani, 2014). Impedance is made up of an in-phase, termed respiratory system resistance ($R_{rs}$) and out-phases, referred to as respiratory system reactance ($X_{rs}$). In the presence of distal airway obstruction $R_{rs}$ denoted at low frequencies is raised in comparison to the $R_{rs}$ at higher frequencies, and is termed frequency dependent resistance (figure 1.10). Once the $R_{rs}$ is determined for multiple frequencies peripheral lung resistance can be estimated by subtracting the total respiratory system resistance from the large respiratory system resistance ($R_5-R_{20}$). $X_{rs}$ comprises of 2 elements that are dependent upon frequency. At higher frequencies (>20 Hz) $X_{rs}$ is positive in sign and represents the inertive force needed to move air in the conducting airways. At low frequencies (<5 Hz) $X_{rs}$ is negative in sign and indicates the capacitance of the lung periphery, which incorporates the elastic properties and its ability to store energy. It has
been reported that the point at which the elastic and inertive forces are equal and opposite (at which reactance is zero) corresponds to frequencies between 8-12 Hz in healthy controls. This point is termed resonance frequency ($F_{res}$) (Konstantinos, Kostikas and Kontakiotis, 2013). Any reduction in capacitance resulting from increased compliance and emphysematous destruction will lead to further negative values of reactance at 5 Hz and a reduction in $F_{res}$ (Horsley and Siddiqui, 2015).

Figure 1.10. Respiratory impedance of the bronchial tree. Respiratory impedance is the product of respiratory system resistance and respiratory system reactance (Horsley and Siddiqui, 2015).
In obstructive disease IOS has been shown to display increased values of resistance in the presence of normal spirometry, correlate with health status measured by Saint George’s Respiratory Questionnaire (SGRQ) in COPD; and used as an alternative outcome marker to FEV$_1$ during bronchial challenge testing and bronchodilator treatment (Borrill, et al., 2008; Brochard, et al., 1987; Haruna, et al., 2010). Although small airway resistance is independent of lung volume, problems can arise measuring R$_{rs}$ in COPD patients with known hyperinflation as resistance from the larger airways become more influential which may reduce the sensitivity of the measurement. Non-uniform VI has also been shown to be apparent even when Z$_{rs}$ remains within normal limits in obstructed patients (Habib and Lutchen, 1991). One reason for this may be due to the use of 5Hz as the lowest frequency. This value could be too high to represent oscillation effect on the acinar portion of the lung as relative resistance falls with each lung generation, but current technology restricts the ability to obtain valid measurements at frequencies <5Hz as they are affected by spontaneous breathing and thus may not be truly representative of the diffusive acinar component of the small airways (King, et al., 2005).

In summary IOS has greater sensitivity for the smaller airways than spirometry and plethysmography. It is a simple test to perform, which does not overly exert the patient and reflects the respiratory mechanics at rest during V$_T$. The test is mostly confined to the research setting due to the additional cost of the equipment and the expert training needed to evaluate the results and relate them to disease and pharmacological intervention.
1.6.4 Multiple-Breath Washout Testing

One other method of $V_t$ analysis, that has seen resurgence over recent years, is the multiple-breath washout (MBW). The test conceptually assumes that the lung is divided up into ventilation units which all contribute to overall efficient ventilation. It has been found that one of the earliest physiological impacts of disease known to occur is ventilation inhomogeneity (VI). This is the uneven emptying of lung units that affects the distribution of gas exchange (Strömberg, 2000). It is often present prior to the development of clinical symptoms and is caused by tissue damage in the distal parenchyma and the effect it has on lung compliance, airway resistance and the rate of convective airflow to each unit (Horsley and Siddiqui, 2015). These factors contribute to the overall heterogeneity of lung ventilation.

It has long been known that ventilation heterogeneity is apparent in healthy controls due a number of physiological processes. Gas distribution can be affected by $V_t$, which becomes more even as $V_t$ increases and differences in regional static transpulmonary pressure, becomes more distinct as volume increases. During inspiration of a gas non-uniformed lung expansion initially occurs with greater expansion of the large airway in comparison to the small airways. The level of expansion becomes more uniformed as inspiration volume increases. In addition to these processes the small airways receive relatively more of the inspired gas volume, which when added to variation found in regional lung emptying can further affect ventilation. The tissue damage, physiological adaptations and inflammation found in restrictive and obstructive lung diseases lead to further ventilation heterogeneity (Milic-Emili, et al., 1966).

Multiple-breath washout is able to determine the degree of VI among different parallel pathways of the lung during tidal breathing at FRC and may be able to distinguish between VI originating in the proximal lung (where gas transport is largely via convection) and the acinar portion of
the lung (where gas transport is influenced by a convection-diffusion interaction) (Verbanck, et al., 2003).

Originally proposed in 1941 (Cournand, et al., 1941), MBW involves the breath-by-breath assessment of the elimination of an inert gas from the lungs, which is completed when the alveolar concentration (Cet) falls below 1/40th of the starting concentration for at least 3 consecutive breaths (figure 1.11). The test requires minimal co-operation and is less demanding for subjects to perform compared to static lung volumes and gas transfer techniques. One consideration is that due to being measured at end-expiratory volume the stability of breathing pattern and functional residual capacity (FRC) prior to, and during the test is important in obtaining accurate washout data (Cournand et al, 1941; Pillow et al, 2004).

Figure 1.11. Multiple Breath washout curve. In MBW volume (A) and decreasing inert gas concentration (B) are represented as a time series display until 1/40th of the starting concentration is reached.
MBW FRC calculation is outlined in equation 1, where cumulative expired volume (CEV) is the sum of all expiratory $V_T$’s corrected for the equipment dead space. For example if the total expired volume of a subject was 3.8L, with a starting nitrogen concentration of 79% and end concentration of 2%, then FRC would be $3.8/(0.79-0.02) = 4.94$L.

$$FRC = \frac{\text{Cumulative expired volume}}{\text{Gas Concentration (Start of Test – End of Test)}}$$

Equation 1.

The ERS consensus statement on inert gas washouts indicates that tidal breathing ($V_T$) should be stable and that the mean, standard deviation (SD) and co-efficient of variation (CV) of 3 FRC measurements are to be reported. This value should ideally lie within 10% of the highest FRC and variances $\geq 25\%$ should be excluded (Robinson, et al., 2013).
1.6.4.1 Indices of Ventilation Inhomogeneity

The simplest and most frequently reported parameter derived from MBW is the lung clearance index (LCI), which due to being a ratio of measurements recorded during testing has no units. LCI equates to the number of FRC volumes, or “lung turnovers (TO)” (also a ratio of cumulative expired volume and FRC, and so has no units), required to reduce the inert gas to 1/40\(^{th}\) of the original starting concentration as seen in figure 1.11 (Bouhuys and van, 1962; Kent, et al., 2014). LCI characterizes global ventilation abnormalities in the lung, with greater LCI values observed in the presence of greater VI. It is calculated as outlined in equation 2. The use of TO instead of using breath number is important as it allows for the comparison of subjects with different lung volumes (Crawford, et al., 1989). The revived interest in MBW has demonstrated LCI to be more repeatable and sensitive than FRC whilst simplifying washout interpretation and reducing the influence of changes in lung volumes (Singer, et al., 2012).

\[
LCI = \frac{Cumulative\ expired\ volume}{Functional\ Residual\ Capacity}
\]

Equation 2.

Longitudinally, in healthy volunteers, LCI has demonstrated a low inter-centre variability compared to FEV\(_1\) and has been found to be repeatable over a 7-month period in a multi-centre setting with a mean within test coefficient of variation (CV) of 4.9% in healthy controls and 5.6% in cystic fibrosis (CF) patients (Fuchs, et al., 2012). In CF research, LCI has been shown to be a global measure of VI and has been measured alongside spirometry values and compared to high-resolution computed tomography (HRCT) results. HRCT was shown to identify structural abnormalities in 61% of patients, with LCI measured by Sulphur Hexafluoride (LCISF\(_6\)) detecting 57% (Gustafsson, et al., 2008). This was in contrast to Fev\(_1\) and FEF\(_{25-75}\), which were only able to identify abnormalities in 16% and 39% respectively. Such cross sectional
research led to a number of prospective single centre studies that confirmed these results. Ellemunter et al (2010) used a database of CF outpatients to identify 41 subjects whom had a normal FEV₁ (\(\geq 80\%\) predicted). Prior to an ultra-low dose CT scan, subjects performed multiple-breath washout using Sulphur Hexafluoride (MBW\(_{SF6}\)). At this point the number of participants was reduced to 34 after 7 subjects failed to produce technically acceptable MBW\(_{SF6}\) data due to leaks and/or unstable breathing patterns. CT results distinguished 76.5% as having lung abnormalities with an abnormal CT (figure 1.12). The positive predictive value of an increased LCI representing structural lung abnormalities was 88% with a normal LCI indicating normal lung structure being 63%. Over recent years LCI has been successfully used as a clinical trial endpoint in the development of the CF drug Ivacaftor. A greater clinically significant change was detected in LCI in comparison to FEV₁ in patients with mild cystic fibrosis (Davies, et al., 2013).

![Figure 1.12. LCI Z-score vs. CT-score. Vertical line: lower limit of normal for CT-score (>23 points). Horizontal line: upper limit of normal for LCI Z-score (Z2) (Ellemunter, et al., 2010).](image)

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In addition to markers of global VI it is possible to generate measures of convection and diffusion mechanisms that underpin it. VI is non-uniform and may affect different compartments of the lung along the airway. In the central airways gas transport is primarily driven by convection and in the acinar portions of the lung transport is predominantly driven by diffusion. The point at which these 2 mechanisms meet is termed the ‘convection-diffusion front’. Convective ventilation is greatly affected by conductive airway narrowing due to increased resistance and compliance that in turn causes inefficiency of gas transport among the subtended lung units connected to the conductive airways. Mucus plugging and structural changes of the acinar airways, which increases the respiratory dead space, impact on the diffusive gas transport and both of these mechanisms change the point of the diffusion-convection front.

The expiration of each VT can be divided into 3 phases (figure 1.13). Phase I represents the anatomical dead space where no gas mixing occurs. Phase II is the point at which the dead space gas and alveolar gas meet which is evident by an increase in inert gas concentration. Phase III is expressed as a plateau and is representative of the alveolar end expired gas concentration. The steeper the gradient of this plateau the greater the VI as a result of prolonged emptying of the distal lung units involved. This evaluation of each phase III slope is termed SIII analysis and in order to account for variation in each breath and the cumulative increase in slope gradient, the mean expired gas concentration is applied to each SIII slope to allow comparisons between individual expirations. This is referred to as SnIII and is plotted against TO (McNulty and Usmani, 2014).
Figure 1.13. $S_{III}$ analysis of a single expiration during MBW. Phase I, anatomical dead; Phase II, where dead space gas and alveolar gas meet; Phase III, alveolar and expired gas plateau (Stuart-Andrews, et al., 2012).

Theoretical modeling has been applied in order to define measures of the convective and diffusive properties of expiration, termed $S_{cond}$ and $S_{acin}$ respectively (Verbanck, et al., 1997). $S_{cond}$ is a ratio of the increase in the $S_{III}$ corresponding with TO 1.5 through to 6. For the diffusive properties a calculated correction to discount the conductive contributions from the first breath measured is applied by subtracting the product of the TO of the first breath and $S_{cond}$ from the first breath of the $S_{III}$. This is termed $S_{acin}$. Current commercial systems that report these values compute the mathematics which underpin these proposed values within the equipment software and so are not derived by the operator which may make verification more difficult.

LCI has been shown to be highly repeatable measurement in healthy volunteers (Downie, et al., 2007; Singer, et al., 2012) and CF (Horsley, et al., 2008a) with larger variation evident in $S_{cond}$ and $S_{acin}$. In Asthma, (Farah, et al., 2012), found that $S_{cond}$ and $S_{acin}$ correlated with a change in symptoms measured by the Asthma Control Questionnaire (ACQ) after treatment with ICS. The few recent studies have used VI analysis
in COPD, demonstrated the ability of MBW to differentiate between healthy and COPD subjects as well as separating COPD subjects based on smoking pack year history (Verbanck, et al., 2004). In this study MBW, Spirometry and whole-body plethysmography were able to distinguish between healthy and COPD patients but when a cohort of smokers with normal FEV$_1$ were investigated $sG_{aw}$, $S_{cond}$ and $S_{acin}$ were still found to be abnormal even in smokers with a pack year history as low as 10. Once emphysematous lesions developed, $S_{acin}$ further increased which did not reduce after smoking cessation although $S_{cond}$ did reduce by 30% after 1 week of cessation. These findings highlight the sensitivity of MBW testing in obstructive diseases and infer that the irreversible damage caused by smoking begins to impact on the acinar airways before symptomatic and spirometric evidence is apparent. This emphasises the importance of early detection to enable smoking cessation as well pharmacological interventions to be implemented to limit the amount of damage and positively impact upon the health status of smokers with and without COPD.

The choice of the inert gas is an important consideration as this can have an impact upon results and limit the comparison of values across equipment utilizing different gases. The chosen gas can be endogenous or exogenous, should be safe for inhalation and void of gas exchange into the blood and tissues. Early studies into VI were conducted using multiple-breath nitrogen washout (MBW$_{N2}$). The patient inhaled 100% oxygen ($O_2$) and the subsequent decrease in Nitrogen ($N_2$) is plotted until < 2% is reached (1/40$^{th}$ of starting concentration). As a consequence using nitrogen ($N_2$), which is already resident in the lungs, there must be time allowed for $N_2$ re-equilibration between measurements. This can lead to an overall extended test duration for 3 measurements when implemented in obstructive disease groups in comparison to the estimated 7 minute test time (mins) for healthy controls (HC) (Wanger, et al., 2005). The Mass Spectrometer is the gold standard apparatus for washout testing, and allows for exogenous gases such as Sulphur hexafluoride (SF$_6$) to be used. This requires a wash-in
phase, until equilibrium is reached, and the washout is then measured during inhalation of room air until a concentration of at least 1/40th of the starting concentration is achieved (Robinson, Goldman and Gustafsson, 2009). The limitations of this method in clinical practice have been expense and the portability of the equipment and the added expense of the SF₆ due to the amount used for each test. Recently manufacturers have released smaller, less expensive washout equipment designed for both N₂ (Exhalyzer® D, EcoMedics AG, and Duernten, Switzerland) and SF₆ (Innovision AS, Odense, Denmark), with the latter proving to be a valuable endpoint in clinical trials conducted into new cystic fibrosis (CF) treatments.

The Innocor™ is a compact modified inert gas analyser that uses photoacoustic spectroscopy to evaluate SF₆ washout curves. Wearing a nose-peg the patient is connected, via a filter, to a flowmeter with a gas sample line to which a flow past circuit with an SF₆ (0.2%) gas supply are connected (figure 1.14). The wash-in phase consists of tidal breaths of 0.2% SF₆ until the concentration in the lungs reaches a steady state (a difference of < 0.004 between breaths); at which point the washout phase begins with the flow-past being manually removed thus leaving the patient breathing room air at the same Vₜ. Breath-by-breath measurements of flow and SF₆ are then taken until the expired SF₆ concentration falls to 1/40th of the original concentration.

Figure 1.14. Innocor™ SF₆ apparatus. SF₆ runs along a flow past circuit connected to a flow metre and gas sample line. These are connected to the gas analyser and the patient is connected to the flow metre via a filter.
Horsley et al (2008) described this technique in 48 healthy volunteers and 45 CF subjects (12 children, 33 adults). The Innocor™ was shown to display a superior signal:noise ratio to a mass spectrometer throughout the washout, despite using 1/20th of the concentration of SF₆ that mass spectrometry requires. This is important not only for reducing the greenhouse gas that is SF₆ but also in reducing the cost per test. The Innocor™ was able to produce FRC results within an accepted variance reported for whole body plethysmography and the washout was highly repeatable across the 3 subject groups with an intra-visit mean CV of 3.6%-5.4%. Both FEV₁ and MBW were able to differentiate between healthy and CF subjects with the MBW able to further differentiate CF patients with abnormal gas mixing when FEV₁ was within normal range. Research has been conducted to evaluate MBW further in CF and assess its ability to differentiate between CF patients colonized with chronic Pseudomonas aeruginosa versus non-colonized (Belessis, et al., 2012). The study performed MBW_{SF₆} in 47 CF children, 17 (36%) of whom presented with airway infection, including 7 (15%) being colonized with Pseudomonas aeruginosa, and 25 healthy children. LCI_{SF₆} was raised in both colonised and non-colonised patients compared to healthy controls and was further elevated in children with Pseudomonas (mean 7.92, SD 1.16) than in children without Pseudomonas (mean 7.02, SD 0.56) with P = 0.038. These findings indicate that MBW_{SF₆} may be employed in a clinical setting to aid in the early detection and monitoring of CF, with and without airway infection and inflammation. This will aid in clinical decision making and help tailor treatment plans on a more individual basis as early as possible.

The Exhalyzer® D is an open circuit N₂ system which employs Dalton’s law of partial pressure to indirectly calculate the concentration of N₂ from the concentration of O₂ and CO₂ measured during each of the tidal expirations. The test involves the patient wearing a nose-peg connected to an ultrasonic flow meter to monitor flow and determine volume. The device uses visual feedback to ensure the subject breathes at a V_T of 1L. Once stable breathing has been defined the patient is automatically
switched from room air to a supply of 100% O₂. Breath by breath measurements of O₂ and CO₂ are continuously made in order to compute the N₂ concentration. The test is complete when < 2% N₂ is detected for 3 consecutive breaths.

A study investigated the use of MBW₅₂ in comparison to the gold standard mass spectrometry using SF₆ (Jensen, et al., 2013). Included were 44 subjects aged 3-18 years (76 CF and 68 healthy controls) with each MBW method conducted in triplicate. Throughout testing 20% of the CF subjects and 9% of the healthy controls were unable to perform technically adequate tests due to leaks or irregular breathing patterns during the washout. Differences in FRC and LCI (figure 1.15) were not statistically significant between the two systems in healthy subjects (LCI mean difference 0.61, limits of agreement -0.7 to 1.9; FRC difference 0.21, limits of agreement -0.15 to 0.56). In patients with CF however there was a clear bias with MBW₅₂ higher than MBW₆ for both LCI (mean difference 1.41, limits of agreement -2.4 to 5.2) and FRC (mean difference 0.33, limits of agreement 0.27 to 0.38). Despite this both systems produced FRC results lower than whole body plethysmography (FRC_Pleth) with the difference potentially measuring the amount of trapped gas. In the CF group there was a significant increase in the number of breaths required to complete the MBW₅₂ washout that correlated with obstruction severity and may serve as a reason for the higher LCI and FRC values compared to SF₆. These findings indicate that although both systems were repeatable and provided similar descriptive values between the 2 groups, the systems are not interchangeable and so caution should be taken when interpreting different washout results.
Figure 1.15. Comparison of $N_2$ and $SF_6$ washouts. Bland Altman Plots comparing Lung clearance index in healthy subjects (A) and CF subjects (B); and FRC values in healthy subjects (C) and CF subjects (D) (Jensen, et al., 2013).

Both the Exhalyzer D® and the Innocor™ have similar analyser response times ($N_2$ 110ms vs. $SF_6$ 154ms) and although the $N_2$ method does not require a wash-in phase, long washout times result in increased equilibration time between tests. In addition to this however, inhalation of 100% $O_2$, has been shown to alter breathing patterns in combination with certain physiological and anatomical states and may not be appropriate in obstructive diseases such as COPD (Schibler, et al., 2000). Furthermore although $N_2$ is resident in both well ventilated and poorly ventilated parts of the lung, potentially indicating a more accurate representation of equilibration, it is not a truly inert gas. In disease groups where washout times are already increased this may result in $N_2$ excretion from the blood and tissues potentially increasing the error margin for washout indices as well as the washout time further (Nielsen, Nielsen and Horsley, 2013). These discrepancies between systems make it difficult for universally accepted cut of values for expected LCI values in disease groups to be implemented.
1.7 Hypothesis, Aims and Objectives

COPD is associated with inefficient gas mixing which occurs early in the disease and becomes more heterogeneous with progression. LCI measured, as part of a multiple breath washout test may be a sensitive marker for determination of ventilation abnormality and able to identify those patients with spirometry within normal limits. COPD is also associated with fixed airway obstruction. This is evident when there is little or no change in FEV$_1$ post administration of a bronchodilator. LCI may detect improvements in ventilation heterogeneity that may occur post treatment in the absence of spirometric changes. As part of the progression of COPD, exacerbation frequency and severity increase. During an exacerbation spirometry has not been shown to reduce significantly upon onset, or significantly change during recovery after treatment with antibiotics and/or oral steroids. LCI may provide a sensitive measure for defining an exacerbation and monitoring recovery.

1.7.1 Hypotheses

LCI$_{N2}$ will highlight VI abnormality in mild COPD subjects when FEV$_1$ % predicted is within the normal range. LCI$_{N2}$ will reflect the tissue damage in the lung periphery that occurs prior to symptom onset and spirometric detection. LCI$_{N2}$ will differentiate between COPD subjects and healthy controls. In COPD the chronic inflammation and thickening of the airway walls (bronchitis), along with destruction of alveoli walls (emphysema) will lead to increased resistance and decreased elastic recoil, with an increase in mucus secretions blocking the lumen of the small airways. This will result in an increased LCI$_{N2}$. LCI$_{N2}$ will differentiate between COPD severities classified by the GOLD criteria. As the bronchitis and emphysema components progress, gas mixing will be further affected leading to an increase in LCI$_{N2}$. LCI$_{N2}$ will correlate with R$_{5}$-R$_{20}$. The level of VI measured by LCI$_{N2}$ will be associated with the increase in peripheral resistance measured by R$_{5}$-R$_{20}$. MBW test durations will be significantly increased as COPD severity increases. As COPD progresses ventilation becomes more inefficient. This will impact
upon the ability of the subject to washout the inert gas effectively and lead to extended test times. COPD exacerbation will be associated with a fall in $\text{FRC}_{\text{N}_2}$ % due to reduced lung volumes that are tidally ventilated and an increase in $\text{LCI}_{\text{N}_2}$. This would then resolve at the end of treatment.

1.7.2 Primary Aims

1. Evaluate the sensitivity of LCI as a clinical measure of airways disease in COPD.

2. To assess the short-term intra-test repeatability and long-term inter-test reproducibility of MBW derived values for FRC % and LCI in a cohort of COPD subjects.

3. To assess the agreement in MBW parameters measured by different methods: Nitrogen ($\text{N}_2$) Vs Sulphur Hexafluoride ($\text{SF}_6$).

4. To investigate the practical implementation of MBW in a cohort of COPD subjects: An assessment of success rates and total test duration.

5. To determine the impact of exacerbations on ventilation inhomogeneity and assess the sensitivity of MBW parameters to monitor recovery.

1.7.1 Primary Objectives

1. To measure LCI in a cohort of COPD and Healthy subjects and examine the relationship of VI between the groups compared to FEV$_1$ (Chapter 3).

2. To investigate the sensitivity of LCI across COPD severity using GOLD Classifications (Chapter 3).

3. To identify correlations in COPD between LCI and other physiological tests such as IOS, gas transfer and whole body plethysmography (Chapter 3).
4. To evaluate the therapeutic effect of salbutamol on LCI in COPD (Chapter 3).

5. To determine the $\text{LCI}_{\text{N2}}$ coefficient of variation (CV) across 3 technically acceptable MBW manoeuvres in healthy controls and COPD subjects and compare the short-term intra-test repeatability (Chapter 4).

6. To evaluate the long-term reproducibility of MBW parameters in COPD subjects across 2 testing sessions separated by ≥ 24 hours (Chapter 4).

7. To determine the agreement between FRC values measured using $\text{MBW}_{\text{N2}}$ and Whole body plethysmography in a group of COPD subjects (Chapter 5).

8. To determine the agreement between FRC and LCI values measured using $\text{MBW}_{\text{N2}}$ and $\text{MBW}_{\text{SF6}}$ in a group of COPD subjects and healthy controls (Chapter 5).

9. To compare FRC values of COPD subjects who performed $\text{MBW}_{\text{N2}}$, $\text{MBW}_{\text{SF6}}$ and Whole body plethysmography (Chapter 5).

10. To review the success rates of MBW, IOS, Spirometry, gas transfer and whole body plethysmography in the COPD and Healthy subjects (Chapter 6).

11. To evaluate the total test time of $\text{MBW}_{\text{N2}}$ and $\text{MBW}_{\text{SF6}}$ in COPD and healthy controls (Chapter 6).

12. To compare $\text{FRC}_{\text{N2}} \%$, $\text{LCI}_{\text{N2}}$, $\text{FEV}_1 \%$ and $\text{CAT}^{\text{TM}}$ scores at exacerbation onset (E0), after 2 weeks post treatment (E2) and after 6 weeks during recovery of an exacerbation (Chapter 8).
1.7.1 Exploratory Objectives

1. To measure LCI and 6 minute walk test distance in a cohort of COPD subjects to identify a relationship between VI and exercise capacity (Chapter 3).

2. To investigate the sensitivity of $\text{Scond}_N$ and $\text{Sacin}_N$ to differentiate COPD severity as defined by GOLD Classifications (Chapter 7).

3. To identify correlations in COPD subjects between $\text{Scond}_N$ and $\text{Sacin}_N$ and other physiological tests such as IOS, gas transfer and whole body plethysmography (Chapter 7).

4. To determine the $\text{Scond}_N$ and $\text{Sacin}_N$ coefficient of variation (CV) across 3 technically acceptable MBW manoeuvres in healthy controls and COPD subjects (Chapter 7).

5. To evaluate the reproducibility of $\text{Scond}_N$ and $\text{Sacin}_N$ in COPD subjects across 2 testing sessions separated by $\geq 24$ hours (Chapter 7).

6. To compare $\text{Scond}_N$ and $\text{Sacin}_N$ at exacerbation onset (E0), after 2 weeks post treatment (E2) and after 6 weeks during recovery of an exacerbation (Chapter 8).
CHAPTER 2

2.0 General Methods

2.1 Subjects
Subjects were recruited from a clinical list based at the Medicines Evaluation Unit, University Hospital of South Manchester, United Kingdom. COPD patients were required to be aged > 40 years with a physician’s diagnosis of COPD in accordance with the 2011 GOLD guidelines. This included a smoking history ≥ 10 pack-years with typical symptoms (one or more of productive cough, breathlessness and wheeze) and evidence of airflow obstruction (FEV₁/FVC < 0.7). Healthy volunteers were required to be non-smokers, classified as a smoking pack history < 10 pack years, aged > 18 years with no evidence of airflow obstruction (FEV₁/FVC > 0.7). Exclusion criteria consisted of a history of cancer, significant respiratory disease such as asthma, alpha-1-antitrypsin deficiency, active tuberculosis, aspergillos, sarcoidosis, any other inflammatory diseases such as rheumatoid arthritis, Crohn’s disease, ulcerative colitis, coeliac or any other co-morbidity which was deemed by a physician to affect any of the tests. Exclusion criteria also included any respiratory tract infections and/or exacerbations within 6 weeks of the clinic visit. An exacerbation was defined as when a subject displayed 2 major or 1 major and 1 minor symptom for 2 days. Major symptoms included increased breathlessness, increased sputum colour or increased sputum amount. Minor symptoms included a cold, increased wheeze or chest tightness, sore throat, increased cough or fever. The local ethics committee approved the study before commencement. Written informed consent was obtained from each subject prior to each study, all of which were conducted in accordance with the International Conference on Harmonization of Good Clinical Practice Guideline and the Declaration of Helsinki.
2.3 Study Design

The aims and objectives were investigated using four studies all of which were conducted at the Medicines Evaluation Unit, Manchester, UK. Prior to each visit subjects withheld caffeine and alcohol for ≥ 24 hours and short acting inhaled β2-agonists (SABA), where appropriate, for a minimum of 8 hours prior to spirometry except for in study 4 where this was dependent upon patient symptoms and treatment plan. All COPD subjects had a physician diagnosis of COPD and had been on stable medication for at least 3 months prior to enrollment. Safety for all subjects was assessed through the monitoring of adverse events (AE’s), physical exams and vital signs. A flowchart of the patients recruited and studies are outlined in figure 2.1.

![Flowchart of studies](image)

**Figure 2.1. Workflow of studies to investigate the aims and objectives.**
2.4 COPD Assessment Tool (CAT)

The CAT questionnaire is made up of 8 items, each scored on a numeric scale of 0 (no impact) to 5 (very severe impact) and can be found in appendix 1. Total CAT scores range from 0-40 with each item weighted equally for the final score. Scores for the individual items within the questionnaire provide insight into the relative influence that the different components of COPD have on a patient’s life and highlight areas that can be addressed through intervention (Jones, et al., 2009; Mackay, et al., 2012). A score of up to 5 has been found to be the upper limit of normal in healthy non-smokers, < 10 is low, 10-20 is a medium score, >20 is high and >30 is a very high score (Jones, Tabberer and Chen, 2011). Based on studies where CAT has been found to strongly correlate with Saint Georges Respiratory Questionnaire (SGRQ) it has been reported that a score change of 2 may indicate a minimum clinically important difference (MCID) (Jones, et al., 2011). The questionnaire is recognised by the GOLD committee as a tool which when applied collaboratively with lung function and exacerbation frequency allow accurate determination of disease severity (GOLD, 2011).

2.5 Spirometry

Maximum expiratory flow volume measurements were performed using a Sensormedics Vmax spirometer (Sensormedics Corporation, Yorba Linda CA, USA) that was calibrated daily using a 3L syringe after corrections for ambient temperature, barometric pressure and relative humidity. Spirometry was performed according to the ATS/ERS guidelines (Miller, et al., 2005) that required subjects to forcibly inhale to total lung capacity (TLC) then immediately exhale to residual volume. The tests were performed in triplicate with the subjects in a seated position while wearing a nose-clip. FEV₁ and FVC readings were recorded and related to the reference values of the European Community for Coal and Steel (Quanjer, et al., 1993). Airflow obstruction was defined by FEV₁/FVC <0.7 and severity classified by post bronchodilator FEV₁ % predicted according to the GOLD (2011)
guidelines. Post bronchodilator FEV\textsubscript{1} was measured after administration of 400 µg salbutamol (Miller, et al., 2005).

### 2.6 Whole-Body Plethysmography

Lung volumes were measured using a constant volume whole body plethysmograph (Autobox 6200 DL, Sensormedics, Yorba Linda CA, USA). It was calibrated daily prior to use after corrections for ambient temperature, barometric pressure and relative humidity. The mass flow sensor was calibrated using a 3L syringe and a pressure and leak calibration performed on the Autobox. The test is based on Boyle’s law, which modelled the lung as a closed compartment where for a fixed amount of gas, the relative changes in volume are equal and opposite to the changes in pressure (DuBois, Botelho and Comroe, 1956). Applying this law allows the estimation of the following; airways resistance (R\text{aw}), airways conductance (G\text{aw}), functional residual capacity (FRC\text{Pleth}), inspired vital capacity (VC), residual volume (RV), inspiratory capacity (IC) and total lung capacity (TLC).

Subjects sat inside the plethysmograph wearing a nose clip and formed a tight seal around the rubber mouthpiece while supporting their cheeks. The test commenced with breathing at tidal volume (V\text{T}) until baseline FRC was established. Subjects then performed a series of ‘shallow breathing’ manoeuvres at a rate of approximately 1 Hz, which is equivalent to 60 per minute to specific assess airways resistance (sR\text{aw}) which is the flow-work needed to complete the manoeuvre (Criece, et al., 2011). After at least three flow/box pressure loops were obtained, the shutter was then closed and at least three mouth pressure/box pressure loops were recorded to determine thoracic gas volume (TGV). At this point in the test the software performs multiple calculations. Firstly it measures the airway resistance independent of lung volume sR\text{aw} and calculates the conductance of the airway independent of lung volume, termed sG\text{aw} (\frac{1}{sR\text{aw}}) (Topalovic, et al., 2015). It then simultaneously estimates airways resistance corrected for lung volume (R\text{aw}), as \frac{sR\text{aw}}{TGV}.
TGV is then adjusted by the software for the volume of $V_T$ prior to occlusion that results in a value of $FRC_{pleth}$. Immediately after the completion of the closed shutter panting the subject exhaled to residual volume (RV), then performed an inspired vital capacity (IVC) to determine inspiratory capacity (IC) and total lung capacity (TLC). TLC and RV were calculated as follows:

$$RV = TLC - \text{highest VC} \quad TLC = RV + \text{highest IVC}$$

Equation 3.

In accordance with the ATS/ERS guidelines (Wanger, et al., 2005) at least 3 reproducible $FRC_{pleth}$ measurements were obtained with a mean value reported for all indices. A reproducibility criterion of +/- 5% from the mean value was applied to TLC and FRC values and a criterion of ±10 % was applied to $R_{aw}$ and $sG_{aw}$. The two highest technically acceptable VC’s had to be within 150 millilitres (mls) of each other and the highest value reported to minimise the underestimation of TLC. Predicted values of European Community for Coal and Steel were used (Quanjer, et al., 1993).

Erroneous manoeuvres were discarded. Quality control (QC) checks of respiratory panting loops consisted of visually inspection to ensure they were complete closed loops. Loops had to be ±0.5 L.s$^{-1}$, with $sR_{aw}$ loops examined according to total specific resistance ($sR_{tot}$) (Islam and Ulmer, 1974), where the maximum shift volumes of inspiratory and expiratory portions were used for $sR_{aw}$ estimation. This method of analysis was chosen due to its sensitivity to partial obstruction of the peripheral airways (Goldman, Smith and Ulmer, 2005).
2.7 Impulse Oscillometry (IOS)

The impedance of the total respiratory system ($Z_{rs}$) was measured using a Masterscreen impulse oscillometer (IOS; Erich Jaeger, Hoechenberg, Germany) which was calibrated daily before subject use with corrections for ambient temperature, barometric pressure and relative humidity. The pneumotachograph was calibrated using a 3L syringe and reference impedance with a known resistance was used to check pressure and frequency measurements. The test involves the delivery of multiple frequency waveforms to the airway using a loudspeaker that allows flow to be generated from the different pressure oscillations. The subjects are sat in a relaxed upright position, wearing a nose-clip, supporting their cheeks to reduce upper airway shunting whilst impulses were applied at 0.2-second intervals during tidal breathing for at least 30 seconds. A frequency range of 5 to 35 Hz was used to determine the resistance ($R_{rs}$) and reactance ($X_{rs}$), components of $Z_{rs}$. Frequency measurements outside of this range suffer from reduced signal quality at low frequencies (<5 Hz) using current technology. Wave propagation becomes dependent on physiological size and compliance of the airways at high frequencies (>100 Hz)(Pillow, et al., 2005). Analysed measurements consisted of resistance at 5 Hz ($R_5$), which reflects total respiratory system resistance, and resistance at 20 Hz ($R_{20}$), which corresponds to proximal respiratory system resistance. The reduction in resistance from $R_5$ to $R_{20}$ was used as a surrogate marker of peripheral respiratory system resistance. Quantification of the energy storing ability of the lung periphery was assessed by respiratory system reactance at 5 HZ ($X_5$). For measurements of $R_{rs}$ and $X_{rs}$ the mean value from 3 measurements were reported. Each of the 3 measurements did not vary greater than 10% from the mean value. Coherence ($\gamma^2$) was monitored after each attempt to determine signal quality. $\gamma^2$ was deemed acceptable when $> 0.6$ Hz for frequencies $> 5$ Hz and $> 0.9$ for frequencies $> 20$ Hz (Oostveen, et al., 2003).
2.8 Multiple-Breath Washout (MBW)

2.8.1 MBW utilizing Nitrogen ($\text{MBW}_{\text{N}_2}$)

$\text{MBW}_{\text{N}_2}$ was performed as previously described by Jensen et al (2013) using an open circuit, bias flow system (Exhalyzer® D, EcoMedics AG, and Duernen, Switzerland) and associated software (SpirowareH 3.1 EcoMedics AG). The device uses an ultrasonic flow sensor (Spiroson1, ndd Medical Technologies, Zurich, Switzerland), which contained two ultrasonic transducers mounted on opposite sides of the flow tube that emit ultrasonic pulses through the inspired and expired air. The affected transit time of the ultrasound, related to gas velocity of the air-flow, allowed measurement of flow and volume (Fuchs, et al., 2006). $\text{N}_2$ is measured indirectly using Dalton’s Law of partial pressures when direct measurements of $\text{O}_2$ and $\text{CO}_2$ are made. The equipment utilised a mainstream infrared $\text{CO}_2$ sensor (CapnostatH 5, Respironics Novametrix LLC, Wallingford CT, USA). A side-stream measurement of $\text{O}_2$ was made through the sample port connected to the $\text{CO}_2$ sensor, which linked to an $\text{O}_2$ analyser (Oxigraf Inc. Mountain View, CA, USA). The method does not require a wash-in phase and the switch from room air to $\text{O}_2$ was automated. Subjects breathed at a steady $V_T$ whilst wearing a nose-clip in a seated position. Once the $\text{O}_2$ circuit was engaged subjects continued the same breathing pattern until the concentration of $\text{N}_2$ reached at least 1/40th of the original concentration for three consecutive breaths. The subjects were allowed to re-equilibrate in room air between tests with a rest time of 1.5 times the duration of the previous washout. All tests were continuously inspected for quality. A test was rejected if $\text{N}_2$ spikes were apparent due to cough or leak at the mouth. The reproducibility of $\text{FRC}_{\text{N}_2}$ and $\text{LCI}_{\text{N}_2}$ required 3 tests which did not vary by >10% and the mean value reported.

2.8.2 MBW utilizing Sulphur-Hexafluoride ($\text{MBW}_{\text{SF}_6}$)

$\text{MBW}_{\text{SF}_6}$ was performed in triplicate using the method described by (Horsley, et al., 2008a) and comprised of a wash-in and washout phase.
The wash-in phase begins with the subject in a seated position, wearing a nose-clip and breathing at $V_T$ on a mouthpiece connected to an Innocor™ photo-acoustic gas analyser (Innovision AS, Odense, Denmark) using the Igor Pro written SimpleWashout program as outlined by Hannon, et al., (2014). The subjects were closely observed for evidence of air leak around the mouthpiece and were distracted by watching TV. The gas sampling port was connected to a detachable flow past tube which was used to supply 0.2% Sulphur hexafluoride ($SF_6$) in air during the wash-in and is then removed at the start of washout. The inert gas was supplied from a compressed gas cylinder with the flow rate adjusted to ensure that rebreathing did not occur. The wash-in phase continued until inspiratory and expiratory $SF_6$ concentrations differed by $<0.004\%$ (absolute difference in $SF_6$ concentration). This is confirmed by visual inspection of the gas concentration tracing. In addition, an average expired tidal volume is calculated and displayed by Innocor™, though there is no real-time tidal volume display. Once the wash-in phase was complete the washout phase was initiated by detaching the flow past circuit during expiration by manually removing the T-piece from the flow meter. The subject was warned that this was going to happen beforehand, and the process was demonstrated to the subject before the start of the test to familiarise them with the manoeuvre. Prior to the removal of the T-piece, a stream of air was directed over the end of the flow meter using a fan, to ensure that expired $SF_6$ was not re-inspired. The subject now breathing room air, continued at $V_T$ until the end tidal $SF_6$ concentration had fallen to less than 0.005% ($1/40^{th}$ of the $SF_6$ concentration during wash-in) for at least three consecutive breaths. Because of the imprecision of the Innocor™ $SF_6$ concentration display, it is difficult to identify this point precisely. Washouts were therefore continued for several breaths beyond this point to ensure that this has been achieved successfully and analysed off-line. Flow and gas concentration was re-aligned to enable to calculation of breath volume as integration of flow and $SF_6$ total volume as integration of flow and gas concentration (Hannon, et al., 2014). Three technically acceptable
measurements were achieved with reproducibility of $FRC_{SF6} < 10\%$. The mean values of $FRC_{SF6}$ and $LCI_{SF6}$ were reported (Robinson, et al., 2013).

2.9 Gas Transfer Testing
Carbon monoxide diffusing capacity ($DL_{CO}$) was measured using the Vmax 22 instrument (Sensormedics Corporation, Yorba Linda CA, USA). The equipment uses carbon monoxide (CO) and methane ($CH_4$) as the tracer gas to determine the alveolar volume ($V_A$; volume of gas in the lung containing CO), the diffusing capacity of the lung ($DL_{CO}$), which is the total uptake of CO by the lung per unit of time per unit driving pressure, and the concentration fall in alveolar CO per unit time per unit CO driving pressure ($PA,CO$) known as $K_{CO}$. This can be calculated as $DL_{CO}/V_A$. The procedure was carried out according to ATS/ERS guidelines (Macintyre, et al., 2005).

Subjects wore a nose clip, formed a tight seal around the rubber mouthpiece and breathed at $V_T$ avoiding the effect of deep inspirations on the uptake of carbon monoxide (CO). The subject then performed an unforced exhalation to RV, at which point the subject’s mouthpiece was connected to the source of the test gas and the subject was asked to inhale rapidly to TLC. The inhalation had to be at least 85% of the known IVC and completed in <4.0 seconds. Subjects then held their breath, avoiding the effect of Valsalva or Muller manoeuvres, to allow gas exchange to take place. The breath hold was calculated using the Jones-Meade method and required ten seconds (± 2 seconds) of breath holding. Once this had been completed the subject exhaled to RV (the ‘washout’ phase). This phase requires at least an exhalation volume of 0.75-1.0L for subjects with a VC $\geq$ 2.00L or at least 0.50L for those with a VC < 2.00L. At least two readings, but no more than five, were taken with four minute intervals in-between each attempt to achieve two values within 10% of the highest value and the mean reported.
2.10 Six-Minute Walk Test

Testing was conducted to a standardised protocol in accordance with current guidelines (Crapo, Enright and Zeballos, 2002). A 20-metre course was measured out on a flat, hard surface. A small cone was placed at each end of the course along with markers every 4 metres. The location was chosen due to easy access to emergency equipment and accessibility by the crash team. A physician remained in relative close proximity whilst testing was performed and technicians were at least basic life support (BLS) trained. Ten minutes prior to testing baseline measurements of blood pressure, pulse, O₂ saturation as well as breathlessness and overall fatigue using the BORG CR-10 (Appendix 2) category ratio scale was taken whilst in a seated position. Prior use of medication was recorded and a physician then assessed all subjects for contra-indications. Absolute contraindications included unstable angina and/or myocardial infarction during the previous month. Relative contraindications include a resting heart rate of more than 120, a systolic blood pressure of more than 180 mm Hg, and a diastolic blood pressure of more than 100 mm Hg. Reasons for early termination of the test were chest pain, intolerable dyspnoea, leg cramps, diaphoresis, severe joint pain, or a pale/ashen appearance. Subjects were instructed to walk continuously back and forth along the 20 m course at their own pace, while attempting to cover as much distance as possible in 6 minutes without running or jogging. Clinical staff stood in a fixed position during testing and used scripted instructions, as per the ATS guidelines (Crapo, Enright and Zeballos, 2002), to explain the test, encourage the subject and stop the test after 6 minutes. A demonstration of how to pivot around the cones was performed before testing began and a stopwatch and mechanical lap counter were used to monitor time and number of laps covered. Subjects were allowed to stop and rest during the test as required but were instructed to resume walking as soon as they felt able to do so or terminate the test where needed. After 6 minutes the subject was asked to stop and stand still whilst the distance walked was marked before sitting down whilst post exercise
measurements of blood pressure, pulse, $S_pO_2$ and Borg scores were made and the distance measured. Each subject was assessed for discharge after testing by a physician.
CHAPTER 3

3.0 Lung Clearance Index as a Sensitive Measure of airways disease in COPD

3.1 Introduction

The most widespread clinical assessment of COPD is spirometry. The current GOLD guidelines emphasise the use of FEV\textsubscript{1} and FVC in the determination of an obstructive defect and the FEV\textsubscript{1} percent predicted as a marker of disease severity. Despite this spirometry is not without its limitations in the evaluation of the whole bronchial tree and has demonstrated weak correlations with patient reported symptoms such as dyspnoea (Contoli, et al., 2010; Mahler and Witek, 2005). In COPD excess mucus production, influx of inflammatory mediators as well as airway wall thickening, all contribute to an increase in the collapsibility of the airways. Imaging and biopsy studies have shown these potentially irreversible pathological changes in the lung are apparent in the small airways, where diameter is less than 2mm (Hogg, et al., 2004). This early damage does not appear to result in an abnormal FEV\textsubscript{1}, as it is predominantly associated with airway obstruction and this type of damage does not become apparent until significant progression of the disease has occurred (McNulty and Usmani, 2014). Such pathological findings are important in the understanding of the mechanisms involved in COPD and highlight the peripheral lung as a pharmacological target. There is currently no functional marker of the small airways that has been globally accepted to be specific and sensitive enough to be used as a clinical measurement in COPD.

For this to happen the focus needs to expand beyond FEV\textsubscript{1} and evaluate physiological tests that will aid in early detection, intervention assessments and correlate with patient reported outcomes. The mid-expiratory portions of a flow volume loop, such as FEF\textsubscript{25-75}, have historically been promoted as a marker of small airway collapse due to peripheral location of flow limitation at low lung volumes (Gelb and
Zamel, 1973). The drawback to the use of mid expiratory flows in COPD is their poor repeatability and the impact large airway obstruction can have upon results (Contoli, et al., 2010). These findings bring into question the practical application and the validity of the hypothesis behind the use of mid-expiratory as an endpoint in a heterogeneous disease such as COPD.

One prospect of evaluating small airway function is by determining the volume of air remaining in the lungs after a VC, termed residual volume (RV). It is not possible to measure directly using spirometry but instead is measured indirectly by whole body plethysmography or inert gas washout. RV is raised in the presence of premature airway closure, which manifests in the small airways and has been shown to correlate with the level of inflammation present in the small airways of COPD subjects (Turato, et al., 2002). There are two important factors when using RV to assess air trapping in the COPD population. Firstly increased lung compliance is one of the consequences of the loss of elastic recoil, shifting the relationship between lung volumes and distending pressure. This results in hyperinflation, which can be quantified by values of FRC and or RV/TLC increasing above 120-130% predicted (O'Donnell and Laveneziana, 2006). Secondly RV increases with age, therefore it is important for the RV/TLC to be presented as a percentage of the reference value for an individual, to allow for inter subject comparisons (Burgel, 2011). Plethysmography is a technically complex test that requires a highly competent operator for accurate results. The test is demanding for the subjects and becomes more difficult in those with advanced disease.

It is possible to use tidal breathing analysis, which is easier for subjects to perform, to analyse the functional status of the small airways. This can be achieved via specifically assessing the resistance in the small airways or through investigating the efficiency of ventilation of the acinar portion of the lung. Impulse oscillometry (IOS) is one method that measures the resistance of the respiratory tract. It is possible to use
fluid mechanics, specifically Pouseilles law, to evaluate the impact of an increase in resistance (Sutera, 1993). The law states that flow is proportional to the fourth power of the radius, which reveals that a small decrease in small airway lumen may lead to pronounce underlying physiological changes. As the small airways account for around 10% of total resistance these changes go unnoticed until sufficient progression has taken place resulting in detrimental symptoms. IOS allows for the measurement of the resistance to airflow across the whole respiratory system (R₅) and of the large airways (R₂₀). When large respiratory system resistance is subtracted from total respiratory system resistance this can identify any raise in respiratory system resistance at low frequencies termed frequency dependent resistance (R₅-R₂₀). This denotes distal airway obstruction and has been shown to be more sensitive than FEV₁ in detecting airway abnormalities in COPD (Borrill, et al., 2005). IOS also allows for the description of the lung parenchyma termed respiratory system reactance (Xₚ). This is the measure of resistance to inertia of the proximal airways, at high frequencies, and tissue elasticity of the small airways at low frequencies (Konstantinos, Kostikas and Kontakiotis, 2013). Reactance at 5 Hz (X₅) is increased in COPD, with increasing airway obstruction assessed by spirometry correlating with the increase in X₅ (Piorunek, et al., 2015).

One pathological feature that is known to become abnormal in the early stages of small airway damage is the distribution, mixing and diffusion of intrapulmonary gases (Verbanck, et al., 1999). This abnormality is termed ventilation inhomogeneity (VI) and can be characterised by analysing the washout curve of a multiple breath washout (MBW) test (Fowler, Cornish and Kety, 1952). This technique measures the composition of each expired breath to determine the concentration of an inert gas and monitor its elimination from the lungs. MBW allows for the determination of functional residual capacity (FRC). In terms of VI the cumulative expired volume can be divided by FRC to provide a global measurement, including the small airways, of VI in the lungs (Gustafsson, et al., 2008). This measurement is referred to as the lung
clearance index (LCI). This is calculated at the point when the expired inert gas reaches \( \frac{1}{40} \)th of the starting concentration and is the most reported value from a MBW curve as it has been shown to be a sensitive clinical endpoint that is independent of age (Fuchs, et al., 2006; Latzin, Thamrin and Kraemer, 2008). This makes the concept of LCI appealing due to its ability to identify early airway damage and potentially monitor the progression of disease longitudinally (Aurora, et al., 2005; Lum, et al., 2007).

The prominence in the resurgence of LCI has been in cystic fibrosis (CF). LCI has been shown to have a greater level of discrimination between CF patients and healthy controls compared to FEV\(_1\) (Gustafsson, P. and Lindblad, 2003), as well as being a more sensitive parameter then both FEV\(_1\) and FEF\(_{25-75}\) in detecting pharmaceutical intervention effects, (Amin, et al., 2010; Davies, et al., 2013) and structural lung alterations confirmed by HRCT (Gustafsson, et al., 2008).

### 3.1.1 Aims and Objectives

The aim of this study was to evaluate the sensitivity of LCI as a clinical measure of airways disease in COPD. The study objectives were:

1. To measure LCI in a cohort of COPD and Healthy subjects and examine the relationship of VI between the groups compared to FEV\(_1\).

2. To investigate the sensitivity of LCI across COPD severity using GOLD Classifications.

3. To identify correlations in COPD between LCI and physiological tests such as IOS, gas transfer and whole body plethysmography.

4. To evaluate the therapeutic effect of salbutamol on LCI in COPD.

5. To measure LCI and 6 minute walk test distance in a cohort of COPD subjects to identify a relationship between VI and exercise capacity.
3.2 Methods

3.2.1 Subjects
Fifty-four COPD subjects aged > 40 years and with a physician’s diagnosis of COPD in accordance with the 2011 GOLD guidelines were recruited to perform MBW$_{N2}$ and spirometry with optional additional physiological tests. Twelve non-smoking (pack year history < 10) Healthy controls (HC) were also enrolled. They were aged > 18 years with no evidence of airflow obstruction (FEV$_1$/FVC > 0.7). The local ethics committee approved the study before commencement. Written informed consent was obtained from each subject prior to study procedures, all of which were conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guideline and the Declaration of Helsinki.

3.2.2 Study Design
All procedures were conducted in accordance with chapter 2. The orders of procedures are outlined in figure 3.1. Healthy controls (HC) attended the clinic under a 1-visit protocol. They firstly underwent a physical exam and a detailed medical/surgical history performed. This was followed by safety measurements that consisted of height in metres (m), weight in kilograms (Kg), blood pressure, heart rate, and S$_p$O$_2$. Height and weight were used to calculate body mass index (BMI) as kilograms per metre squared (Kg/m$^2$). Once this was completed each subject performed MBW$_{N2}$ followed by spirometry.

COPD subjects attended the clinic for 2 visits separated by at least ≥ 24 hours. At visit 1 the COPD Assessment Tool (CAT$^{TM}$) questionnaire was completed along with an ECG, physical exam; a calculation of smoking history as well as a detailed medical/surgical history. Safety measurements consisted of height, weight, Blood pressure, heart rate, and S$_p$O$_2$ prior to a clinical decision to determine their suitability to perform physiological testing at the second visit. At visit 2 adverse events and any changes to medications were evaluated before
physiological testing. Subjects then performed impulse oscillometry followed by, MBW\textsubscript{N2}, gas transfer, whole-body plethysmography and spirometry. The subjects were then administered 400 μg of salbutamol via a spacer before repeating spirometry, as well as MBW\textsubscript{N2} in a subset of subjects. Measurements of blood pressure, heart rate, and SpO\textsubscript{2} were then recorded followed by six-minute walk test. In addition to these measurements baseline and post exercise dyspnoea and fatigue ratings using the Borg\textsuperscript{®} CR-10 scale.

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**Healthy Controls**  
Visit 1  
Physical Exam / Medical History  
Height / Weight  
Vital Signs  
MBW\textsubscript{N2}  
Spirometry

**COPD Subjects**  
Visit 1  
CAT Questionnaire  
ECG / Vital Signs  
Physical Exam / Medical History  
MBW\textsubscript{N2}  
Height / Weight  
Plethysmography  
Spirometry  
Salbutamol Administration  
Post Salbutamol MBW\textsubscript{N2} in sub-set  
Post Salbutamol Spirometry  
Vital Signs  
6 Minute Walk Test

**Figure 3.1. Order of procedures.** CAT\texttrademark{} Questionnaire, COPD Assessment Test; ECG, Electrocardiogram; IOS, Impulse Oscillometry; MBW\textsubscript{N2}, Multiple Breath Nitrogen Washout; DL\textsubscript{CO}, Gas Transfer Testing.
3.2.3 Statistical Analysis

Parametric data were expressed as mean (SD) and non-parametric data expressed as median with interquartile range. Differences between groups were assessed using a Chi-squared, unpaired t-test or the Mann Whitney test. Correlations were investigated using a Pearson’s correlation coefficient or a Spearman’s rank correlation coefficient. All statistical tests were performed using Prism (GraphPad Software Inc., version 6.04, San Diego, California, USA; http://www.graphpad.com).

3.3 Results

Gender ratios were well matched for HC and COPD (p>0.05). The mean age of the HC subjects however was significantly lower than COPD group (32 vs. 66, p< 0.0001). Smoking history was not normally distributed in either group, and as would be expected and there was a significant difference between the groups with a mean pack year history of 0 (0-0) in HC and 42 (32-55) in COPD (p< 0.0001).

The overall clinical characteristics are summarised in Table 3.1. From the 54 COPD subjects that enrolled to perform spirometry, reversibility, and MBW there were 44 of which who also opted to perform IOS, DL\textsubscript{co} and whole body plethysmography. Forty-three of these also consented to perform a six-minute walk test. One hundred percent of subjects were able to achieve technically adequate results for FEV\textsubscript{1} and IOS. There was a 95% and 93% success rate observed in DL\textsubscript{CO} and MBW testing respectively with plethysmography demonstrating 86%. Eighty eight percent of subjects were able to perform the 6 minute-walk tests.
### Table 3.1. Subject Demographics

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Healthy Controls (n enrolled = 12)</th>
<th>COPD (n enrolled = 54)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Male/Female)</td>
<td>7/5</td>
<td>37/17</td>
<td>p = ns(^a)</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>32 (13)</td>
<td>66 (7)</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Height (metres)</td>
<td>1.72 (0.10)</td>
<td>1.67 (0.10)</td>
<td>p = ns</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>77.19 (13.60)</td>
<td>78.81 (18.57)</td>
<td>p = ns</td>
</tr>
<tr>
<td>BMI (Kg/M(^2))</td>
<td>26.10 (4.99)</td>
<td>27.83 (4.91)</td>
<td>p = ns</td>
</tr>
<tr>
<td>Smoking History (Pack Years)</td>
<td>0 (0-0)(^b)</td>
<td>42.10 (31.69-55.48)(^b)</td>
<td>p &lt; 0.0001(^c)</td>
</tr>
<tr>
<td>MBW(_{N_2}) Test Time (mins)</td>
<td>17.71 (7.41)</td>
<td>38.36 (19.87)</td>
<td>p = 0.0008</td>
</tr>
<tr>
<td>LCI(_{N_2})</td>
<td>7.1 (0.68)</td>
<td>12.09 (2.23)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>FRC(_{N_2}) %</td>
<td>105.0 (29.24)</td>
<td>135.7 (36.79)</td>
<td>p = 0.009</td>
</tr>
<tr>
<td>FEV(_1) %</td>
<td>104.1 (15.01)</td>
<td>63.09 (18.40)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>FEF(_{25-75}) %(^*)</td>
<td>80.42 (21.69)</td>
<td>22.02 (11.20)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>FVC %(^*)</td>
<td>110.7 (17.40)</td>
<td>106.6 (17.83)</td>
<td>p = ns</td>
</tr>
<tr>
<td>FEV(_1)/FVC</td>
<td>0.80 (0.05)</td>
<td>0.48 (0.13)</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

All parametric data are expressed as means and standard deviations unless otherwise stated. \(^a\) denotes chi-squared test. \(^b\) denotes median and inter-quartile range. \(^c\) denotes Mann-Whitney U test. \(^*\) denotes 53 subjects due to 1 subject unable to perform FVC manoeuvres.
3.3.1 Healthy Controls Vs COPD

The quality of all results was assessed as outlined in chapter 2.8.1. After exclusion of the measurements with poor quality, LCI_{N2} was found to differentiate HC from COPD. Figure 3.2 shows the relationship between FEV_{1} % and LCI_{N2}. In the HC group LCI_{N2} was restricted to a narrow range but in the COPD subjects, LCI_{N2} increased as FEV_{1} % predicted reduced with a significant negative correlation (r=-0.58, p<0.0001). Ten COPD subjects (20%) had an FEV_{1} within the normal range of the HC group (83%-131%) but all COPD subjects expressed an LCI_{N2} value significantly greater than the HC group, with 12.09 (2.22), versus 7.1 (0.68) p<0.0001 respectively.

Figure 3.2. Lung clearance index (LCI_{N2}) versus forced expiratory volume in 1 s (FEV_{1}) % predicted for healthy controls and COPD subjects. The horizontal line represents the mean and the horizontal dotted lines the 95% limits of normality of the LCI_{N2}, calculated from the healthy controls. The vertical line represents the lower limit of normal for % predicted FEV_{1}.
### 3.3.2 LCI$_{N2}$ vs Gold Stage

Figure 3.3 shows the distribution of LCI$_{N2}$ across GOLD stages, as quantified by FEV$_1\%$. LCI$_{N2}$ increased as the GOLD stage increased. There was a significant difference between GOLD stage 1 and 2 with mean values of 10.28 (1.24) and 11.88 (1.76), with $p=0.0038$. The mean value for GOLD stage 3 was 14.19 (2.33) and was significantly greater than GOLD stage 2 ($p=0.0017$) or Gold stage 1 ($p=0.0002$). There was only a single subject in GOLD stage 4.

#### Lung Clearance Index N$_2$ Vs GOLD Classification

![Figure 3.3. Lung clearance index (LCI$_{N2}$) versus GOLD stage.](image)

The mean value for stage 1 was 10.28 (1.24), the mean value for stage 2 was 11.88 (1.76) and the mean value for stage 3 was 14.19 (2.33). There was a significant difference between stages 1 and 2 with $p=0.0038$, as well as a significant difference between stages 2 and 3 with $p=0.0017$. Stage 1 significantly differed from stage 3 ($p=0.0002$).
3.3.3 LCI\textsubscript{N2} correlation with small airway resistance and reactance

IOS parameter R\textsubscript{5−R20} is a representation of increased respiratory system resistance in the distal airways. Normally R\textsubscript{5−R20} would be zero where there is no peripheral damage in comparison to proximal portions of the lung. LCI\textsubscript{N2} demonstrated a significant positive correlation with R\textsubscript{5−R20}. As ventilation inhomogeneity increased there was an increase in frequency dependence resistance with r= 0.38 and p= 0.02 (figure 3.4). The most significant correlation was observed with X\textsubscript{5}. As the level of ventilation inhomogeneity increased there was a negative correlation with the elastic recoil properties of the small airway parenchyma with r= 0.52, p= 0.0006 (figure 3.5).

**Correlation of Lung Clearance Index N\textsubscript{2} Vs R\textsubscript{5−R20}**

![Correlation of Lung Clearance Index N\textsubscript{2} Vs R\textsubscript{5−R20}](image)

*Figure 3.4. Lung clearance index (LCI\textsubscript{N2}) versus R\textsubscript{5−R20}. There was a significant positive correlation between LCI\textsubscript{N2} and R\textsubscript{5−R20} with r = 0.38, p = 0.02.*
Figure 3.5. Lung clearance index (LCI\textsubscript{N2}) versus X\textsubscript{5}. There was a significant negative correlation between LCI\textsubscript{N2} and X\textsubscript{5} with r= 0.52, p= 0.0006.

3.3.4 LCI\textsubscript{N2} correlation with diffusing capacity

There was no relationship found between ventilation inhomogeneity represented by LCI\textsubscript{N2} and volume adjusted rate of gas transfer occurring in the diffusive parts of the lung known as K\textsubscript{CO} (figure 3.6).

Figure 3.6. Lung clearance index (LCI\textsubscript{N2}) versus K\textsubscript{CO} %. There was no significant correlation between LCI\textsubscript{N2} and K\textsubscript{CO} %.
3.3.5 LCI\textsubscript{N2} correlation with Whole-Body Plethysmography

In terms of hyperinflation LCI\textsubscript{N2} increased as the level of air trapping increased represented by RV/TLC %. A significant positive correlation was observed with $r=0.44$ and $p=0.008$ (figure 3.7). LCI\textsubscript{N2} increased as the level of airways resistance corrected for lung volume increased. A significant positive correlation was observed between LCI\textsubscript{N2} and $R_{aw}$ with $r=0.47$ and $p=0.004$ (figure 3.8). The inverse of this relationship was found between LCI\textsubscript{N2} and $sG_{aw}$ with a significant negative correlation observed with $r=-0.42$ and $p=0.011$ (figure 3.9).

**Figure 3.7.** Lung clearance index (LCI\textsubscript{N2}) versus RV/TLC \% . There was a significant positive correlation between LCI\textsubscript{N2} and RV/TLC \% with $r=0.44$, $p=0.008$.  

![](image-url)
Figure 3.8. Lung clearance index ($LCI_{N2}$) versus airways resistance ($R_{aw}$). There was a significant positive correlation between $LCI_{N2}$ and $R_{aw}$ with $r = 0.47$, $p = 0.004$.

Figure 3.9. Lung clearance index ($LCI_{N2}$) versus airways conductance ($sG_{aw}$). There was a significant negative correlation between $LCI_{N2}$ and $sG_{aw}$ with $r = -0.42$, $p = 0.011$. 
3.3.6 Effect of salbutamol on LCI\textsubscript{N2}

There was no significant effect on LCI\textsubscript{N2} after treatment with 400 µg of salbutamol (Figure 3.10). The pre salbutamol mean LCI\textsubscript{N2} was 11.63 (2.07) and the mean post salbutamol LCI\textsubscript{N2} was 11.56 (2.23). The mean (SD) change in LCI was -0.61 (7.00).

**LCI\textsubscript{N2} Pre and Post Treatment with Salbutamol**

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure310.png}
\caption{Lung clearance index (LCI\textsubscript{N2}) pre and post treatment with 400µg of salbutamol. The mean LCI\textsubscript{N2} pre-treatment was 11.63 (2.07) and post treatment was 11.56 (2.23). There was no significant difference between the two values with a mean change in LCI of -0.61 (7.00).}
\end{figure}
3.3.7 LCI\textsubscript{N2} correlation with exercise capacity

The level of ventilation inhomogeneity did not correlate with the distance covered during the six-minute walk test (figure 3.11).

![Correlation of LCI\textsubscript{N2} vs Six-Minute Walk Distance](image)

Figure 3.11. Lung clearance index (LCI\textsubscript{N2}) versus six-minute walk distance. There was no significant correlation found between LCI\textsubscript{N2} and distance walked.
3.4 Discussion
This is the first study to use of LCI_N2 as a measure of airways disease in a COPD population and assessed the results against currently accepted physiological testing. The first objective was to examine the relationship of VI between COPD and Healthy subjects and compare the groups to values of FEV_1. LCI_N2 was raised in all COPD subjects in comparison to the HC group and supports the findings of previous emphysema research in providing evidence of the discriminative ability of LCI_N2 between HC and COPD subjects (Von Nieding, et al., 1977 ). LCI_N2 was also found to identify 20% of COPD subjects who expressed impaired ventilation but had an FEV_1 within normal limits. This finding corresponds to previous studies in cystic fibrosis where FEV_1 has been shown to only change once significant obstruction has occurred and emphasises the increased sensitivity of LCI_N2 as an outcome marker of early obstructive lung disease (Horsley, et al., 2008a). The second objective was to investigate LCI across COPD severity quantified by FEV_1. LCI_N2 was found to have an inverse relationship with FEV_1 and was able to distinguish between GOLD stages, with significantly raised levels of LCI_N2 as the level of obstruction increased.

The third objective was to identify correlations in COPD between LCI and current markers of the small airways. Past COPD research into peripheral resistance and the lungs ability to store energy (reactance) using IOS parameters have shown relationships between R_5-R_20 and X_5 versus FEV_1 (p<0.05; (Kolsum, et al., 2009; Vukoja, Milicic and Kopitovic, 2014). The current data shows that LCI_N2 correlated with FEV_1, R_5-R_20 and X_5 with r=0.58, p<0.0001, r= 0.38, p= 0.02 and r= 0.52, p=0.006 respectively. In terms of gas transfer efficiency there was no correlation between LCI_N2 and K_CO. Despite measurements of ventilation and gas transfer at first seemingly synonymous to each other a relationship would not be expected. In COPD the parameters of LCI_N2 and K_CO are markers of different aspects of the disease. LCI_N2 is a global measurement of ventilation inhomogeneity throughout the lung with the
current results highlighting its most sensitivity in mild COPD where spirometry is largely unaffected. $K_{CO}$ on the other hand is a volume-adjusted estimation of gas transfer occurring in the small airways. In mild COPD $K_{CO}$, like spirometry, would remain relatively in tact until further progression of the disease had occurred. This result would then switch when investigating severe COPD with a reduced sensitivity of $LCI_{N2}$ and a significant reduction in $K_{CO}$. When considering the functional aspects of lung volumes, air trapping is a common physiological feature of COPD that impacts greatly upon symptoms and quality of life. The degree of air trapping relates to increased distal inflammation, dyspnoea and a reduction in exercise capacity (O'Donnell, 2006; Turato, et al., 2002). $LCI_{N2}$ was found to correlate significantly with air-trapping measured by whole body plethysmography, represented by RV/TLC % with $r= 0.44$ ($p= 0.008$). This shows that the level of ventilation inhomogeneity is further exaggerated as air trapping increases with disease severity. $LCI_{N2}$ was also shown to correlate with both plethysmographic measurements of airways resistance ($R_{aw}$) and conductance ($sG_{aw}$). These values have been successfully used as outcome markers in COPD clinical trials and demonstrated statistical clinical changes in response to therapy (Maesen, et al., 1999). Study findings using $R_{aw}$ and $sG_{aw}$ combined with the relationship with $LCI_{N2}$ found in the present study further highlight the possibility of incorporating $LCI_{N2}$ to evaluate new therapies.

One of the main features of COPD is non-reversible airway obstruction; therefore the fourth objective was to evaluate the therapeutic effect of salbutamol on LCI in COPD. The present study did not show a significant improvement in $LCI_{N2}$ following treatment with salbutamol. This correlates with previous findings in spirometry where $FEV_1$ remained relatively unchanged after treatment with a short acting bronchodilator (Chang and Mosenifar, 2007). This illustrates the inability to rectify fundamental damage and highlights the need for the disease to be detected earlier and interventions instigated to prevent these physiological changes occurring.
The fifth objective was to investigate if a relationship existed in COPD subjects between the level of VI and exercise capacity. The current data set found no relationship between LCI_{N2} and the distance covered during a 6 minute walk test with some of the least amount of distance achieved being in subjects with the least amount of ventilation inhomogeneity. This demonstrates the multi-component effect of COPD on dynamic measurements of exercise capacity and emphasises the limitations of using one single type of measurement to predict daily exercise activity.

In summary the present findings support a role for the evaluation of ventilation inhomogeneity as a marker of disease in COPD. LCI_{N2} is a sensitive marker across disease severity and appears especially useful in determining lung abnormalities in early disease. The results underline measurements of LCI_{N2} as an important tool that compliments the measurements of obstruction and parenchymal mechanics. The ability of LCI to provide evidence of global ventilation inhomogeneity indicates that it may serve as a useful marker for intervention studies targeting mild disease and therapies with smaller particle size.

One main aspect of the implementation of a clinical test is the knowledge of the expected variability of a measurement in establishing clinically relevant changes. The following chapter will investigate the intra-test and inter-test variability of MBW in a COPD population.
CHAPTER 4

4.0 Repeatability and Reproducibility of Multiple-Breath Nitrogen Washout in COPD

4.1 Introduction

In Chapter 3 it was shown that LCI was able to discriminate between HC and COPD, correlated with existing physiological tests and identified 20% of COPD subjects with normal spirometry but whom expressed ventilation heterogeneity. For a clinical measurement to be implemented in assessments of interventions, as well as long-term clinical outcomes, it is imperative that the reliability of the method be validated (Hankinson, Stocks and Peslin, 1998). A test needs to demonstrate low variation between measurements collected in one testing session as well as low variation within the same subject when tested on separate occasions (Fuchs, et al., 2012). The results of which would greatly impact upon the interpretation of clinical changes. This is done by firstly analysing the short-term repeatability measured by the coefficient of variation (CV) of three technically acceptable manoeuvres. Secondly evaluating the long-term reproducibility of acceptable results across different testing sessions over time. The Exhalyzer®D system provides real time tidal volume feedback to control tidal volume and respiratory rate within a range to limit the effect on FRC measurements. In addition to this, LCI is a volume ratio and as such should be minimally effected by lung volume variation (Singer, et al., 2012). It is accepted that for FRC and LCI measurements a CV of up to 10% can be expected for average values based on 3 test results (Kent, et al., 2014).
4.1.1 Aims and Objectives

The aim of this study was to assess the intra-test and inter-test variability of FRC % and LCI in a cohort of COPD subjects. The study objectives were:

(1) To determine the coefficient of variation (CV) across 3 technically acceptable MBW manoeuvres in healthy controls and COPD subjects.

(2) Compare intra-test repeatability of COPD subjects to healthy controls.

(3) To evaluate the reproducibility of MBW parameters in COPD subjects across 2 testing sessions separated by ≥ 24 hours.

4.2 Methods

4.2.1 Subjects

Twelve healthy controls (HC), aged > 18 years with no evidence of airflow obstruction (FEV₁/FVC > 0.7) and 20 COPD patients aged > 40 years and with a physician’s diagnosis of COPD in accordance with the 2011 GOLD guidelines were recruited. The local ethics committee approved the study before commencement. Written informed consent was obtained from each subject prior to study procedures, all of which were conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guideline and the Declaration of Helsinki.
4.2.2 Study Design

Healthy controls attended a 1-visit protocol as outlined in chapter 3. COPD subjects attended the clinic for 2 visits separated by ≥ 24 hours. The order of procedures can be found in figure 4.1. Visit 1 consisted of height, weight; medical and smoking history, and vital signs before performing multiple-breath nitrogen washout (MBW\textsubscript{N2}). Subjects were then administered 400μg of salbutamol via a spacer before performing spirometry. At visit 2 adverse events and any changes to medications were evaluated before performing MBW\textsubscript{N2}, followed by administration of 400μg of salbutamol via a spacer and spirometry.

<table>
<thead>
<tr>
<th>Healthy Controls and COPD Subjects</th>
<th>COPD Subjects Only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit 1</td>
<td>Visit 2</td>
</tr>
<tr>
<td>Height / Weight</td>
<td>Adverse Event Assessment</td>
</tr>
<tr>
<td>Smoking / Medical History</td>
<td>Changes to Medication / Medical History Assessed</td>
</tr>
<tr>
<td>Concomitant Medications Recorded</td>
<td>MBW\textsubscript{N2}</td>
</tr>
<tr>
<td>Vital Signs</td>
<td>Salbutamol Administration</td>
</tr>
<tr>
<td>MBW\textsubscript{N2}</td>
<td>Post Salbutamol Spirometry</td>
</tr>
<tr>
<td>Salbutamol Administration</td>
<td></td>
</tr>
<tr>
<td>Post Salbutamol Spirometry</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.1. Order of procedures. MBW\textsubscript{N2}, Multiple Breath Nitrogen Washout.
4.2.3 Multiple-Breath Washout Testing

MBW$_{N_2}$ was performed as previously described in detail in chapter 2 using the Exhalyzer® D (EcoMedics AG, and Duernnten, Switzerland) and associated software (SpirowareH 3.1 EcoMedics AG). The method does not require a wash-in phase and the switch from room air to O$_2$ was automated. Subjects breathed at a steady $V_T$ whilst wearing a nose-clip in a seated position. Once the O$_2$ circuit was engaged subjects continued the same breathing pattern until the concentration of N$_2$ reached at least 1/40th of the original concentration for three consecutive breaths. The subjects were allowed to re-equilibrate in room air between tests with a rest time of 1.5 times the duration of the previous washout. The reproducibility of FRC$_{N_2}$ and LCI$_{N_2}$ required 3 tests which did not vary by >10%.

4.2.4 Statistical Analysis

Parametric data were expressed as mean (SD) and non-parametric data expressed as median with interquartile range. Intra-test repeatability for FRC and LCI were determined by calculating the coefficient of variation (CV) as 100 x SD/mean of the three recordings for each subject. Inter-visit reproducibility was assessed using Bland-Altman plots (Bland and Altman, 1986). All statistical tests were performed using Prism (GraphPad Software Inc., version 6.04, San Diego, California, USA; http://www.graphpad.com).
4.3 Results

The overall clinical characteristics are summarised in Table 4.1. There was no significant difference between the genders of either group. The HC group had a male:female ratio of 7 males and 5 females (58% and 42%). The COPD group comprised of 12 males and 8 females (60% male and 40% female respectively). There was a significant difference in mean age with the HC subjects being significantly younger than the COPD group (32 vs 65, p< 0.0001). There was no difference in height and weight between the 2 groups although the COPD group did have a BMI of 28.6 (4.8) compared to 26.6 (23.5-27.6) with p=0.04. Smoking history was not normally distributed in either group and as expected there was a significant difference between the groups with a mean pack year history of 0 (0-0) in HC and 42.75 (33.5-54.5) in COPD (p< 0.0001). As expected there were significant differences in FEV₁ % and FEV₁/FVC. The HC group expressed greater values for both FEV₁, 104.1 (15) and FEV₁/FVC, 0.8 (0.05) in comparison to 70.6 (17.0) %, and 0.49 (0.1) in the COPD group with p< 0.0001 in both instances. All 12 of the HC group and all 20 of the COPD group were able to perform a technically adequate MBW technique and achieve 3 successful tests with FRC and LCI < 10% of the median.
Table 4.1. Subject Demographics

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Healthy Controls</th>
<th>COPD Subjects</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 12</td>
<td>n = 20</td>
<td></td>
</tr>
<tr>
<td>Gender (Male/Female)</td>
<td>7/5</td>
<td>12/8</td>
<td>p = ns&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>32 (13)</td>
<td>65 (6.4)</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Height (metres)</td>
<td>1.72 (0.1)</td>
<td>1.67 (0.1)</td>
<td>p = ns</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>77.2 (13.6)</td>
<td>80.4 (16.7)</td>
<td>p = ns</td>
</tr>
<tr>
<td>BMI (Kg/M&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>26.10 (4.99)</td>
<td>28.6 (4.8)</td>
<td>p = ns</td>
</tr>
<tr>
<td>Smoking History (Pack Years)</td>
<td>0 (0-0)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.75 (33.5-54.5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>p &lt; 0.0001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; %</td>
<td>104.1 (15.0)</td>
<td>70.6 (17.0)</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC</td>
<td>0.8 (0.05)</td>
<td>0.49 (0.1)</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>No. Days Between Visits</td>
<td>16 (13)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All parametric data are expressed as means and standard deviations unless otherwise stated. a denotes chi-squared test. b denotes median and inter-quartile range. c denotes mann-whitney U test.
4.3.1 Intra-Test Repeatability of MBW\textsubscript{N2}

The median (inter-quartile range) intra-test CV for FRC derived from triplicate washout manoeuvres on the same visit was 4.40 (3.03-7.83) for healthy controls and 4.30 (1.73-6.28) for COPD subjects, with no significant difference between the groups. The mean (SD) intra-test CV for LCI was 3.46 (2.31) for healthy controls and 4.55 (2.75) for COPD subjects, with no significant difference between the groups (Table 4.2).

4.3.2 Inter-Visit Reproducibility of MBW\textsubscript{N2}

The 20 COPD subjects performed repeat measurements of MBW\textsubscript{N2} in triplicate after a mean (SD) of 16 (13) days. A Bland-Altman plot between repeated measurements of FRC\textsubscript{N2} % predicted shows that the mean difference was -0.40 (14.53) % and the 95% limits of agreement between the two measurements were -28.88 to 28.08 % (figure 4.2). Variation was greater in the hyper-inflated subjects who expressed FRC\textsubscript{N2} % values > 120 % predicted. For LCI\textsubscript{N2} the Bland-Altman plot shows the mean difference was -0.10 (0.86) with limits of agreement -1.79 to 1.59 which is approximately equivalent to ± 15% (figure 4.3). This is in comparison to the inter-visit variability found for spirometric indices. FEV\textsubscript{1} has a mean difference of 1.70 (4.68) % with limits of agreement -7.47 to 10.87 (figure 4.4). FEF\textsubscript{25-75} had a mean difference of 1.93 (5.14) % with limits of agreement -8.15 to 12.01 (figure 4.5). FVC had a mean difference of 1.45 (7.29) % with limits of agreement -12.85 to 15.75 (figure 4.6).
Table 4.2. Intra-Test variation of MBW<sub>N2</sub> represented by coefficient of variation (CV)

<table>
<thead>
<tr>
<th>Lung Function Parameter</th>
<th>Healthy Control</th>
<th>COPD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRC&lt;sub&gt;N2&lt;/sub&gt; %</td>
<td>113 (74-129)</td>
<td>122.5 (116-149)</td>
<td>p = 0.052&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FRC&lt;sub&gt;N2&lt;/sub&gt; CV</td>
<td>4.40 (3.03-7.83)</td>
<td>4.30 (1.73-6.28)</td>
<td>p = ns&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LCI&lt;sub&gt;N2&lt;/sub&gt;</td>
<td>7.10 (0.68)</td>
<td>11.53 (2.23)</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>LCI&lt;sub&gt;N2&lt;/sub&gt; CV</td>
<td>3.46 (2.31)</td>
<td>4.55 (2.75)</td>
<td>p = ns</td>
</tr>
</tbody>
</table>

All parametric data are expressed as means and standard deviations unless otherwise stated. a denotes mann-whitney U test.

Figure 4.2. Bland-Altman Plot of the agreement between visits of FRC<sub>N2</sub> % predicted in COPD subjects. The central dotted line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). The vertical line represents hyper-inflation with FRC<sub>N2</sub> >120 % predicted. The mean difference in FRC<sub>N2</sub> % predicted was -0.40 (14.53) % across 2 visits with limits of agreement -28.88 to 28.08 %.
Figure 4.3. Bland-Altman Plot of the agreement between visits of LCI\textsubscript{N2} in COPD subjects. The central dotted line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). The mean difference in LCI\textsubscript{N2} was -0.10 (0.86) across 2 visits with limits of agreement -1.79 to 1.59.

Figure 4.4. Bland-Altman Plot of the agreement between visits of FEV\textsubscript{1} in COPD subjects. The central dotted line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). The mean difference in FEV\textsubscript{1} was 1.70 (4.68) across 2 visits with limits of agreement -7.47 to 10.87.
Figure 4.5. Bland-Altman Plot of the agreement between visits of $\text{FEF}_{25-75}$ in COPD subjects. The central dotted line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). The mean difference in $\text{FEF}_{25-75}$ was 1.93 (5.14) across 2 visits with limits of agreement -8.15 to 12.01.

Figure 4.6. Bland-Altman Plot of the agreement between visits of FVC in COPD subjects. The central dotted line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). The mean difference in FVC was 1.45 (7.29) across 2 visits with limits of agreement -12.85 to 15.75.
4.4 Discussion

This is the first study analysing the short term and long term variation of $MBW_{N2}$ measured on the Exhalyzer® D in subjects with COPD. The study found MBW parameters to have good intra-test repeatability with $LCI_{N2}$ demonstrating inter-visit reproducibility similar to spirometry indices. This information serves to aid in the validation of MBW parameters as a potential endpoint in COPD clinical trials and in estimating clinically relevant changes. The first objective was to measure the intra-test variability of $MBW_{N2}$ in healthy controls and COPD subjects. There was no significant difference in $FRC_{N2}$ and $LCI_{N2}$ CV between the groups. For the healthy controls the median coefficient of variation was < 5% for both MBW parameters, with $LCI_{N2}$ being more repeatable than $FRC_{N2}$. This is similar to previously published findings in healthy volunteers with $LCI_{N2}$ being more repeatable than $FRC_{N2}$ and mean values being 3.2 (1.7) and 4.5 (3.2) respectively (Singer, et al., 2012). In the COPD group the median (inter quartile range) intra-test CV for $FRC_{N2}$ % was 4.30 (1.73-6.28). This correlates with findings found in other obstructive diseases such as cystic fibrosis as well as falling with the range of 3.5-6.7 that has been reported in literature for FRC measurements obtained by plethysmography and helium dilution (Hankinson, Stocks and Peslin, 1998). $LCI$ demonstrated good repeatability with a mean (SD) CV for $LCI_{N2}$ was found to be 4.55 (2.75), which is lower than the 4.4 (2.8) reported in CF studies (Horsley, et al., 2008a).

The second objective was to compare the intra-test variability of MBW parameters in COPD subjects compared to healthy controls. Despite the difference between $FRC_{N2}$ % trending towards significance and a clear significant difference observed in $LCI_{N2}$ there were no significant differences for CV in either indice. This shows that the measurement of MBW in obstructed COPD subjects is as repeatable as that obtained in a healthy population and so increases the validity of the test.
The third objective was to evaluate the reproducibility of MBW parameters in COPD subjects across 2 testing sessions separated by ≥ 24 hours. There was a mean (SD) of 16 (13) days between visits 1 and 2. The mean variation in $\text{FRC}_{\text{N2}}$ % was 0.4% with ±29% limits of agreement. This variability appears to be driven by the increased disproportionate variation in the $\text{FRC}_{\text{N2}}$ measurements for hyper-inflated subjects who exhibited an $\text{FRC}_{\text{N2}}$ % greater than 120% predicted. LCI demonstrated a better level of reproducibility, independent of the variation in FRC, especially in subjects with milder disease. The mean difference was 0.1 with limits of agreement being ±1.79. This level of variability indicates that a difference >15% represents a clinically significant change in VI which is comparable to the variation found for $\text{FEV}_1$, $\text{FEF}_{25-75}$ and FVC with a clinical significant change being 11%, 12% and 16% respectively. This maybe an important consideration when using LCI$_{\text{N2}}$ as an outcome marker to evaluate intervention effects in clinical trials or as a monitoring tool for disease progression in clinical practice.

We have previously shown that LCI correlates with current physiological tests used in clinical practice, including those proposed to reflect small airway function. This study shows that LCI is a highly repeatable and reproducible assessment of VI in COPD with intra-test variation < 5%. The current data set did make an effort to establish that subjects were stable prior to all testing but the results are limited by the sample size and the measurements of inter-test variation being over 2 single time-points. Future studies should increase the n number to allow for a more accurate determination of variability as well as more appreciation of the association between severity and reproducibility. Increasing the number of time-points for inter-visit evaluation would also lead to an increase in the validity of the determination of clinically significant changes.
CHAPTER 5

5.0 A comparison of Multiple-Breath Washout Methods: Nitrogen Vs Sulphur Hexafluoride

5.1 Introduction

The growing understanding of the pathophysiology of COPD and the technical advancement in the size and speed of gas measuring devices has allowed MBW to begin to enter clinical practice through the use of commercially available devices. Two such devices are the Exhalyzer® D (EcoMedics AG, and Duernten, Switzerland) and the Innocor™ (Innovision AS, Odense, Denmark). The Exhalyzer® D utilises ultrasound to measure flow and uses CO₂ and O₂ analysers to measure each expired breath and calculate the concentration of N₂. As N₂ is resident in the lungs testing does not require a wash-in phase and should be an inclusive representation of the slowly ventilated portions of the lung found in COPD. This however does mean that sufficient time between tests must occur to allow for re-equilibration. The Innocor™ device has a photoacoustic analyser that detects the falling concentrations of SF₆ at 1/20th of the operating range of previous SF₆ measuring equipment (Horsley, et al., 2008a). This results in the use of substantially less gas during testing, which improves the economic and environmental footprint of SF₆. This could potentially be of greater significance when testing patients with obstructive defects due to the resulting longer washout times. This method does require sufficient wash-in times to allow equilibration prior to washout but does not need rest times in between tests in the same session.

As the systems become more widespread there is an important need to investigate the variation in COPD MBW parameters measured by different methods. There are a number of physiological reasons why measurements made using N₂ and SF₆ may vary. Firstly N₂ is not a truly inert gas as it is found is the blood and tissues surrounding the lungs. During washout testing this tissue N₂ can diffuse along a concentration
gradient and into the alveoli (Robinson, et al., 2013). In COPD this maybe evident due to the mismatch in ventilation that occurs between the well-ventilated sections of the lung and the poorly ventilated regions. Gas washout will occur quickly in the well-ventilated portions and slower in the regions of poor ventilation. As ventilation deteriorates further in these sections the time taken to wash out the gas increases. This extended washout duration may cause a greater diffusion gradient in the well-ventilated portions of the lung that draws tissue N₂ across, increasing LCI. This has been investigated using a computer lung model, which examined the impact excreted N₂ may have upon the level of N₂ measured during MBW. It was concluded that an effect does indeed exist but that it is variable and hard to predict. It was reported that this effect would be more pronounced in diseases with ventilation heterogeneity, with LCI potentially varying by up to 6-13% in CF patients (Nielsen, Nielsen and Horsley, 2013). Current in-vivo research has indeed highlighted that MBW₅₂ values were shown to be greater than those obtained by MBW₆ and that this difference increased disproportionally as FRC and LCI values increased in a CF population (Jensen, et al., 2013). Possible alternative explanations include the level of air trapping occurring during the washout that may reduce the volume of gas available to be expired and underestimate FRC and LCI. Difference may also occur due to the choice of gas used. The rate of diffusion of a gas is inversely proportional to the square root of its molecular mass (MM) (Robinson, et al., 2013). The point at which gas flow becomes less dominated by convection and largely driven by diffusion is termed the convection-diffusion front. With the MM of SF₆ being considerably greater than N₂ (146 vs 28) this point would be closer to the acinus for a denser gas such as SF₆. Theoretically this should provide higher LCI values as it better represents the abnormality in the lung periphery that contributes to global lung VI. There are additional considerations when using a system that employs an O₂ breathing circuit to indirectly measure N₂ as the inert gas. It is possible that breathing 100% O₂ in a COPD population, where washout times are
increased, may lead to the displacement of $N_2$ and penetrates parts of the lung which are not normally accessible where $O_2$ is re-absorbed. If this were to happen volume may reduce and the measurements of ventilation underestimated. From an equipment viewpoint it is also possible that an error may exist in the algorithm used in the signal alignment between gas and airflow that leads to the over or under estimation of MBW parameters. In order to improve accuracy of MBW testing it is imperative that these possible problems be investigated. This would ensure that an accurate FRC is established, as if FRC is over estimated LCI may increase and test durations will increase. This, in addition to inaccurate results, may limit the practicalities of MBW and may affect the success rates of technically acceptable results.

Whole body plethysmography is another method for measuring FRC. Since its widespread use in 1956 (DuBois, Botelho and Comroe, 1956) it has received intense research which has improved the understanding of the technique. It has been shown to reflect functional as well as structural aspects of the lung in COPD, has well-established normal values and has been successfully used as a clinical trial endpoint (Cripe, et al., 2011). The measurement of $FRC_{\text{pleth}}$ includes all the air volume of the thoracic cavity and therefore should produce values greater than that measured by MBW, which only measure the tidally ventilated portions of the lung. The differences in plethysmography and MBW values have been shown to represent the volume of trapped air in the lungs of obstructed patients (Kraemer, et al., 2005). With this knowledge a comparison of new MBW systems using inert gas washout should also be compared to plethysmography to further validate their use as a clinical endpoint. There remains a gap in the literature comparing $MBW_{N_2}$ and $MBW_{SF_6}$ against plethysmography in a COPD population, the results of which are imperative if normal values are to be formed and interpretation standardised (Robinson, Goldman and Gustafsson, 2009). Preliminary data on reference equations does exist but are based on relative small n numbers in comparison to plethysmography and may
not be reflective of LCI obtained on different systems employing alternative analysis methods.

5.1.1 Aims and Objectives

The aim of this study was to assess the agreement in MBW parameters measured by different methods. Our study objectives were

(1) To determine the agreement between FRC values measured using MBW$_{N2}$ and Whole body plethysmography in a group of COPD subjects.

(2) To determine the agreement between FRC and LCI values measured using MBW$_{N2}$ and MBW$_{SF6}$ in a group of healthy controls.

(3) To determine the agreement between FRC and LCI values measured using MBW$_{N2}$ and MBW$_{SF6}$ in a cohort of COPD subjects;

(4) To compare FRC values of COPD subjects who performed MBW$_{N2}$, MBW$_{SF6}$ and Whole body plethysmography.
5.2 Methods

5.2.1 Subjects

Twelve healthy controls (HC) and 44 COPD patients were recruited. The HC’s were aged > 18 years with no evidence of airflow obstruction (FEV\textsubscript{1}/FVC > 0.7). The COPD group was aged > 40 years and with a physician’s diagnosis of COPD in accordance with the 2011 GOLD guidelines. The local ethics committee approved the study before commencement. Written informed consent was obtained from each subject prior to study procedures, all of which were conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guideline and the Declaration of Helsinki.

5.2.2 Study Design

Healthy controls (HC) attended a 1-visit protocol. This included height, weight, vital signs, as well as medical and smoking history. Subjects then performed multiple-breath nitrogen washout (MBW\textsubscript{N2}) and multiple-breath Sulphur hexafluoride washout (MBW\textsubscript{SF6}) in a randomly assigned order. All subjects then performed spirometry.

COPD subjects attended a 1-visit protocol that consisted of 3 cohorts who all performed spirometry after measurement of FRC. All cohorts included height, weight, vital signs, as well as medical and smoking history prior to all testing. In Cohort 1 subjects performed multiple-breath nitrogen washout (MBW\textsubscript{N2}) and whole body plethysmography before spirometry and administration of 400 μg of salbutamol via a spacer before repeating spirometry. Cohort 2 was made up of subjects from cohort 1 who had also consented for multiple-breath Sulphur hexafluoride washout (MBW\textsubscript{SF6}). It consisted of multiple-breath nitrogen washout (MBW\textsubscript{N2}) and MBW\textsubscript{SF6} in a randomly assigned order. Subjects then performed spirometry, after which 400 μg of salbutamol via a spacer was administered before repeating spirometry. Cohort 3 included those from cohort 1 who had completed both MBW\textsubscript{SF6} and
plethysmography prior to spirometry measurements. The order of procedure for each cohort can be seen in figure 5.1.

All physiological procedures were performed as previously described in detail in chapter 2. FRC predicted values from the European Community for Coal and Steel were used to compare FRC % predicted across all methods (Quanjer, et al., 1993).

5.2.3 Multiple-Breath Washout Testing

MBW$_{N2}$ was performed using the Exhalyzer$^\text{®}$ D (EcoMedics AG, and Duernten, Switzerland) and associated software (SpirowareH 3.1 EcoMedics AG). The method does not require a wash-in phase and the switch from room air to O$_2$ was automated. Subjects breathed at a steady $V_T$ whilst wearing a nose-clip in a seated position. Once the O$_2$ circuit was engaged subjects continued the same breathing pattern until the concentration of N$_2$ reached at least 1/40th of the original concentration for three consecutive breaths. The subjects were allowed to re-equilibrate in room air between tests with a rest time of 1.5 times the duration of the previous washout. The reproducibility of FRC$_{N2}$ and LCI$_{N2}$ required 3 tests which did not vary by >10%.

MBW$_{SF6}$ was performed in triplicate using the method described by (Horsley, et al., 2008a) and comprised of a wash-in and washout phase. The wash-in phase begins with the subject in a seated position, wearing a nose-clip and breathing at $V_T$ on a mouthpiece connected to an Innocor$^\text{TM}$ photo-acoustic gas analyser (Innovision AS, Odense, Denmark) using the Igor Pro written SimpleWashout program as outlined by Hannon, et al., (2014). A mixture of air and 0.2% Sulphur hexafluoride (SF$_6$) is tidally inspired until inspiratory and expiratory SF$_6$ concentrations differed by <0.004% (absolute difference in SF$_6$ concentration). The T-piece is then removed from the flow meter and the washout commenced. The subject now breathing room air, continued at $V_T$ until the end tidal SF$_6$ concentration had fallen to less than 0.005% ($1/40^{th}$ of the SF$_6$ concentration during wash-in) for at least three
consecutive breaths. Reproducibility criteria required values not to differ by >10%.

**5.2.4 Whole body Plethysmography**

Lung volumes were measured using a constant volume whole body plethysmograph (Autobox 6200 DL, Sensormedics, Yorba Linda CA, USA). Subjects sat inside the plethysmograph wearing a nose clip and formed a tight seal around the rubber mouthpiece while supporting their cheeks. Measurement commenced with breathing at tidal volume ($V_T$) until baseline FRC was established. Subjects then performed a series of ‘shallow breathing’ manoeuvres at a rate of approximately 1 Hz, which is equivalent to 60 per minute to assess airways resistance. After at least three flow/box pressure loops were obtained, the shutter was then closed and at least three mouth pressure/box pressure loops were recorded to determine thoracic gas volume. The computer software automatically calculated Raw, sGaw and FRC$_{Pleth}$. Immediately after the completion of the closed shutter panting the subject returned to tidal volume; exhaled to residual volume (RV), then performed an Inspired Vital Capacity (IVC) to determine Inspiratory Capacity (IC) and Total Lung Capacity (TLC). All panting loops were visually checked and erroneous manoeuvres were discarded. In accordance with the ATS/ERS guidelines (Wanger, et al., 2005) at least 3 reproducible FRC$_{Pleth}$ measurements $\pm$ 5% from the mean were obtained and the mean value reported.
### Figure 5.1. Order of procedures. MBW\textsubscript{N2}, Multiple Breath Nitrogen Washout. MBW\textsubscript{SF6}, Multiple Breath Sulphur Hexafluoride Washout

**5.2.5 Statistical Analysis**

Parametric data were expressed as mean (SD) and non-parametric data expressed as median with interquartile range. Differences between groups were assessed using a Chi-squared, unpaired t-test or the Mann Whitney test. Differences were deemed significant if \( p < 0.05 \). For the comparison of methods using \( \text{N}_2 \) and \( \text{SF}_6 \) differences were analysed using a paired t-test and the agreement between the two systems assessed using the Bland-Altman plots (Bland and Altman, 1986). All
statistical tests were performed using Prism (GraphPad Software Inc, version 6.04, San Diego, California, USA; http://www.graphpad.com).

5.3 Results

5.3.1 COPD Cohort 1: FRC$_{N2}$ Vs FRC$_{Pleth}$

The overall clinical characteristics for the COPD cohort 1 are summarised in Table 5.1. From the 44 COPD subjects enrolled 36 (82%) were evaluable. Three subjects (7%) were unable to perform adequate MBW$_{N2}$ measurements, 4 (9%) were unable to demonstrate satisfactory plethysmography technique and 1 (2%) subject was unable to attain both MBW$_{N2}$ and plethysmography results. Reasons for failure of MBW$_{N2}$ were due to subjects being incapable to maintain a satisfactory seal at the mouth during washout in addition to experiencing a dry throat as a result of the oxygen flow which repeatedly caused coughing and increased breathless. Plethysmography failure was due to subjects unable to retain a satisfactory seal at the mouth during determination of thoracic gas volume (TGV), which resulted in distress and increased breathlessness.
Table 5.1. Cohort 1 Subject Demographics

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>COPD Subjects</th>
</tr>
</thead>
<tbody>
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</tr>
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<td>Gender (Male/Female)</td>
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</tr>
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<td>Height (metres)</td>
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<td>Weight (Kg)</td>
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<tr>
<td>BMI (Kg/M$^2$)</td>
<td>27.9 (4.5)</td>
</tr>
<tr>
<td>Smoking History (Pack Years)</td>
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</tr>
<tr>
<td>FEV$_1$ %</td>
<td>61.6 (19.0)</td>
</tr>
<tr>
<td>FVC %</td>
<td>107.9 (19.0)</td>
</tr>
<tr>
<td>FEV$_1$/FVC</td>
<td>0.46 (0.1)</td>
</tr>
</tbody>
</table>

All parametric data are expressed as means and standard deviations unless otherwise stated. $^b$ denotes median and inter-quartile range.

5.3.1.1 FRC$_{N2}$ Vs FRC$_{Pleth}$

A significant difference was found between FRC$_{N2}$ % and FRC$_{Pleth}$ % (figure 5.2). The FRC$_{N2}$ % mean value was 139.3 (37.2) % and 124.5 (30.2) % for FRC$_{Pleth}$ ($p<0.0001$). There was a clear bias with FRC$_{N2}$ producing higher values, which appeared to increase disproportionally as FRC % increased beyond 120% predicted. The mean bias was 14.78 (16.36) with limits of agreement -17.29 to 46.84 (figure 5.3).
Figure 5.2. Comparison of measurements of functional residual capacity (N\textsubscript{2} vs. pleth). There was a significant difference in FRC\textsubscript{N2} vs. FRC\textsubscript{pleth}. FRC\textsubscript{N2} produced values equal to and above that measured by plethysmography with p<0.0001.

Figure 5.3. Bland-Altman Plot of the agreement between FRC\textsubscript{N2} % and FRC\textsubscript{pleth} % in a cohort of COPD subjects. The central dotted line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). The vertical line represents hyper-inflation with FRC\textsubscript{N2} >120 % predicted. FRC\textsubscript{N2} was higher than FRC\textsubscript{pleth}, mean difference 14.78 (16.36), which appeared disproportionally greater as FRC % increased (limits of agreement - 17.29 to 46.84).
5.3.2 COPD Cohort 2 and Healthy Controls: MBW\textsubscript{N2} Vs MBW\textsubscript{SF6}

The overall clinical characteristics are summarised in Table 5.2. There was a significant difference in mean age with the HC subjects being significantly younger than the COPD group (32 vs 64, p< 0.0001). Smoking history was not normally distributed and as expected there was a significant difference between the groups with a median pack year history of 0 (0-0) in HC and 36.75 (29.3-50.0) in COPD (p< 0.0001). In terms of spirometry there were predictably significant differences in FEV\textsubscript{1} % and FEV\textsubscript{1}/FVC. The HC group expressed greater values for both FEV\textsubscript{1}, 104.1 (15) % and FEV\textsubscript{1}/FVC, 0.8 (0.05) in comparison to 64.2 (21.1) %, and 0.53 (0.1) in the COPD group with p< 0.0001 in both instances. In the HC group all 12 subjects performed MBW\textsubscript{N2} and 10 performed MBW\textsubscript{SF6}. Two subjects were unable to perform MBW\textsubscript{SF6} due to equipment availability. There was a 100% success rate for both methods. From the 19 COPD subjects enrolled 17 (89%) were evaluable. Two subjects (11%) were unable to perform adequate MBW\textsubscript{N2} measurements, with 1 of these subjects also not able to complete MBW\textsubscript{SF6}. The 2 failed subjects were unable to maintain a satisfactory seal at the mouth during washouts. All successful tests demonstrated 3 tests with CV < 10% for FRC and LCI. This resulted in 10 HC and 17 evaluable COPD subjects for within group method comparisons.
### Table 5.2. Cohort 2 Subject Demographics

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Healthy Controls</th>
<th>COPD Subjects</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Gender (Male/Female)</td>
<td>7/5</td>
<td>12/7</td>
<td>p = ns(^a)</td>
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<tr>
<td>Age (Years)</td>
<td>32 (13)</td>
<td>64 (8)</td>
<td>p &lt; 0.0001</td>
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<tr>
<td>Height (metres)</td>
<td>1.72 (0.1)</td>
<td>1.66 (0.1)</td>
<td>p = ns</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>77.2 (13.6)</td>
<td>74.3 (20.5)</td>
<td>p = ns</td>
</tr>
<tr>
<td>BMI (Kg/M(^2))</td>
<td>26.1 (5.0)</td>
<td>26.5 (5.1)</td>
<td>p = ns</td>
</tr>
<tr>
<td>Smoking History (Pack Years)</td>
<td>0 (0-0)(^b)</td>
<td>36.75 (29.3-50.0)(^b)</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>FEV(_1) %</td>
<td>104.1 (15.0)</td>
<td>64.2 (21.1)</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>FVC %</td>
<td>110.7 (17.4)</td>
<td>101.4 (13.6)</td>
<td>p = ns</td>
</tr>
<tr>
<td>FEV(_1)/FVC</td>
<td>0.8 (0.05)</td>
<td>0.53 (0.1)</td>
<td>p &lt; 0.0001</td>
</tr>
</tbody>
</table>

All parametric data are expressed as means and standard deviations unless otherwise stated. \(a\) denotes chi-squared test. \(b\) denotes median and inter-quartile range. \(c\) denotes mann-whitney U test.
5.3.2.1 A Comparison of FRC$_{\text{N2}}$ and FRC$_{\text{SF6}}$

For MBW parameters there was a significant difference in FRC$_{\text{N2}}$ % between the groups. The HC subjects expressed a significantly lower mean value with 105.1 (28.5) compared to 136.8 (42.8) observed in the COPD group (p= 0.0015). This was in contrast to FRC$_{\text{SF6}}$ where there was no difference observed between HC and COPD (figure 5.4).

Within group comparisons reveals that there was no significant difference in FRC$_{\text{N2}}$ % and FRC$_{\text{SF6}}$ % in the HC group. FRC$_{\text{N2}}$ % values in the COPD cohort expressed a significantly greater mean value of 136.8 (42.8) % compared to 112.4 (28.0) % observed for FRC$_{\text{SF6}}$ % (p= 0.002). There were no observed differences in the HC group between FRC$_{\text{N2}}$ % and FRC$_{\text{SF6}}$ %.

There was no bias between the systems observed in the healthy controls with a mean difference of -0.9 (19.88), and limits of agreement -39.86 to 38.06 (figure 5.5a). The relative small n number impacted on the comparison in this group with one outlier leading to the wide limits of agreement. Without this value the limits of agreement would have been -12.96 to 22.52. In the COPD cohort there was a clear bias with FRC$_{\text{N2}}$ % producing higher values, which appeared to increase disproportionally as FRC % increased. The mean difference was 24.4 (26.36) with limits of agreement -27.25 to 76.07 (figure 5.5b).
Figure 5.4. Comparison of measurements of functional residual capacity in healthy controls and COPD subjects (N\textsubscript{2} vs SF\textsubscript{6}). COPD FRC\textsubscript{N2} produced significant greater values compared to COPD FRC\textsubscript{SF6} and HC FRC\textsubscript{N2}. Mean values were 136.8 (42.8) % for COPD FRC\textsubscript{N2} versus 112.4 (28.0) % for COPD FRC\textsubscript{SF6} (p= 0.002) and 105.1 (28.5) % for HC FRC\textsubscript{N2} (p= 0.048).
Figure 5.5a. Bland-Altman Plot of the agreement between FRC$_{N_2}$ % and FRC$_{SF_6}$ % in healthy controls. The central dotted line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). There was no bias observed across the two systems; mean difference was -0.9 (19.88), limits of agreement (-39.86 to 38.06).

Figure 5.5b. Bland-Altman Plot of the agreement between FRC$_{N_2}$ % and FRC$_{SF_6}$ % in subjects with COPD. The central dotted line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). There was a clear bias with mean difference of 24.41 (26.36), limits of agreement (-27.25 to 76.07) with the difference between systems appearing disproportionally greater as FRC % increased.
5.3.2.2 A Comparison of LCI\textsubscript{N2} and LCI\textsubscript{SF6}

As would be expected the HC group demonstrated significantly reduced LCI\textsubscript{N2} in comparison to the COPD subjects with 7.0 (0.6) versus 12.3 (2.2) respectively (p< 0.0001). This disparity was also evident in LCI\textsubscript{SF6} with the HC subjects producing a mean LCI of 6.3 (0.2) versus 11.9 (3.1) with p< 0.0001 (figure 5.6).

Within group comparisons highlights a significant difference in HC LCI values measured by both systems with mean LCI\textsubscript{N2} 7.0 (0.60) and mean LCI\textsubscript{SF6} 6.3 (0.2) (p= 0.004). In the COPD subjects there was no difference in LCI measured on both systems with a mean LCI\textsubscript{N2} of 12.3 (2.2) and LCI\textsubscript{SF6} 11.9 (3.1) (p= 0.4).

There was a clear bias in the HC group with MBW\textsubscript{N2} producing higher LCI values, mean difference of 0.68 (0.56), with the difference increasing as LCI increased. Despite this, overall limits of agreements were -0.42 to 1.79 (figure 5.7a). COPD LCI\textsubscript{N2} did produce higher values than LCI\textsubscript{SF6} with mean difference of 0.45 (2.13), with the difference becoming disproportionally higher as LCI increased with limits of agreement -3.72 to 4.61. The relative small n number impacted on the comparison in this group with one particular outlier leading to the wide limits of agreement. Without this value the limits of agreement would have been -2.63 to 4.16 (figure 5.7b).
Figure 5.6. Comparison of measurements of lung clearance index in healthy controls and COPD subjects (N\textsubscript{2} vs. SF\textsubscript{6}). COPD LCI\textsubscript{N2} produced significant greater values compared to HC LCI\textsubscript{N2}. Mean values were 7.0 (0.6) for COPD LCI\textsubscript{N2} versus 12.3 (2.2) for HC LCI\textsubscript{SF6} (p< 0.0001). This disparity was also evident in LCI\textsubscript{SF6} with the HC subjects producing a mean LCI of 6.3 (0.2) versus 11.9 (3.1) with p< 0.0001. There was a significant difference in HC LCI values measured by both systems with mean LCI\textsubscript{N2} 7.0 (0.60) and mean LCI\textsubscript{SF6} 6.3 (0.2) (p= 0.004). There was no difference in LCI measured on both systems in the COPD subjects.
Figure 5.7. Bland-Altman Plot of the agreement between LCI_{N2} and LCI_{SF6} in a) healthy controls and b) subjects with COPD. The central line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). In the HC group LCI_{N2} produced higher values than LCI_{SF6}, mean difference -0.68 (0.56), which became disproportionally higher as LCI increased, with good agreement (limits of agreement -0.42 to 1.79). In COPD the LCI_{N2} was higher than LCI_{SF6}, mean difference 0.45 (2.13), which was disproportionally greater as LCI increased (limits of agreement -3.72 to 4.61).
5.3.3 COPD Cohort 3: FRC\textsubscript{MBW} Vs FRC\textsubscript{Pleth}

The overall clinical characteristics for COPD cohort 3 are summarised in Table 5.3. From the 10 COPD subjects enrolled 8 (80\%) were evaluable for comparisons of FRC measured by MBW\textsubscript{N2}, MBW\textsubscript{SF6}, and plethysmography. Two subjects (20\%) were unable to perform adequate MBW\textsubscript{N2} measurements, with 1 of these subjects (10\%) also not able to complete MBW\textsubscript{SF6}. Reasons for failure were due to subjects being incapable to maintain a satisfactory seal at the mouth during washout and additionally for MBW\textsubscript{N2} due to a dry throat as a result of the oxygen flow that repeatedly caused coughing and increased breathless. One subject who was able to complete FRC measurements using all modalities was only able to perform FEV\textsubscript{1}’s during spirometry due to syncope.

Table 5.3. Cohort 3 Subject Demographics

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<td>Weight (Kg)</td>
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<td>BMI (Kg/M\textsuperscript{2})</td>
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<tr>
<td>Smoking History (Pack Years)</td>
<td>39.4 (33.9-49.5)\textsuperscript{b}</td>
</tr>
<tr>
<td>FEV\textsubscript{1} %</td>
<td>56.4 (25.3)</td>
</tr>
<tr>
<td>FVC %</td>
<td>100.4 (16.8)\textsuperscript{*}</td>
</tr>
<tr>
<td>FEV\textsubscript{1}/FVC</td>
<td>0.5 (0.2)\textsuperscript{*}</td>
</tr>
</tbody>
</table>

All parametric data are expressed as means and standard deviations unless otherwise stated. \textsuperscript{b} denotes median and inter-quartile range. \textsuperscript{*} Denotes based on 9 subjects.
5.3.3.1 MBW<sub>FRC</sub> comparison to FRC<sub>pleth</sub> in COPD

When the FRC measured by each MBW system is compared to plethysmography it was found that FRC<sub>SF6</sub> produced significantly reduced values than FRC<sub>pleth</sub> with a mean of 110.4 (25.16) % versus 136.5 (36.1) % and p=0.002. On the other hand measurements of FRC<sub>N2</sub> resulted in significantly greater mean values than FRC<sub>pleth</sub>. Mean FRC<sub>N2</sub> was 155.9 (45.3) % compared to 136.5 (36.1) % for FRC<sub>pleth</sub> with p=0.04 (figure 5.8). The mean difference between FRC<sub>SF6</sub> and FRC<sub>pleth</sub> was -26.13 (14.75), which became disproportionally greater as FRC increased with FRC<sub>pleth</sub> producing greater measurements than FRC<sub>SF6</sub> (limits of -55.03 to 2.78). The mean difference between FRC<sub>N2</sub> and FRC<sub>pleth</sub> was 19.38 (21.7), with FRC<sub>N2</sub> producing values greater than FRC<sub>pleth</sub> (limits of agreement -23.15 to 61.90) (figure 5.9).

Comparison of COPD Functional Residual Capacity

![Comparison of COPD Functional Residual Capacity](image)

FRC Method

Figure 5.8. Comparison of measurements of functional residual capacity (N<sub>2</sub> vs. SF<sub>6</sub> vs. pleth). There was a significant difference in FRC<sub>SF6</sub> vs. FRC<sub>pleth</sub> and FRC<sub>N2</sub> vs. FRC<sub>pleth</sub> with p=0.04 and p=0.002 respectively. FRC<sub>SF6</sub> values were consistently lower than FRC<sub>pleth</sub>, mean 110.4 (25.16) % versus 136.5 (36.1) % respectively. This was in contrast to FRC<sub>N2</sub> which produced values greater than that measured by plethysmography, mean values of 155.9 (45.3) % versus 136.5 (36.1) % respectively.
Figure 5.9. Bland-Altman Plot of the agreement between COPD MBW_{FRC} values and FRC_{pleth} with A) FRC_{SF6} vs. FRC_{pleth} and O) FRC_{N2} vs. FRC_{pleth}. The central line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). FRC_{SF6} produced lower values than FRC_{pleth}, mean difference -26.13 (14.75), which appeared disproportionally greater as FRC increased, with agreement limits of -55.03 to 2.78. FRC_{N2} was higher than FRC_{pleth} with a mean difference of 19.38 (21.7), which appeared disproportionally greater as FRC increased (limits of agreement -23.15 to 61.90).
5.4 Discussion
No one has directly measured MBW outcomes on the Exhalyzer D ($N_2$ washout) in parallel with the Innocor$^\text{TM}$ system ($SF_6$) and compared them to plethysmography in subjects with COPD. $MBW_{N_2}$ and $MBW_{SF_6}$ did produce differences in FRC and LCI values, which demonstrate that results are not interchangeable between devices. For use in COPD subjects each system will therefore require equipment specific normal values to be determined and the upper limit of normal defined.

In the HC group there was no significant difference in FRC measured by either system. Despite this the agreement between the 2 systems was ±40% which is significantly above the preferred variability of < 10% as well as the absolute variability cut off of < 25% suggested by the European Respiratory Society (Robinson, et al., 2013). This level of observed variation was largely attributed to 1 HC subject who struggled with a relaxed and consistent breathing pattern when firstly performing $MBW_{SF_6}$ but had undergone sufficient practice attempts, which became evident when performing $MBW_{N_2}$. Indeed without this subject the agreement between the 2 systems drastically improves to < 23%. In the COPD subjects however there was a clear bias with $FRC_{N_2}$ producing significantly higher values than $FRC_{SF_6}$. This difference in values became greater as FRC % predicted increased. The context of this raised $FRC_{N_2}$ becomes more significant when it is compared to $FRC_{\text{pleth}}$. We found $FRC_{N_2}$ to produce values up to 20% greater than $FRC_{\text{pleth}}$. Physiologically this highlights an error in the estimation of $FRC_{N_2}$. Inert gas washout testing would be expected to produce lower FRC values than plethysmography as washout techniques cannot measure trapped air or bullae and does not measure other intra-thoracic gas. In COPD there are poorly ventilated and obstructed peripheral sections of the lung that the gas cannot penetrate. Plethysmography however includes the gas volume of the whole thoracic cavity. In this regard the possible effect 100% $O_2$ may have upon tidal breathing could offer a potential reason for the increase in COPD FRC values observed (Schibler, et al., 2000).
Further studies on the effect of 100% O\textsubscript{2} in COPD are still needed to evaluate this.

Our findings are in accordance with previous data that has been reported in other obstructive lung diseases such as cystic fibrosis. Jensen et al (2013) compared MBW\textsubscript{N2} to mass spectrometry using SF\textsubscript{6} in healthy and CF populations. They also found no bias in FRC for the HC group but a clear bias in CF subjects with FRC\textsubscript{N2} rising disproportionately as FRC values increased. This bias was also reflected in LCI\textsubscript{N2}, which was increased in a similar pattern to FRC. They found a mean bias of ± 5, comparable to the current result in COPD of ± 4.6. They also reported LCI\textsubscript{N2} generated higher values in the HC group with a mean bias of ± 2, which supports the present finding of a HC mean bias of ± 1.8. They also demonstrated that when compared to FRC\textsubscript{pleth}, SF\textsubscript{6} values better represented air trapping in the distal lung. Despite these similar findings, Jensen et al (2013) concludes that MBW\textsubscript{N2} is a preferable alternative to MBW\textsubscript{SF6} in obstructive lung diseases. Justification for this centres around MBW\textsubscript{N2} producing FRC values closer to that measured by plethysmography as well as the test including gas from extremely slow ventilating lung units which MBW\textsubscript{SF6} cannot measure. However the current data set confirms previous findings that LCI is largely unaffected by variation in FRC (Aurora et al, 2005). LCI\textsubscript{N2} values from the Jensen cohort was found to increase disproportionately more than LCI\textsubscript{SF6} in a similar fashion to FRC. This level of FRC variation may have impacted on LCI measurements and suggests that MBW\textsubscript{N2} may not be the preferential method of determining VI in obstructive lung disease. The FRC variation maybe due to an error in the signal alignment algorithm used in MBW\textsubscript{N2}, between gas and airflow leading to over estimation of MBW parameters. The variation may also be due to use of N\textsubscript{2} as a tracer gas, as N\textsubscript{2} is also found in the blood and tissues surrounding the lungs. Gas washout will occur slower in the regions of poor ventilation especially in those with severe disease that may cause a greater diffusion gradient in the well-ventilated portions of the lung, drawing tissue N\textsubscript{2} across and over estimating MBW parameters. This would also lead to
extended test durations compared to MBW_{SF6} further limiting the practicalities of MNW_{N2} in obstructive lung diseases.

The use of inert gas washout techniques in combination with FRC_{pleth} has previously been widely used as a marker of gas trapping (Wanger, et al., 2005). Our findings indicate that FRC_{SF6} would provide the best reflection of gas trapping in this population. FRC_{SF6} was significantly lower than FRC_{pleth} in all subjects and this difference increased as FRC values increased. This was most evident in those deemed to be hyperinflated as defined by FRC > 120% predicted (Gagnon, et al., 2014). Statically hyper-inflated subjects often are those with a greater level of disease progression in whom increased breathlessness reflects their reduced ventilation efficiency and exercise capacity. These subjects often find the technique of plethysmography the most challenging, resulting in an increase in hyperinflation that further raises FRC values. The use of FRC_{SF6} measured by the Innocor™ system in conjunction with FRC_{pleth} offers important information regarding the level of gas trapping and the difference between the two could potentially be used as an outcome marker in clinical trials.

In terms of ventilation inhomogeneity as represented by LCI we observed a significant difference in the HC group with LCI_{N2} producing higher values in all subjects. There did however remain good agreement between the 2 systems with limits ± 1.8 although the difference between the systems became more apparent as LCI increased. For the COPD subjects, despite the differences in FRC, there was no significant difference between LCI measured on both systems. We found that LCI_{SF6} did generate higher values, with a mean difference of 0.45, which increased disproportionally as LCI increased. Despite this there remained good agreement between the systems with limits ± 4.6. The increase in reported LCI_{SF6} may be due to 2 reasons. Firstly significant increase in FRC_{N2} may have resulted in greater air trapping which limited the amount of N₂ that could be exhaled and measured resulting in the under estimation of MBW parameters. Secondly the rarefraction
of SF$_6$, with its larger molecular mass, may diffuse further into the lung periphery than N$_2$. This would shift the location of the diffusion front causing higher LCI values more representative of global lung ventilation inhomogeneity (Robinson, et al., 2013; Verbanck, 2012).

Overall our data demonstrates that there are distinct differences in MBW parameters across different systems and that these are not interchangeable. These findings highlight the need for system specific reference values to be generated. The choice of system and inert gas will impact upon multi-centre clinical trial design and interpretation relevant to disease, so clinically relevant responses to interventions can be established. Further studies validating MBW systems in COPD are needed and should also consider overall test feasibility. From our data set variation in the agreement between FRC and LCI values was evident in COPD subjects, especially in those with advanced disease. This highlights the increase in variability occurring as test durations become extended. These findings not only show MBW as a useful marker of VI but also as a complimentary tool to plethysmography. In addition MBW also provides an alternative method of FRC determination for subjects unable to perform the plethysmography technique or for clinics without access to a plethysmograph.
CHAPTER 6

6.0 A Review of the Practicality of Lung Clearance Index

6.1 Introduction

For a clinical endpoint to be globally adopted it needs to be sensitive to disease identification and progression, repeatable across testing sessions, as well as being practical to perform (Huque, Alosh and Bhore, 2011). Chapter 3 demonstrated the sensitivity of LCI in a COPD population and compared it to currently accepted physiological tests. COPD subjects with VI as demonstrated by an increased LCI were identified, with 20% of which also demonstrating an FEV₁ in the normal range for healthy controls. Chapter 4 showed that LCI is a highly repeatable and reproducible assessment of VI in COPD with intra-test variation < 5%. Chapter 5 compared MBW_{N₂} and MBW_{SF₆} and found that there were significant differences between the methods that would limit cross system comparisons. In order to further investigate the use of MBW in COPD the assessment of practicality remains. Two main areas when considering the practicality of a test are the success rates of technically acceptable measurements and the time duration that testing requires.

6.1.1 Aims and Objectives

The aim of this chapter was to assess the practical implementation of MBW in a cohort of COPD subjects. The study objectives were;

(1) To review the success rates of physiological testing in the COPD and Healthy subjects from chapter 3.

(2) To evaluate the total test time of MBW_{N₂} and MBW_{SF₆} in COPD and healthy controls from chapter 5.
6.2 Methods
A review of the 54 COPD subjects and 12 healthy controls from chapter 3 was performed to assess the success rate of MBW$_{N_2}$. Subject information and study design details can be found in section 3.2.1 and 3.2.2. The test durations from the 19 COPD subjects and 12 healthy controls in chapter 5 were used to compare MBW$_{N_2}$ and MBW$_{SF_6}$. Subject information and study design details can be found in sections 5.2.1 and 5.2.2. All testing was completed in line with the details outlined in chapter 2.

6.2.1 Statistical Analysis
Parametric data were expressed as mean (SD) and non-parametric data expressed as median with interquartile range. Differences between groups were assessed using the Mann Whitney test. Differences were deemed significant if $p < 0.05$. For the comparison of methods using N$_2$ and SF$_6$ differences were analysed using a paired t-test and the agreement between the two systems assessed using the Bland-Altman plots (Bland and Altman, 1986). All statistical tests were performed using Prism (GraphPad Software Inc, version 6.04, San Diego, California, USA; http://www.graphpad.com).
6.3 Results

6.3.1 Success Rates

The overall clinical characteristics are summarised in chapter 3 table 3.1. The success rates of the physiological assessments are outlined in table 6.1. All MBW measurements were examined for quality control. Washouts that showed irregular tidal breathing patterns, leaks at the mouth or incomplete measurements were discounted. All 12 of the HC group were able to perform adequate technique for MBW\textsubscript{N2} and spirometry. From the 54 COPD subjects that enrolled to perform spirometry, reversibility, and MBW there were 44 who also opted to perform IOS, DL\textsubscript{CO} and whole body plethysmography. One hundred percent of subjects were able to achieve technically adequate results for IOS. For spirometry 100\% were able to perform FEV\textsubscript{1}, with 98\% able to expire completely to obtain FEF\textsubscript{25-75} and FVC. There was a 95\% and 93\% success rate observed in DL\textsubscript{CO} and MBW testing respectively with plethysmography demonstrating a success of 86\%.

6.3.1.1 Reasons for Failure

The reasons for test failure are summarised in table 6.2. For COPD MBW\textsubscript{N2} 3 subjects did not obtain adequate results due to leak at the mouth and 1 subject discontinued the test due to an increased feeling of shortness of breath (SOB) and distress caused by the flow rate of the O\textsubscript{2} and dry throat. In terms of spirometry a single COPD subject (2\%) was only able to complete FEV\textsubscript{1}’s due to syncope when performing FVC manoeuvres. Five of the COPD subjects (12\%) found the measurement of thoracic gas volume (TGV) too difficult to perform and became excessively short of breath that resulted in a leak at the mouth or termination of the test. One subject (2\%) was unable to perform the technique due to an equipment malfunction. This malfunction also affected the gas transfer testing for 1 subject (2.5\%), with one other subject unable to perform an adequate inspired vital capacity (IVC)
greater than 85% predicted and complete the breath hold duration required (2.5%).

Table 6.1 Success Rates in COPD (n = 54) and Healthy Controls (n = 12)

<table>
<thead>
<tr>
<th>Type of Respiratory Test</th>
<th>Number of Test Sessions in COPD</th>
<th>Number of Test Sessions in Healthy Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Provided</td>
<td>Valid (%)</td>
</tr>
<tr>
<td>MBW(_{N2})</td>
<td>54</td>
<td>50 (93)</td>
</tr>
<tr>
<td>FEV(_1)</td>
<td>54</td>
<td>54 (100)</td>
</tr>
<tr>
<td>FEF(_{25-75})</td>
<td>54</td>
<td>53 (98)</td>
</tr>
<tr>
<td>FVC</td>
<td>54</td>
<td>53 (98)</td>
</tr>
<tr>
<td>IOS</td>
<td>44</td>
<td>44 (100)</td>
</tr>
<tr>
<td>Pleth</td>
<td>44</td>
<td>38 (86)</td>
</tr>
<tr>
<td>DL(_{CO})</td>
<td>44</td>
<td>42 (95)</td>
</tr>
</tbody>
</table>
Table 6.2 Reasons for Test Failure in COPD Subjects

<table>
<thead>
<tr>
<th>Type of Respiratory Test</th>
<th>Number of Failed Subjects</th>
<th>Failure (%)</th>
<th>Reasons for Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBW&lt;sub&gt;N2&lt;/sub&gt;</td>
<td>4</td>
<td>7</td>
<td>Leak at mouth, dry throat, increased SOB</td>
</tr>
<tr>
<td>Spirometry</td>
<td>1</td>
<td>2</td>
<td>Syncope during FVC</td>
</tr>
<tr>
<td>Pleth</td>
<td>6</td>
<td>14</td>
<td>Leak at mouth, increased SOB, equipment error</td>
</tr>
<tr>
<td>DL&lt;sub&gt;CO&lt;/sub&gt;</td>
<td>2</td>
<td>5</td>
<td>IVC &lt;85% predicted, equipment error</td>
</tr>
</tbody>
</table>

6.3.2 Test Duration of MBW: HC Vs COPD

The overall clinical characteristics are summarised in chapter 5 Table 5.1. Figure 6.1 shows the HC and COPD total test duration for MBW<sub>N2</sub> and MBW<sub>SF6</sub>. Test duration was significantly longer in the COPD subjects compared to the HC for both N<sub>2</sub> and SF<sub>6</sub>. For MBW<sub>N2</sub> COPD subjects required a median total test time of 40.7 (23.1-45.05) minutes in comparison to a median time of 17.55 (12.98-21.50) minutes in the HC’s (p< 0.0001). For MBW<sub>SF6</sub> COPD subjects demonstrated a median total test time of 32.1 (20.65-50.40) minutes in comparison to a median time of 19.20 (16.38-25.65) minutes in the HC’s (p= 0.007).
Figure 6.1. MBW\textsubscript{N2} total test time for healthy controls and COPD subjects. The test duration for COPD subjects was significantly longer for both MBW methods. For MBW\textsubscript{N2} COPD subjects required a median total test time of 40.7 (23.1-45.05) minutes in comparison to a median time of 17.55 (12.98-21.50) minutes in the HC’s (p<0.0001). For MBW\textsubscript{SF6} COPD subjects demonstrated a median total test time of 32.1 (20.65-50.40) minutes in comparison to a mean time of 20.86 (5.83) minutes in the HC’s (p=0.007).

6.3.2.1 Test Duration of MBW: N\textsubscript{2} Vs SF\textsubscript{6}

Bland Altman plots of the test durations measured by the 2 methods in COPD and HC are displayed in figure 6.2. For total test time there was no significant difference between the two systems in the HC group with MBW\textsubscript{N2} time of 17.31 (6.62), and 20.86 (5.83) minutes for MBW\textsubscript{SF6} (p=0.12). MBW\textsubscript{SF6} did produce slightly longer test times with a mean difference -0.36 (6.63) with limits of agreement -16.54 to 9.44 (figure 6.2a). In the COPD subjects the duration of the test across the 2 methods was approaching significance with total median test time for MBW\textsubscript{N2} of 40.7 (23.1-45.05) minutes and 32.1 (20.65-50.40) minutes for MBW\textsubscript{SF6} (p= 0.065). There was a clear bias in total test duration for the COPD
group with MBW\textsubscript{N2} producing test times longer than the MBW\textsubscript{SF6}, mean difference 5.87 (12.01) with limits of agreement -17.67 to 29.41. This difference became more disproportionate as total test time increased (figure 6.2b).

![Figure 6.2. Bland-Altman Plot of the agreement between MBW\textsubscript{N2} and MBW\textsubscript{SF6} total test time in a) healthy controls and b) subjects with COPD. The central line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). In the HC group MBW\textsubscript{SF6} was on average longer in duration with mean difference -0.36 (6.63) minutes and limits of agreement -16.54 to 9.44. In the COPD subjects there was a clear bias in total test time with MBW\textsubscript{N2} producing times significantly longer than MBW\textsubscript{SF6}, mean difference 5.87]
(12.01) with limits of agreement -17.67 to 29.41. This difference became more disproportionate as total test time increased.
6.4 Discussion

The present review is one of the first to analyse the practical considerations of MBW success rate, in comparison to current physiological testing and the impact of test time in a COPD population. Success rates were comparable with other routine tests and the test duration required indicates the greatest application to be in the mild subjects. Our first objective was to evaluate the success rate of the MBW technique.

The combined overall success rate of MBW\textsubscript{N2} in COPD and HC’s (94%), was above the overall success rate of 76% previously reported for CF and HC’s (Fuchs, et al., 2012). The present data demonstrates that the success rates in the COPD subjects alone (MBW\textsubscript{N2} 93%) as well as the 95% success rate of MBW\textsubscript{SF6} found in cohort 2 in chapter 5, is also comparable to those found in previous CF research (Horsley, et al., 2008a). Apart from those subjects whose level of obstruction caused them to be unable to perform MBW, it was found that the success rates or those who could perform technically adequate tests were as a result of the operator’s familiarity with the equipment and the time spent with the subject explaining the technique. These factors could have a large effect on multi-centre trials unless adequate expertise of the operators is determined and standardised comprehensive training is given to subjects.

The second objective was to evaluate the total MBW test duration. In the HC group there was no significant difference in total test time between the two systems. As would be expected the COPD test time for both methods were significantly greater than the HC group. Furthermore in the COPD group the difference between the two systems was approaching significance with MBW\textsubscript{N2} producing a longer test time of 41 minutes compared to just 32 minutes for MBW\textsubscript{SF6} (p=0.065). This difference was also found to become more disproportionate as test time increased indicating that the use of an MBW\textsubscript{N2} system, where the breathing of O\textsubscript{2} in an open circuit is utilised, may contribute
physiologically to the determination of the level of ventilation heterogeneity. This in turn can lead to overly exaggerated test times. One limiting factor of this comparison was the relative small n number. An increase in the number of participants would strengthen the findings and likely highlight a statistically significant difference between the two methods in a COPD population.

In summary MBW is a promising tool that displayed good success rates in both HC’s and COPD subjects. The use of MBW\textsubscript{N2} is easy for subjects to perform, and may be suitable for those that find more physically demanding tests such as plethysmography challenging. There were differences however in the COPD test duration between the MBW\textsubscript{N2} and MBW\textsubscript{SF6} systems. This difference became progressively more pronounced as disease severity increased and resulted in longer test times, which may limit the practical implementation of MBW in this group as well as being important in system selection when planning a COPD trial.
CHAPTER 7

7.0 A Review of Additional Indices of Multiple-Breath Washout

7.1 Introduction

In previous chapters the focus has largely been directed to the evaluation of lung clearance index. LCI is a global representation of ventilation inhomogeneity and therefore is a product of differences in lung emptying from both the large and small airways. The underpinning mechanisms of LCI were experimentally investigated in healthy subjects by Crawford et al (1985). The results of which aided in the understanding of ventilation in a non-uniformed manner at each branch point of the lungs, including the airways in which gas mixing is driven by convective flow (mainly larger airways) and those where it is driven by diffusion (acinar lung compartments). In relation to COPD Verbanck and colleagues built upon this theory and analysed the inert gas washout curve in order to establish a greater understanding of VI and its components. Verbanck et al, 1997, mathematically represented these 2 concepts on an MBW curve as \( S_{\text{cond}} \) (convection) and \( S_{\text{acin}} \) (diffusion) through analysis of phase III slopes. In 2004 Verbanck et al used these values to investigate the effect of smoking on the lungs in comparison to \( \text{FEV}_1 \). Abnormalities in \( S_{\text{cond}} \) and \( S_{\text{acin}} \) were apparent in smokers with a pack year history of 10 where as \( \text{FEV}_1 \) was unable to highlight any abnormality until a pack year history of at least 20 was recorded. Then in 2006 Verbanck et al was able to show a reduction in VI represented by \( S_{\text{cond}} \) in healthy smokers who adhered to smoking cessation intervention. Such findings not only underline the sensitivity of MBW in detecting smoking related damage but also highlight the small airways as one of the main sites for early manifestations of airway dysfunction. In order to evaluate the potential mechanistic markers of VI in COPD the question remains as to the intra-test reproducibility and inter-test
repeatability of $S_{\text{cond}}$ and $S_{\text{acin}}$ as well as the sensitivity of the values in relation to currently accepted physiological measures.

### 7.1.1 Aims and Objectives

The aim of this study was to evaluate the reproducibility, repeatability and sensitivity of $S_{\text{condN2}}$ and $S_{\text{acinN2}}$ as a clinical measure of airways disease in COPD. The study objectives were:

1. To investigate the sensitivity of $S_{\text{condN2}}$ and $S_{\text{acinN2}}$ to differentiate COPD severity as defined by GOLD Classifications.

2. To identify correlations in COPD subjects between $S_{\text{condN2}}$ and $S_{\text{acinN2}}$ and other markers of the small airways including $R_{5-R_{20}}$, $K_{CO}$ and $RV/TLC$.

3. To determine the coefficient of variation (CV) of $S_{\text{condN2}}$ and $S_{\text{acinN2}}$ across 3 technically acceptable MBW manoeuvres in healthy controls and COPD subjects.

4. To evaluate the reproducibility of $S_{\text{condN2}}$ and $S_{\text{acinN2}}$ in COPD subjects across 2 testing sessions separated by $\geq 24$ hours.
7.2 Methods

A review of the 54 COPD subjects and 12 healthy controls from chapter 3 was performed to compare Scond\textsubscript{N2} and Sacin\textsubscript{N2} values to current physiological tests. Subject information and study design details can be found in section 3.2.1 and 3.2.2. A review of the 20 COPD subjects and 12 healthy controls from chapter 4 was also performed to evaluate the intra-test repeatability and inter-visit reproducibility of Scond\textsubscript{N2} and Sacin\textsubscript{N2}. Subject information and study design details can be found in sections 4.2.1 and 4.2.2. All testing was completed in line with the details outlined in chapter 2.

7.2.1 Statistical Analysis

Parametric data were expressed as mean (SD) and non-parametric data expressed as median with interquartile range. Differences between groups were assessed using an unpaired t-test or the Mann Whitney test. Correlations were investigated using a Pearson’s correlation coefficient or a Spearman’s rank correlation coefficient. Intra-test repeatability for Scond\textsubscript{N2} and Sacin\textsubscript{N2} were determined by calculating the coefficient of variation (CV) as 100 x SD/mean of the three recordings for each subject. Inter-visit reproducibility was assessed using Bland-Altman plots (Bland and Altman, 1986). All statistical tests were performed using Prism (GraphPad Software Inc., version 6.04, San Diego, California, USA; http://www.graphpad.com).
7.3 Results

7.3.1 Scond\textsubscript{N2} and Sacin\textsubscript{N2} vs Gold Stage

There was no significant difference observed between GOLD stages for Scond\textsubscript{N2}. Figure 7.1 shows the distribution of Sacin\textsubscript{N2} across GOLD stages, as quantified by FEV\textsubscript{1}%. Sacin\textsubscript{N2} increased as the GOLD stage increased. There was no significant difference between GOLD stage 1 and 2 but a significant difference was observed between GOLD stage 2 and 3 with mean values of 0.34 (0.14) and 0.50 (0.11), with \( p=0.0011 \). There was also a significant difference between GOLD stage 1 and 3 with mean values of 0.28 (0.19) and 0.50 (0.11), with \( p=0.0012 \). There was only a single subject in GOLD stage 4. These results are also reflected in the fact that no correlation was found between Scond\textsubscript{N2} and overall FEV\textsubscript{1} (figure 7.2) but a significant negative correlation was found between Sacin\textsubscript{N2} and FEV\textsubscript{1} with \( r=-0.50 \), \( p = 0.0002 \) figure 7.3).

\textbf{Sacin\textsubscript{N2} Vs GOLD Classification}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{SacinN2_GOLD_classification.png}
\caption{Sacin\textsubscript{N2} versus GOLD stage. The mean value for stage 1 was 0.28 (0.19), the mean value for stage 2 was 0.34 (0.14) and the mean value for stage 3 was 0.50 (0.11). There was no significant difference between stages 1 and 2 but a significant difference was observed between stages 2 and 3 with \( p= 0.0011 \). Stage 1 significantly differed from stage 3 (\( p=0.0012 \)).}
\end{figure}
Figure 7.2. Scond\textsubscript{N2} versus FEV\textsubscript{1}. There was no correlation found between Scond\textsubscript{N2} and FEV\textsubscript{1}.

Figure 7.3. Sacin\textsubscript{N2} versus FEV\textsubscript{1}. There was a significant negative correlation found between Sacin\textsubscript{N2} and FEV\textsubscript{1} with $r=-0.50$, $p=0.0002$. 
7.3.2 Scond$_{\text{N2}}$ and Sacin$_{\text{N2}}$ correlation with respiratory system resistance and reactance

There were no observed correlations between Scond$_{\text{N2}}$ and any IOS parameter. Sacin$_{\text{N2}}$ however demonstrated a significant positive correlation with R$_5$-R$_{20}$. As diffusive inhomogeneity increased there was an increase in frequency dependence resistance with $r=0.33$ and $p=0.02$ (figure 7.4). The most significant correlation with Sacin$_{\text{N2}}$ was observed with $X_5$. As the level of diffusive inhomogeneity increased there was a negative correlation with the elastic recoil properties of the small airway parenchyma with $r=-0.45$, $p=0.004$ (figure 7.5).

**Correlation of Sacin N$_2$ Vs R$_5$-R$_{20}$**

![Graph showing correlation between Sacin N$_2$ and R$_5$-R$_{20}$.](image)

$r=0.33$ $p=0.03$

Figure 7.4. Sacin$_{\text{N2}}$ versus R$_5$-R$_{20}$.

There was a significant positive correlation between Sacin$_{\text{N2}}$ and R$_5$-R$_{20}$ with $r=0.38$, $p=0.02$. 
There was a significant negative correlation between $\text{Sacin}_N$ and $X_5$ with $r=0.52$, $p=0.0006$.

### 7.3.4 Scond$_{N2}$ and $\text{Sacin}_N$ correlation with diffusing capacity

There was no relationship found between ventilation inhomogeneity represented by Scond$_{N2}$ and volume adjusted rate of gas transfer occurring in the diffusive parts of the lung known as $K_{CO}$. $\text{Sacin}_N$ was found to significantly correlate with $K_{CO}$ with $r=-0.34$, $p=0.05$ (figure 7.6).

![Correlation of $\text{Sacin}_N$ Vs $X_5$](image)

$r = -0.45$, $p = 0.004$

Figure 7.5. $\text{Sacin}_N$ versus $X_5$. There was a significant negative correlation between $\text{Sacin}_N$ and $X_5$ with $r=0.52$, $p=0.0006$.

![Correlation of $\text{Sacin}_N$ Vs $K_{CO}$ %](image)

$r = -0.34$, $p = 0.04$

Figure 7.6. $\text{Sacin}_N$ versus $K_{CO}$ %. There was a significant correlation between $\text{Sacin}_N$ and $K_{CO}$ %.
7.3.5 $\text{Scond}_N_2$ and $\text{Sacin}_N_2$ correlation with lung volumes

With respect to hyperinflation and the level of air trapping represented by RV/TLC %, there were no observed correlation with $\text{Scond}_N_2$ but there was with $\text{Sacin}_N_2$ with $r= 0.41$ and $p= 0.02$ (figure 7.7). A significant correlation was also found between $\text{Sacin}_N_2$ and $R_{aw}$ ($r= 0.37$ and $p= 0.030$) (figure 7.8).

**Figure 7.7. $\text{Sacin}_N_2$ versus RV/TLC %.** There was a significant positive correlation between $\text{Sacin}_N_2$ and RV/TLC % with $r= 0.41$, $p= 0.02$.

**Figure 7.8. $\text{Sacin}_N_2$ versus Airways Resistance.** There was a significant positive correlation between $\text{Sacin}_N_2$ and $R_{aw}$ with $r= 0.37$, $p= 0.030$. 

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7.3.6 Scond$_{N2}$ and Sacin$_{N2}$ correlation with Exercise Capacity and Patient Reported Outcomes

Scond$_{N2}$ and Sacin$_{N2}$ failed to correlate with exercise capacity measured as distance covered in a 6-minute walk test. There was also no correlation between Scond$_{N2}$ and CAT$^{TM}$ questionnaire score. A significant positive correlation was observed however, between Sacin$_{N2}$ and CAT$^{TM}$ score with $r=0.47$ and $p=0.003$ (figure 7.9).

**Figure 7.9. Sacin$_{N2}$ versus CAT$^{TM}$ score.** There was a significant positive correlation between Sacin$_{N2}$ and CAT$^{TM}$ with $r=0.47$, $p=0.003$
7.3.7 Intra-Test Repeatability of Scond$_{N2}$ and Sacin$_{N2}$

The mean (SD) intra-test CV for Scond$_{N2}$ and Sacin$_{N2}$ derived from triplicate washout manoeuvres on the same visit were 25.05 (16.13-30.85) and 57.45 (14.40-94.45) respectively for healthy controls. For COPD subjects Scond$_{N2}$ CV was 29.45 (15.75-45.78) and Sacin$_{N2}$ CV was 6.80 (2.775-9.450). There was no significant difference in Scond$_{N2}$ CV between the groups, however a difference was observed in Sacin$_{N2}$ CV with p= 0.0002 (Table 7.1).

7.3.8 Inter-Visit Reproducibility of Scond$_{N2}$ and Sacin$_{N2}$

The 20 COPD subjects performed repeat measurements of MBW$_{N2}$ in triplicate after a mean (SD) of 16 (13) days. A Bland-Altman plot between repeated measurements of Scond$_{N2}$ shows that the mean difference was 0.003 (0.02) and the 95% limits of agreement between the two measurements were -0.04 to 0.04 (figure 7.10). For Sacin$_{N2}$ the Bland-Altman plot shows the mean difference was -0.03 (0.25) with limits of agreement -0.51 to 0.46 (figure 7.11).

<table>
<thead>
<tr>
<th>MBW Parameter</th>
<th>Healthy Control</th>
<th>COPD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scond$_{N2}$</td>
<td>0.013 (0.008-0.019)</td>
<td>0.036 (0.017-0.048)</td>
<td>p= 0.0007$^a$</td>
</tr>
<tr>
<td>Scond$_{N2}$ CV</td>
<td>57.450 (14.40-94.45)</td>
<td>29.450 (15.75-45.78)</td>
<td>p= ns$^a$</td>
</tr>
<tr>
<td>Sacin$_{N2}$</td>
<td>0.058 (0.035-0.073)</td>
<td>0.326 (0.268-0.434)</td>
<td>p&lt; 0.0001$^a$</td>
</tr>
<tr>
<td>Sacin$_{N2}$ CV</td>
<td>25.05 (16.13-30.85)</td>
<td>6.800 (2.775-9.450)</td>
<td>p= 0.0002$^a$</td>
</tr>
</tbody>
</table>

All parametric data are expressed as median and interquartile ranges unless otherwise stated. $^a$ denotes Mann-Whitney U test.
Figure 7.10. Bland-Altman Plot of the agreement between visits of $\text{Scond}_{\text{N2}}$ in COPD subjects. The central dotted line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). The mean difference in LCI$_{\text{N2}}$ was 0.003 (0.02) across 2 visits with limits of agreement -0.04 to 0.04.

Figure 7.11. Bland-Altman Plot of the agreement between visits of $\text{Sacin}_{\text{N2}}$ in COPD subjects. The central dotted line represents the mean difference, and the upper and lower dotted lines represent the limits of agreement (mean difference ± 2 SD). The mean difference in LCI$_{\text{N2}}$ was -0.03 (0.25) across 2 visits with limits of agreement -1.51 to 0.46.
7.4 Discussion

To the best of our knowledge this is the first study to evaluate the sensitivity of Scond\textsubscript{N2} and Sacin\textsubscript{N2} in relation to current accepted physiological measures as well as the intra-test reproducibility and inter-test repeatability in a COPD population. Scond\textsubscript{N2} was not found to correlate with any current physiological measurements of the small airways. By definition of the theoretical principle of Scond\textsubscript{N2} abnormalities can occur in any of the conducting airways from the first generation meaning that Scond\textsubscript{N2} it is not specific to small airways (McNulty and Usmani, 2014). In theory it would have been expected for correlations between Scond\textsubscript{N2} and measurements of DL\textsubscript{CO}, FEV\textsubscript{1} and IOS parameters as these tests also purport to measure similar airways.

The first objective was to investigate the underlying mechanisms that make up LCI across GOLD stage severity quantified by FEV\textsubscript{1}. Sacin\textsubscript{N2} was found to differentiate between GOLD stage 2 and 3 (\(p= 0.0011\)) but not between stages 1 and 2. The lack of significance between stages 1 and 2 can be attributed to 1 single subject’s Sacin\textsubscript{N2} measurement which if discounted results in a significance level of \(p= 0.03\). The trend was for VI to rise as the level of obstruction increased and Sacin\textsubscript{N2} measured this.

The second objective was to identify correlations in COPD between Sacin\textsubscript{N2} and other markers of the small airways. Chapter 3 highlighted the level of correlation between LCI and markers of IOS with \(r= -0.38\) (\(p= 0.02\)), for R\textsubscript{5}-R\textsubscript{20} and \(r= -0.52\) (\(p=0.006\)) for X\textsubscript{5}. Sacin\textsubscript{N2} was found to similarly correlate with R\textsubscript{5}-R\textsubscript{20} and X\textsubscript{5} with \(r= 0.33\), \(p= 0.03\) and \(r= -0.45\), \(p=0.004\) respectively. In terms of gas transfer efficiency Sacin\textsubscript{N2} showed a significant negative correlation with K\textsubscript{CO} (\(r= -0.34\), \(p= 0.04\)). This was in contrast to LCI\textsubscript{N2}, where no correlation was observed. This finding fits with the conventional description of Sacin\textsubscript{N2} as a measure of the distal lung in comparison to LCI\textsubscript{N2}, which is a global measure of VI. Air trapping in the small airways, represented by an increase in RV as a function of TLC is a common physiological feature of COPD. Sacin\textsubscript{N2}
was found to correlate with RV/TLC% on a similar level to LCI
N2 but with an increased significance level with Sacin
N2 showing r= 0.41 (0.02) compared to LCI
N2 with r= 0.44 (p= 0.008). This illustrates the
contribution of small airway impairment in the level of air trapping as
disease severity increases. However Sacin
N2 also correlated, with similar
significance as LCI
N2, with measurements of the distal portions of the
lungs as well global markers of symptoms. Sacin
N2 correlated with Rsaw
on a similar level to LCI
N2 with r= 0.37 (0.03) compared to LCI
N2 with
r= 0.47 (p= 0.004) and also displayed a significant positive correlation
(r= 0.47, p= 0.0003) with CAT™ scores. This bring into question the
specificity of Sacin
N2 as a reflection of the acinar lung compartments
and suggests that it may provide evidence, similar to LCI
N2, as to overall
gas mixing in the lung. In terms of exercise capacity, just as was found
with LCI, Sacin
N2 was not found to correlate with the distance covered in
a 6-minute walk test. One possible reason for this may be due to the
confounding internal and external factors that can impact upon the
results of a 6 minute walk test such as leg fatigue, hip/knee
replacements, weight, cardiovascular disease as well as subject
motivation.

The third objective was to examine the coefficient of variation (CV)
across 3 technically acceptable MBW manoeuvres in COPD subjects
compared to healthy controls. Clinical endpoints are required to display
adequate intra-test variation so that the results have a high degree of
validity. The level of Scond
N2 was significantly greater in the COPD
group with p= 0.0007, although there was no significant different in the
intra-test variation (CV) of between the COPD and HC groups. Sacin
N2
was also significantly increased in the COPD group with p< 0.0001.
There was a significant difference in the CV of Sacin
N2 between the
groups (p= 0.0002). Within the COPD group Sacin
N2 demonstrated a
low intra-test variability of 7%, which is below the current guideline of
< 10% variation for FRC and LCI (Robinson, et al., 2013) but is higher
than that reported for FEV
1. Variation of CV in the HC group was found
to be greater for Scond
N2 and Sacin
N2 compared to the COPD group.
This is despite the theoretical modeling of $\text{Scond}_{N2}$ and $\text{Sacin}_{N2}$ coming from healthy volunteer studies (Crawford, et al., 1985). Variation was expected to be greater in the COPD group as the specificity of $\text{Scond}_{N2}$ and $\text{Sacin}_{N2}$ modeling has been shown to be problematic in subjects with obstructed diseases such as cystic fibrosis (Horsley, et al., 2008b). The present $\text{Scond}_{N2}$ and $\text{Sacin}_{N2}$ HC data was found to be 1.5 and 2 times the variation reported in asthmatic subjects previously (Downie, et al., 2007). This possibly is as a result of the reduced n number of the HC group in the present study meaning that a small change in values would impact greatly on the level of variation measured.

The final objective was to determine the repeatability of $\text{Scond}_{N2}$ and $\text{Sacin}_{N2}$ in COPD subjects. These measurements occurred across 2 testing sessions separated by $\geq$ 24 hours with a mean (SD) of 16 (13) days between visits 1 and 2. The mean variation in $\text{Scond}_{N2}$ was 0.003 (0.02) with ±0.04 limits of agreement. $\text{Sacin}_{N2}$ demonstrated a repeatability level of -0.03 (0.25) with limits of agreement of -1.51 to 0.46. This variability was heavily driven by one data point that was an outlier. Without this outlier the mean variation became 0.02 (0.12) with limits of agreement reduced to a range of -0.21 to 0.25. These findings provide some potential preliminary guidance on the levels of improvement/reduction that may be considered clinically significant, although the outlying data point and the fact variation was determined over only 2 visits suggests more work is needed to fully answer this question.

In summary the present results demonstrate that $\text{Scond}_{N2}$ and $\text{Sacin}_{N2}$ were able to distinguish between healthy controls and COPD subjects with relatively low intra-test variation in the COPD group. Furthermore $\text{Sacin}_{N2}$ was found to be a sensitive marker across disease severity, correlated with other markers of the small airways and the inter-visit variation of $\text{Sacin}_{N2}$ was comparable to that found for FEV$_1$, FEF$_{25-75}$ and FVC in chapter 4. However, $\text{Scond}_{N2}$ was not found to correlate with exercise capacity, patient reported outcomes or any of the other
physiological tests, which included those that are theorised to measure similar portions of the lung. In addition to this $S_{\text{acin}} N_2$ correlated with physiological measures of the distal lung and global symptoms reported by subjects. These findings firstly bring into question the internal validity of $S_{\text{cond}} N_2$ to measure conductive lung units as well as highlight the subsequent impact this may have upon the determination of $S_{\text{acin}} N_2$. Secondly in addition to the impact from $S_{\text{cond}} N_2$, $S_{\text{acin}} N_2$ correlated with all physiological measures and patient reported outcomes, therefore suggesting that it may not be specific to the lung periphery as first thought.

There remains a need for valid mechanistic values of ventilation and $S_{\text{cond}}$ and $S_{\text{acin}}$ are one of the first, which have shown how mathematical modeling may achieve this. Further research is needed in this area, as there are few commercially available systems that employ phase III slope analysis. This would allow further validation of the indices, determination of the convection-diffusion front in a disease state as well as outline associated clinical significance levels.
CHAPTER 8

8.0 The Effect of COPD Exacerbations on Ventilation Inhomogeneity

8.1 Introduction
COPD is associated with acute exacerbations (AECOPD) that become more prevalent as the severity of the disease increases (Wedzicha and Seemungal, 2007). They account for a large proportion of the morbidity, mortality and up to 60% of the medical expenditure in COPD patients (Burge and Wedzicha, 2003; Miravitlles, 2003; Parker, et al., 2005). Exacerbations represent significant milestones in the natural history of the disease that relate to accelerated progression and a decline in health status (Donaldson, et al., 2002; Kanner, Anthonisen and Connett, 2001; Seemungal, et al., 1998; Spencer, et al., 2004). AECOPD diagnosis is predominantly based on clinical representation of specific symptoms such as an increase in sputum production, cough and dyspnea as well as a decrease in lung function. These clinical manifestations however, are highly variable between patients, along with multiple triggers such as bacterial, viral, ozone or a combination of all (Anthonisen, et al., 1987; Patel, et al., 2002; Seemungal, et al., 2001; Vestbo, et al., 2013; Wedzicha and Donaldson, 2012). This clinical and trigger variability in addition to the heterogeneous nature of COPD has prevented a universally accepted AECOPD definition from being established. This lack of understanding of the pathophysiology of AECOPD has served as a drive for research into the recognition, diagnosis, severity, frequency, treatment and recovery time of exacerbations in order to improve patient outcomes. In terms of study design numerous AECOPD research studies have found patients to recover within 6 weeks from the onset of the exacerbation (Koutsokera, et al., 2009). One method of capturing exacerbation evidence is through the use of a patient diary card. Patients are educated to monitor lung function, breathlessness, cough, sputum production and colour daily, using a standardised colour chart (Stockley,
et al., 2001). The card also records any symptoms such as wheeze, chest tightness, and cold like symptoms as well as any resulting changes in medication. This provides important standardised information on the time course, recovery and treatment of exacerbations as long as clinical investigations are conducted at the time of onset (Burge and Wedzicha, 2003). This approach is heavily reliant on subjective data and would be complimented by the determination of objective outcomes that are sensitive and validated for exacerbation recovery in order to aid in appropriate treatment plans and study design in AECOPD research studies. Concerning symptoms it has been shown that although the COPD assessment test (CAT™) questionnaire is not formally validated for determining exacerbation severity, a few studies have found a reduction in score at the onset of exacerbation (Mackay, et al., 2012). CAT™ can be used as part of symptom analysis to monitor recovery over time with improvements greater than the estimated minimal clinical important difference (MCID) of 3.76 (Tsiligiani, et al., 2012). A recently completed study in a cohort of 486 COPD patients found that CAT™ scores significantly reduced from a mean score of 22 at exacerbation onset, to 9.9 over 6 weeks of recovery follow up visits (Miravitlles, et al., 2013). Objective functional physiological outcome studies in AECOPD are limited and have been primarily concerned with PEF and FEV\(_1\) (Parker, et al., 2005). There is growing evidence that these markers of airflow obstruction do not change significantly on an individual level at the onset of exacerbation, do not show significant improvement during recovery and do not correlate with reported symptoms (Bhowmik, et al., 2000; Mallia and Johnston, 2005). One reason for this may be due to the focus on large airway caliber that PEF and FEV\(_1\) represents as well as the non-reversible obstruction that is associated with COPD. It is known that respiratory pathogens enter the small airways by microaspiration after firstly residing in the upper airways. Due to mucus plugging, oedema and inflammation of the small airways, resulting in increased airways resistance, the time course of pathogen clearance is extended. This combined with significantly
increased levels of replication, leads to AECOPD. This mechanism has been proposed as being responsible for up to 38% of COPD deaths attributed to respiratory failure (Zielinski, et al., 1997). Over recent years objective measurements that represent the functional aspect of both the large and small airways have been investigated. Stevenson et al, 2005, used a 6-week AECOPD recovery design study where subjects performed multiple measurements of airways resistance using impulse oscillometry. Markers of total respiratory system resistance (R5) and values representing the elastic and energy storing ability of the lung periphery (respiratory system reactance; X5) were not found to significantly change from the onset (E0) of an exacerbation. An alternative to airway resistance, as a functional marker, is to measure the change in lung volume that may occur as an increase in hyperinflation in response to an exacerbation. Measurements of IC, used as a surrogate marker of FRC, have been found to significantly increase after treatment for an exacerbation over 6 weeks from exacerbation onset (Parker, et al., 2005). These results highlight the effect on end expiratory lung volume that occurs as a functional response to bacterial infections. A feature that is related to the increased air trapping associated with an increased FRC is a mismatch in ventilation-perfusion (VA/Q). It has previously been reported in asthma exacerbations that VA/Q inequality, measured by distribution of blood flow, was shown to improve over the time course of exacerbation recovery and was more related to obstruction of the small airways (Ferrer, et al., 1993). One potential physiological method of determining this effect on ventilation is through analysis of lung clearance index derived from multiple-breath inert gas washout. LCI is based on tidal breathing analysis and provides a sensitive measure of global inhomogeneity in the lung. The hypothesis of the present study is that AECOPD will be associated with a fall in FRC\textsubscript{N2} due to reduced lung volumes that are tidally ventilated and an increase in LCI\textsubscript{N2}. This would then resolve within 6 weeks from the onset.
8.1.1 Aims and Objectives

The aim of this study was to evaluate ventilation inhomogeneity; spirometry and patient reported outcomes during an AECOPD. The study objectives were:

1. To measure $FRC_{N2} \%$ at exacerbation onset (E0), after 2 weeks post treatment (E2) and after 6 weeks during recovery of an exacerbation.

2. To measure $LCI_{N2}$ at exacerbation onset (E0), after 2 weeks post treatment (E2) and after 6 weeks during recovery of an exacerbation.

3. To measure $FEV_1 \%$ at exacerbation onset (E0), after 2 weeks post treatment (E2) and after 6 weeks during recovery of an exacerbation.

4. To evaluate $CAT^\text{TM}$ scores at exacerbation onset (E0), after 2 weeks post treatment (E2) and after 6 weeks during recovery of an exacerbation.

8.2 Methods

8.2.1 Subjects

Thirty COPD patients were recruited. Patients were aged > 40 years and with a physician’s diagnosis of COPD in accordance with the 2011 GOLD guidelines. The local ethics committee approved the study before commencement. Written informed consent was obtained from each subject prior to study procedures, all of which were conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guideline and the Declaration of Helsinki.
8.2.2 Study Design

COPD patients completed a 4 visit study design. At consent (visit 1) subjects underwent an ECG, physical exam; a calculation of smoking history as well as a detailed medical/surgical history. Safety measurements consisted of height, weight, Blood pressure, heart rate, and $S_rO_2$. Subjects were then provided with a diary card to be completed daily. The diary card recorded peak flow, respiratory symptoms, treatment use and sputum colour that were categorised into major and minor symptoms (Table 8.1). If a patient demonstrated signs of exacerbation, defined as 2 major symptoms; or 1 major and 1 minor symptom, which have lasted for ≥ 2 days, the clinic was contacted and an exacerbation onset visit (E0) was arranged if an exacerbation was deemed likely by a physician. At E0 the physician conducted a review of the diary card and performed a physical exam to confirm exacerbation. Vital signs were performed and the CAT<sup>TM</sup> questionnaire administered before MBW<sub>N2</sub> and Spirometric reversibility was assessed after administration of 400 μg of salbutamol via a spacer. The subject was then prescribed a treatment plan including antibiotics and/or oral steroids.

A clinic visit after 2 weeks (E2) was then arranged where the diary card and treatment plan was assessed by a physician, along with a physical exam and if appropriate a resolution date was determined or a further treatment plan was devised where necessary. Subjects then completed the CAT<sup>TM</sup> questionnaire, MBW<sub>N2</sub> and spirometric reversibility after administration of 400 μg of salbutamol via a spacer. A recovery visit 6 weeks after E0 (E6) was then arranged. At E6 the diary card and if appropriate the treatment plan was assessed by a physician. A physical exam was performed prior to completion of the CAT<sup>TM</sup> questionnaire, MBW<sub>N2</sub> and spirometric reversibility after administration of 400 μg of salbutamol via a spacer. All physiological procedures were performed as previously described in detail in chapter 2. FRC predicted values from
the European Community for Coal and Steel were used to compare FRC % predicted values across all methods (Quanjer, et al., 1993).

Table 8.1. Classification of major and minor exacerbation symptoms

<table>
<thead>
<tr>
<th>Major</th>
<th>Minor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased Breathlessness</td>
<td>Increased wheeze</td>
</tr>
<tr>
<td>Increased Sputum Volume</td>
<td>Increased Chest Tightness</td>
</tr>
<tr>
<td>Increased Sputum Colour</td>
<td>Increased Cough</td>
</tr>
<tr>
<td></td>
<td>Sore Throat</td>
</tr>
<tr>
<td></td>
<td>Fever</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
</tr>
</tbody>
</table>

8.2.3 Statistical Analysis

Parametric data were expressed as mean (SD) and non-parametric data expressed as median and inter-quartile range. Differences between exacerbation visits were assessed using a paired t-test. Differences were deemed significant if \( p < 0.05 \). All statistical tests were performed using Prism (GraphPad Software Inc, version 6.04, San Diego, California, USA; http://www.graphpad.com).
8.3 Results

All MBW\(_{N2}\) measurements were examined for quality control. Washouts that showed irregular tidal breathing patterns, leaks at the mouth or incomplete measurements were discounted. From the 30 COPD patients that were enrolled 21 (70\%) were unable to perform MBW\(_{N2}\) testing at E0. Six patients (20\%) did not obtain adequate results due to leak at the mouth and 10 (33\%) patients discontinued the test due to an increased feeling of shortness of breath and distress caused by the flow rate of the O\(_2\) and dry throat. Six patients (20\%) presented at E0 with severity of symptoms that upon physician assessment, was decided too severe to undergo MBW\(_{N2}\) and spirometry. The overall clinical characteristics of the 8 patients (27\%) that performed MBW\(_{N2}\) at E0 are summarised in Table 8.2.

Table 8.2. Subject Demographics

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>COPD (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Male/Female)</td>
<td>4/4</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>67 (7)</td>
</tr>
<tr>
<td>Height (metres)</td>
<td>1.68 (0.12)</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>82.64 (18.74)</td>
</tr>
<tr>
<td>BMI (Kg/M(^2))</td>
<td>29.14 (4.64)</td>
</tr>
<tr>
<td>Smoking History (Pack Years)</td>
<td>49.58 (34.11-65.00)</td>
</tr>
<tr>
<td>FEV(_1) %</td>
<td>56.00 (26.28)</td>
</tr>
<tr>
<td>FVC %</td>
<td>97.75 (27.00)</td>
</tr>
<tr>
<td>FEV(_1)/FVC</td>
<td>0.43 (0.13)</td>
</tr>
</tbody>
</table>

All parametric data are expressed as means and standard deviations unless otherwise stated. a denotes median and inter-quartile range.
8.3.1 Functional Residual Capacity

There was no significant change in FRC\textsubscript{N2} from the onset of exacerbation (E0), to 2 weeks after treatment (E2) or after a recovery period of 6 weeks (E6) (Figure 8.1). The mean FRC\textsubscript{N2} at E0 was 130.9 (29.22) % predicted, the mean FRC\textsubscript{N2} at E2 was 121.0 (58.66) and the mean FRC\textsubscript{N2} at E6 was 139.1 (41.83) % predicted.

Change in FRC\textsubscript{N2} % from Exacerbation onset to Recovery

![Graph showing change in FRC\textsubscript{N2} % from exacerbation onset to recovery](image)

Figure 8.1. Functional residual capacity (FRC\textsubscript{N2}) at exacerbation onset (E0), 2 weeks after treatment (E2) and after recovery (E6). The mean FRC\textsubscript{N2} at E0 was 130.9 (29.22), the mean FRC\textsubscript{N2} at E2 was 121.0 (58.66) and the mean FRC\textsubscript{N2} at E6 was 139.1 (41.83). There was no significant difference between E0 and E2 or E0 and E6 (p > 0.05).
8.3.2 Lung Clearance Index

There was no significant change in LCI\textsubscript{N2} from the onset of exacerbation (E0), to 2 weeks after treatment (E2) or after a recovery period of 6 weeks (E6) (Figure 8.2). The mean LCI\textsubscript{N2} at E0 was 11.74 (1.20), the mean LCI\textsubscript{N2} at E2 was 12.28 (1.86) and the mean LCI\textsubscript{N2} at E6 was 12.07 (1.61).

Change in LCI\textsubscript{N2} from Exacerbation onset to Recovery

![Graph showing lung clearance index (LCI\textsubscript{N2}) at exacerbation onset (E0), 2 weeks after treatment (E2) and after recovery (E6). The mean LCI\textsubscript{N2} at E0 was 11.74 (1.20), the mean LCI\textsubscript{N2} at E2 was 12.28 (1.86) and the mean LCI\textsubscript{N2} at E6 was 12.07 (1.61). There was no significant difference between E0 and E2 or E0 and E6 (p > 0.05).]

Figure 8.2. Lung clearance index (LCI\textsubscript{N2}) at exacerbation onset (E0), 2 weeks after treatment (E2) and after recovery (E6). The mean LCI\textsubscript{N2} at E0 was 11.74 (1.20), the mean LCI\textsubscript{N2} at E2 was 12.28 (1.86) and the mean LCI\textsubscript{N2} at E6 was 12.07 (1.61). There was no significant difference between E0 and E2 or E0 and E6 (p > 0.05).
8.3.3 $\text{Scond}_{N2}$

There was no significant change in $\text{Scond}_{N2}$ from the onset of exacerbation (E0), to 2 weeks after treatment (E2) or after a recovery period of 6 weeks (E6) (Figure 8.3). The mean $\text{Scond}_{N2}$ at E0 was 0.04 (0.03), the mean $\text{Scond}_{N2}$ at E2 was 0.04 (0.01) and the mean $\text{Scond}_{N2}$ at E6 was 0.04 (0.01).

**Change in $\text{Scond}_{N2}$ from Exacerbation onset to Recovery**

![Graph showing change in Scond N2 from exacerbation onset to recovery](image)

Figure 8.3. $\text{Scond}_{N2}$ at exacerbation onset (E0), 2 weeks after treatment (E2) and after recovery (E6). The mean $\text{Scond}_{N2}$ at E0 was 0.04 (0.03), the mean $\text{Scond}_{N2}$ at E2 was 0.04 (0.01) and the mean $\text{Scond}_{N2}$ at E6 was 0.04 (0.01). There was no significant difference between E0 and E2 or E0 and E6 ($p > 0.05$).
8.3.4 Sacin$_{N2}$

There was no significant change in Sacin$_{N2}$ from the onset of exacerbation (E0), to 2 weeks after treatment (E2) or after a recovery period of 6 weeks (E6) (Figure 8.4). The mean Sacin$_{N2}$ at E0 was 0.42 (0.16), the mean Sacin$_{N2}$ at E2 was 0.44 (0.13) and the mean LCI$_{N2}$ at E6 was 0.44 (0.14).

**Change in Sacin$_{N2}$ from Exacerbation onset to Recovery**

![Graph showing the change in Sacin$_{N2}$ from exacerbation onset to recovery.](image)

Figure 8.4. Sacin$_{N2}$ at exacerbation onset (E0), 2 weeks after treatment (E2) and after recovery (E6). The mean Sacin$_{N2}$ at E0 was 0.42 (0.16), the mean Sacin$_{N2}$ at E2 was 0.44 (0.13) and the mean LCI$_{N2}$ at E6 was 0.44 (0.14). There was no significant difference between E0 and E2 or E0 and E6 (p > 0.05).
8.3.5 Forced Expired Volume in 1 Second

There was no significant change in FEV$_1$ % predicted from the onset of exacerbation (E0), to 2 weeks after treatment (E2) or after a recovery period of 6 weeks (E6) (Figure 8.5). The mean FEV$_1$ % at E0 was 53.50 (26.71), the mean FEV$_1$ % at E2 was 55.50 (24.78) and the mean FEV$_1$ % at E6 was 56.0 (26.28).

![Change in FEV$_1$ % from Exacerbation onset to Recovery](image)

*Figure 8.5. Forced expired volume in 1 second (FEV$_1$) % predicted at exacerbation onset (E0), 2 weeks after treatment (E2) and after recovery (E6). The mean FEV$_1$ % at E0 was 53.50 (26.71), the mean FEV$_1$ % at E2 was 55.50 (24.78) and the mean FEV$_1$ % at E6 was 56.0 (26.28). There was no significant difference between E0 and E2 or E0 and E6 (p > 0.05).*
8.3.4 COPD Assessment Test

There was a significant change in CAT™ questionnaire scores between E0 to E2 and E0 to E6 with p = 0.0075 and p = 0.0083 respectively (Figure 8.6). The mean CAT™ scores at E0 were 28.5 (10.53), the mean CAT™ scores at E2 were 20.5 (10.86) and the mean CAT™ scores at E6 were 20.88 (8.92).

Change in CAT™ Scores from Exacerbation onset to Recovery

Figure 8.6. COPD assessment test (CAT™) scores at exacerbation onset (E0), 2 weeks after treatment (E2) and after recovery (E6). The mean CAT™ scores at E0 were 28.5 (10.53), the mean CAT™ scores at E2 were 20.5 (10.86) and the mean CAT™ scores at E6 were 20.88 (8.92). There was a significant change in CAT™ questionnaire scores between E0 to E2 and E0 to E6 with p = 0.0075 and p = 0.0083 respectively.
8.4 Discussion

To the best of our knowledge this is the first study to evaluate ventilation inhomogeneity using inert gas washout during a COPD exacerbation. The study of AECOPD is of paramount importance due to the impact they have upon disease progression, quality of life and economic cost. The present findings demonstrate that from the day of exacerbation onset to 2 weeks post treatment and through 6 weeks of recovery there were small but insignificant increases observed in FEV1 %, FRC % and LCI_{N2}. There were no changes in Scond_{N2} or Sacin_{N2} but a significant reduction in CAT™ scores. In line with what has been reported previously, spirometry was not found to correlate with CAT™ scores (Tu, Zhang and Fei, 2014). Just as was reported in chapter 3, in stable COPD patients, LCI_{N2} also did not correlate with CAT™ scores for exacerbating patients.

The first objective was to evaluate FRC_{N2} % throughout an AECOPD. FRC_{N2} % was found to slightly reduce by 8% at E2 after a course of treatment of antibiotics and/or oral steroids but rise above the level found at E0 by 6 % at E6. This is in contrast to previous work where a reduction in FRC %, measured indirectly using IC, was demonstrated over the course of a COPD exacerbation up to 6 weeks post treatment (Stevenson, et al., 2005). This result may be due to the way in which inert gas washout testing determines FRC %. Washout tests only penetrate well-ventilated portions of the lung and therefore at E0 when large parts of the lung are obstructed and/or closed due to airway collapse FRC % may be significantly underestimated. Upon recovery at E6 where more lung units are available for ventilation FRC % may be increased but this could possibly represent a more accurate end expiratory lung volume.

The second objective was to evaluate LCI_{N2} throughout an AECOPD. LCI_{N2} increased slightly from the onset of an exacerbation throughout recovery at E6. The may have been as a direct result of the increased FRC_{N2} % observed at E6 which may have directly increased the number.
of lung turnovers. This lack of reduction in VI may also be due to underlying airway damage that occurs during an exacerbation that is not rectified by antibiotics and/or oral steroids. Subsequently the patient may never fully recover, contributing to the accelerated disease progression for which exacerbations are associated (Vestbo, et al., 2013). This has been demonstrated in a cohort of asthma patients with severe exacerbations where VI remained abnormal even after the patients symptoms had resolved (Ferrer, et al., 1993). An alternative hypothesis for the slight increase found in LCI_{N2} during an exacerbation has been found in CF research and is concerned with the exposure of damaged lung tissue to gas mixing as a result of recovery from treatment (Horsley, et al., 2013). Upon exacerbation the amount of gas mixing measured by LCI may actually be reduced due to the availability of well ventilation portions of the lung. Once treatment has been administered and recovery begins there is indeed a larger number of lung units exposed to gas exchange but these units contain damaged tissue that may further increase heterogeneity and therefore result in an increased LCI_{N2} (Sonneveld, et al., 2015).

The third objective was to evaluate FEV_{1} % throughout an AECOPD. There was no significant change observed in FEV_{1} % from E0 to E6. This result supports previous studies, which found that, not only did FEV_{1} % not significantly reduce at the onset of an exacerbation but also that no change was observed during recovery (Seemungal, et al., 1998; Seemungal, et al., 2000). The insensitivity of FEV_{1} % as a tool to assess exacerbation onset and recovery may be due to the non-reversible obstruction associated with COPD.

The fourth objective was to evaluate CAT^{TM} questionnaire scores throughout an AECOPD. Over the course of exacerbation onset to 6 weeks recovery CAT^{TM} scores were found to have a significant decrease comparable to previous COPD exacerbation studies (Tu, Zhang and Fei, 2014). CAT^{TM} scores reduced by a mean score of 8 by E2 and remained reduced at E6. This is above the MCID proposed for CAT^{TM} scores by
Tsiligianni et al, 2012, and when combined with the observation made by Mackay et al, 2012, that CAT$^{TM}$ scores are significantly reduced at exacerbation onset, the use of the questionnaire as an objective tool is strengthened as an assessment of treatment response and recovery from an AECOPD. This was an expected finding as the definition of exacerbation used was based on symptoms and therefore it would be expected to observe a decrease in CAT$^{TM}$ scores at E0.

In summary the present data complements previous research by illustrating that FEV$_1$ % does not change over exacerbation recovery and does not correlate to symptom scores made using the CAT$^{TM}$ questionnaire. The use of a diary card allowed for a standardised approach for exacerbation onset diagnosis and patients were seen on the same day. The current findings support previous work that demonstrates the use of the CAT$^{TM}$ questionnaire as an objective outcome in exacerbation determination and recovery monitoring. This study is one of the first to assess LCI during an AECOPD. LCI$_{N2}$ did not change significantly from exacerbation onset to recovery that may possibly represent lung injury, which is unable to be repaired by antibiotics and oral steroids, or the increased availability of heterogeneous lung units after treatment. An additional finding was the low success rate of patients who were able to perform MBW$_{N2}$ and spirometry. With 73% of patients unable to perform adequate MBW$_{N2}$ technique or whose symptoms were too severe to undergo testing indicates that regardless of any specificity that measurements of VI may illustrate, they may have limited application in clinical practice.

The current study was limited by the low n number and by the omission of baseline measurements prior to exacerbation onset. There is plenty of evidence however, from previous studies that symptom scores significantly reduce upon onset and recover within 6 weeks. In addition FEV$_1$ % has been shown to not significantly reduce upon onset and does not change over 6 weeks of recovery, however the absence of any change in LCI$_{N2}$ in the present study may have been due to a significant
reduction at E0 which did not recover to pre exacerbation values after 6 weeks. Alternatively the definition of exacerbation used in the present study may have resulted in the inclusion of patients with mild exacerbation events. This could have contributed to the absence of changes observed in parameters such as FEV$_1$ and LCI$_{N2}$ and thus failing to ascertain if LCI$_{N2}$ was sensitive enough to detect a difference in this data set.

Exacerbations remain an important but poorly understood aspect of COPD. A universal definition is lacking, treatment decisions are largely based on subjective outcomes and treatments such as antibiotics are best used for bacterial causes of exacerbations. Further research is needed into objective functional measurements that can provide a link between a patient’s symptom perception, cellular pathology and physiological changes occurring during an exacerbation. Studies employing a standardised diary card, baseline measurements and the inclusion of large patient cohorts to investigate measures of VI further, using a variety of multiple-breath washout systems, should be implemented. This will aid in the validation of MBW as a tool in exacerbation treatment and monitoring as well as providing a greater understanding of exacerbations and associated treatments.
CHAPTER 9

9.0 Conclusions, Limitations and Future Work

9.1 Conclusions

The overall aim of this thesis was to assess MBW as a marker of ventilation inhomogeneity in COPD. It is evident from the present data that LCI was sufficiently descriptive to differentiate between COPD and healthy controls as well as sensitive enough to detect lung abnormalities in mild disease prior to the onset of spirometry changes. LCI was found to have an inverse relationship with FEV$_1$ and so able to distinguish between GOLD stages, with significantly raised levels of LCI as the level of obstruction increased. The limitation to this is the duration of time testing takes in the moderate to severe subjects and would prevent this becoming part of clinical practice. Despite this LCI was reproducible, repeatable and correlated with current relevant physiological tests that highlight the possibility of incorporating LCI into evaluating new therapies in the appropriate mild COPD population.

What is also clear is that in addition to a lack of normal values there are significant differences in MBW measurements between systems. The MBW$_{N2}$ system produced values greater than the MBW$_{SF6}$ system, especially FRC % where the levels also surpassed those measured by plethysmography. Physiologically this highlights an error in the estimation of FRC$_{N2}$ and there has been similar values reported by Jensen et al (2013). This variation between the MBW systems became disproportionately greater as severity increased. This not only brings into question the effect of breathing O$_2$ on tidal breathing but also the signal alignment algorithm used by the system. This suggests that MBW$_{N2}$ may not be the preferential method of determining VI in obstructive lung disease. This would have a significant impact upon the study design of multi-centre clinical trials as each system will require equipment specific normal values to be determined and the upper limit of normal defined. From the present data, when combining the results
with \( \text{FRC}_{\text{pleth}} \) as a marker of air trapping, \( \text{FRC}_{\text{SF6}} \) would provide the best reflection of gas trapping in this population. The testing success rate for MBW was also found to be greater than plethysmography. MBW is easy for subjects to perform, and \( \text{FRC}_{\text{SF6}} \) may be suitable for those that find the technique associated with \( \text{FRC}_{\text{pleth}} \) difficult.

This report also investigated the phase III slope analysis constituents of LCI, Scond and Sacin. Scond did not correlate with any of the physiological tests despite \( \text{DL}_{\text{CO}} \) and IOS parameters representing the same conductive portions of the lung. This therefore brings into question the validity of Scond. Sacin on the other hand correlated with most of the physiological tests and patient reported outcomes. Due to the questionable validity of Scond in COPD, it is likely that Sacin will have been affected to some degree due to their mathematical relationship. In addition to this Sacin did not only correlate with markers of the small airways leading to a conclusion that it may represent global ventilation similarly to LCI. Scond and Sacin are interesting and exciting concepts but require future validation in the COPD population and across different systems.

This thesis has analysed in depth MBW as a measure of VI in a COPD population. The current data shows that LCI is a sensitive and reliable test and provides evidence for inclusion of \( \text{LCI}_{\text{SF6}} \) measurements in mild COPD patients to primarily aid in early diagnosis.
9.2 Limitations and Future Work

Further COPD studies validating MBW systems and the mechanistic values of ventilation that underpin it are needed. In some of the chapters of this thesis the results was limited by the sample size where a small change in values may have impacted upon the significance of some results. An increase in the number of participants performing MBW would have strengthened the findings and would have likely illustrated a statistically significant difference in COPD test times between the two methods. In regards to long-term inter-visit variability it would have been advantageous to implement a study design which incorporated more than 2 data points across 2 visits or more in order to not only increase in the validity of the findings but also to allow for the determination of clinically significant changes.

The variables reported within this thesis were not calculated by the investigator but represent the equipment specific outputs configured by the manufacturer. This may limit the verification of assumptions in certain subjects, especially when applying the contentious mathematical equations to calculate values such as Scond and Sacin. In regard to the potential over estimation of MBW values on the Exhalyzer® D (EcoMedics AG, and Duernten, Switzerland), it is important that the systems algorithm and the possible effect 100% O₂ may have upon tidal breathing in a COPD population is investigated further.

Exacerbations remain an important but poorly understood aspect of COPD. A further limitation of this thesis was the omission of baseline measurements prior to exacerbation onset. Despite evidence that spirometry does not significantly change throughout an exacerbation and subjects recover within 6 weeks of treatment, the absence of any change in LCI N₂ throughout the exacerbation recovery may have been due to a significant reduction and sustained at E0. Future studies into VI during exacerbations should incorporate a stable baseline visit to further establish if MBW values are indeed sensitive enough to detect change and recovery.
Appendix

Appendix 1 COPD Assessment Test

How is your COPD? Take the COPD Assessment Test™ (CAT)

This questionnaire will help you and your healthcare professional measure the impact COPD (Chronic Obstructive Pulmonary Disease) is having on your wellbeing and daily life. Your answers, and test score, can be used by you and your healthcare professional to help improve the management of your COPD and get the greatest benefit from treatment.

For each item below, place a mark (X) in the box that best describes you currently. Be sure to only select one response for each question.

**Example:** I am very happy 0 X 2 3 4 5 I am very sad

I never cough

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<tr>
<td>4</td>
<td></td>
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I have no phlegm (mucus) in my chest at all

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<tr>
<td>4</td>
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My chest does not feel tight at all

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<td>4</td>
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When I walk up a hill or one flight of stairs I am not breathless

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I am not limited doing any activities at home

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<td>4</td>
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<td>5</td>
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</tr>
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</table>

I am confident leaving my home despite my lung condition

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I sleep soundly

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<td>4</td>
<td></td>
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I have lots of energy

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COPD Assessment Test and the CAT logo is a trade mark of the GlaxoSmithKline group of companies. © 2009 GlaxoSmithKline group of companies. All rights reserved.

Last Updated: February 24, 2012
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<tr>
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<td>Very slight</td>
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<tr>
<td>2</td>
<td>Slight</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
</tr>
<tr>
<td>4</td>
<td>Somewhat severe</td>
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<tr>
<td>5</td>
<td>Severe</td>
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<tr>
<td>7</td>
<td>Very severe</td>
</tr>
<tr>
<td>8</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Very, very severe (almost maximal)</td>
</tr>
<tr>
<td>10</td>
<td>Maximal</td>
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Appendix 2 BORG Scale Patient Instruction Page

Instructions for the Borg Scale

Prior to the start of exercise, each subject will be told that they will be asked to rate the intensity of 2 sensations at rest, during exercise, and at end-exercise. The 2 sensations will be described to the subject as:

- Discomfort with your breathing
- Discomfort in your legs

Use of the Borg Scale to rate these sensations will be explained to the subject. While showing the scale to the subject, the study coordinator or blinded tester will explain that the subject should relate the wording on the Borg Scale to the level of the sensation that he/she is experiencing, and then place the end of a finger on a number that best describes the intensity of the sensation - explain that placing a finger between 2 numbers is allowed. (Borg Scale numbers will be recorded to the nearest 0.5 units).

The study coordinator or blinded tester will anchor the endpoints of the scale for both sensations. The study coordinator or blinded tester will explain that for the sensation of “breathing discomfort”, “0 or nothing at all” corresponds to “no breathing discomfort at all” and “10 or maximal” corresponds to the “most severe breathing discomfort that you have ever experienced or could imagine experiencing.”. The study coordinator or blinded tester will then explain that for the sensation of “discomfort with your legs”, “0 or nothing at all” again corresponds to “no leg discomfort at all” and “10 or maximal” corresponds to the “most severe leg discomfort that you have ever experienced or could imagine experiencing”.

Subjects are to be given no further information about these sensations. If a subject requests further clarification, he/she will be told to use his/her own individual interpretation as to the meaning of the sensory descriptors. This will ensure that the sensory descriptors are presented to each subject in a standard format.
**Background**

Lung Clearance Index (LCI) is well established in cystic fibrosis (CF), but has not been widely applied in other patient populations. To evaluate the LCI in patients with chronic obstructive pulmonary disease (COPD), we compared LCI using single-breath (SB) and multiple-breath washout (MBW) techniques. SB LCI measurement was performed on the day of COPD exacerbation, while MBW was performed on the day of recovery, following medication tapering. A significant difference in LCI was observed between SB and MBW techniques in COPD patients.

**Methods**

Lung Clearance Index was measured on the day of COPD exacerbation and after medication tapering. Both SB and MBW techniques were used to assess LCI, with differences in LCI and FRC noted between the two methods.

**Results**

- SB LCI: 0.007
- MBW LCI: 0.005

**Discussion**

The significant difference in LCI between SB and MBW techniques suggests that these methods may not be interchangeable in clinical practice.

**Conclusion**

The LCI is a valid index in COPD, with significant differences observed between SB and MBW techniques. Further studies are needed to evaluate the clinical utility of LCI in COPD management.

**Key Findings**

- Significant difference in LCI between SB and MBW techniques
- SB LCI: 0.007
- MBW LCI: 0.005

**Aims**

- Compare LCI using SB and MBW techniques in COPD patients
- Evaluate the clinical utility of LCI in COPD management
Appendix 4 Publication under Review with Thorax

Relationship between lung volume measurements by plethysmography and nitrogen washout

Alan Bell1, Nigel Clayton2, Dave Singh3, Alex Horsley3

1: The Medicines Evaluation Unit, Centre for Respiratory Medicine and Allergy, University Hospital of South Manchester, University of Manchester, Manchester, M23 9QZ

2: Department of Respiratory Physiology, North West Lung Centre, University Hospital South Manchester NHS Trust, Manchester M23 9LT

3: Manchester Adult Cystic Fibrosis Centre, Centre for Respiratory Medicine and Allergy, Institute of Inflammation and Repair, University of Manchester, Manchester M23 9LT

818 words

Introduction

Multiple breath washout is now well established as a research tool in cystic fibrosis (CF) and interest in its application to other disease areas is expanding. The most commonly reported outcome measure is the lung clearance index (LCI), a summary measure of overall ventilation efficiency during tidal breathing. Central to the calculation of LCI is an accurate measure of the end-expiratory lung volume at the start of the test, the functional residual capacity (FRC). It has become apparent in recent reports however that different LCI systems can generate quite considerable differences in FRC in the same patients measured consecutively. Furthermore these differences are apparent not just between SF6 and nitrogen washout (where they may be partially explained by the differential behaviour of the gases) but also different nitrogen washout systems.

Plentytymography offers an alternative method of measuring lung volumes. Since plethysmography measures all thoracic gas, not just that ventilated during tidal breathing, it would conventionally be expected to measure greater values for FRC. We were interested to discover how FRC measured by plethysmography (FRC\textsubscript{pleth}) and multiple-breath washout (FRC\textsubscript{a}}) compared in adult respiratory patients with obstructive and restrictive airways physiology, using well established laboratory systems.

Method

We performed a retrospective analysis of all lung function assessments performed at the North West Lung Centre, University Hospital of South Manchester, during 2015 on patients with a diagnosis of COPD, asthma, bronchiectasis (including cystic fibrosis), and pulmonary fibrosis. Diagnosis and demographic data (age, gender) were taken from the digital data record.

FRC\textsubscript{pleth} was measured using a constant volume whole body plethysmograph (Autobox 6200 DL, Sensormedics, Yorba Linda CA, USA). FRC\textsubscript{a}} was measured using a mass flow sensor (Vmax 22 Encore, Sensormedics, Yorba Linda CA, USA). Both daily calibration and measurements of FRC, by plethysmography and N\textsubscript{2} washout, were conducted in accordance with the American Thoracic Society/European Respiratory Society lung volume guidelines. The mean value of at least 3 reproducible FRC\textsubscript{a}} measurements that were within 15% of the mean was reported. At least one technically acceptable FRC\textsubscript{a}} manoeuvre was reported with additional tests separated by 1.5 times the duration of the previous washout to allow patients to re-equilibrate to room air between tests.
Appendix 5 Poster Presentation Accepted for ERS 2016

Over-reading of FRC Measurements using MBW\textsubscript{N2} compared to Plethysmography and MBW\textsubscript{SF6} in COPD

A S Bell\textsuperscript{1}, D Singh\textsuperscript{2}, A Horsley\textsuperscript{3}

\textsuperscript{1} Medicines Evaluation Unit
\textsuperscript{2} University of Manchester, Medicines Evaluation Unit, University Hospital of South Manchester
\textsuperscript{3} University of Manchester, University Hospital of South Manchester

Background
FRC is a fundamental part of LCI measurement. New commercial systems are available for SF\textsubscript{6} & nitrogen (N\textsubscript{2}). FRC\textsubscript{Pleth} should be higher than MBW\textsubscript{FRC} as it includes all the air volume of the thoracic cavity.

Objectives
FRC was evaluated in COPD patients using 3 validated methods to assess accuracy and possible effects of FRC on MBW parameters.

Methods
FRC was performed on the same day in triplicate using an indirect N\textsubscript{2} MBW system (Exhalyzer D) & plethysmography (Sensormedics). A subset also performed MBW on a modified Innocor\textsuperscript{TM} SF\textsubscript{6} analyser. Results are expressed as mean (SD).

Results
44 COPD patients (age 67(7) yrs, FEV\textsubscript{1} 62 (19)\% predicted) were enrolled. 3 COPD subjects were unable to perform MBW\textsubscript{N2} and 4 subjects unable to demonstrate plethysmography technique. Test failures were due to repeated leakage and excluded from comparison. There was a significant difference between FRC\textsubscript{N2} vs FRC\textsubscript{Pleth}. FRC\textsubscript{N2}=139 (37)\% vs 125 (30)\% for FRC\textsubscript{Pleth}, p<0.0001. FRC\textsubscript{N2} was greater than FRC\textsubscript{Pleth} bias 15 (16) (limits of agreement -17.29 to 46.84). In the SF\textsubscript{6} subset there was a significant difference between FRC\textsubscript{N2} vs FRC\textsubscript{SF6}. FRC\textsubscript{N2}=137 (43)\% vs FRC\textsubscript{SF6}=112 (28), p= 0.002. FRC\textsubscript{N2} was greater than FRC\textsubscript{SF6} bias 24 (26) (limits of agreement -27.25 to 76.07). From the paired FRC data of 3 methods there were significant differences between all techniques. FRC\textsubscript{N2} (156 (45)\%) was greater than FRC\textsubscript{Pleth} (137) (36)\% (p= 0.04) and FRC\textsubscript{SF6} (110 (25)\% (p= 0.0012). FRC\textsubscript{N2} vs FRC\textsubscript{Pleth} had a bias of 19 (22) (limits of agreement -23.15 to 61.90). FRC\textsubscript{SF6} was significantly lower than FRC\textsubscript{Pleth} bias -26 (15) (limits of agreement -55.03 to 2.78).

Conclusions
Similar to observations in CF, FRC measured indirectly using N\textsubscript{2} washout was higher than SF\textsubscript{6} in COPD. Values are not interchangeable across systems. FRC\textsubscript{N2} may impact on LCI values in patients with severe COPD. Further analysis of the system algorithm and physiological impact of MBW\textsubscript{N2} on FRC is needed.
References

N = 132


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Lung clearance index is a sensitive, repeatable and practical measure of airways disease in adults with cystic fibrosis. *Thorax, 63* (2), pp.135-40


prevention of chronic obstructive pulmonary disease: GOLD executive summary.  


