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Validation and application of a photo-acoustic gas analyser for multiple breath inert gas washout in children

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Abbreviations

ACBT	Active cycle of breathing technique
ANOVA	Analysis of variation
ATP	Ambient temperature, pressure
ATS	American Thoracic Society
BAL	Broncho-alveolar lavage
BTPS	Body temperature, pressure, saturated
BTS	British Thoracic Society
CDI	Convection dependent inhomogeneity
Cet	End-tidal concentration
CEV	Cumulative expired volume
CF	Cystic fibrosis
CFGTC	Cystic Fibrosis Gene Therapy Consortium
CFTR	Cystic fibrosis transmembrane regulator
Cinit	Initial concentration
CO2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
L	Litres
L/s	Litres per second
LCI	Lung clearance index
MBNW	Multiple breath nitrogen washout
MBW	Multiple breath washout
ml/s	Millilitres per second
MRI	Magnetic resonance imaging
N2O	Nitrous oxide
O2	Oxygen
PGA	Photoacoustic gas analyser
PNT	Pneumotachograph
RVRTC	Raised volume, rapid thoracic compression
Sacin	Progression of phase III slope corresponding to inhomogeneity in the acinar region
Scond	Progression of phase III slope corresponding to inhomogeneity in the conductive region
SD	Standard deviation
SF6	Sulphur Hexafluoride

CV%	Coefficient of variability
DCDI	Diffusion/convection dependent inhomogeneity
ERS	European Respiratory society
FeNO	Fraction of expired Nitric Oxide
FEV0.5	Forced expired volume in 0.5 seconds
FEV1	Forced expired volume in 1 second
FRC	Functional residual capacity
FVC	Full vital capacity
HRCT	High resolution computed tomography
SIGN	Scottish Intercollegiate Guideline Network
SnIII	Normalised phase III slope
SOP	Standard operating procedure
V/Q	Ventilation/perfusion quotient
Vd/Vt	Dead space volume/ tidal volume ratio
VDI	Ventilation defects per image
Vexp	Expired volume
Vt	Tidal volume

Declaration

This thesis has been composed entirely by me. The work presented in this thesis was primarily conducted by me, however where planning, execution and analysis was conducted in collaboration with other researchers this has been clearly indicated.

This work has not been submitted for any other degree or professional qualification. Some of the results of the studies described in this thesis have been previously presented elsewhere; a list of publications is presented.

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Kenneth Macleod

Abstract

Multiple breath washout (MBW) of inert gas for assessment of airway disease in children is an emerging technique. In many studies Lung Clearance Index (LCI), derived from multiple breath washout of SF_6, is more able to detect early or mild lung disease than standard lung function measurements. It is also able to detect very early lung disease in progressive conditions such as Cystic Fibrosis (CF). Where infants born with this condition were thought to have minimal lung disease activity, LCI is higher in these children than healthy controls. Lack of available commercial devices has hampered expansion of this technique to centres other than specialist research teams.

Innocor (Innovision, Dk), a photoacoustic mass spectrometer capable of performing multiple breath washout, was adapted within this research group for use in adults. This thesis describes the setup, adaptation and validation of Innocor for use in children.

In 4 studies, healthy controls, children with asthma and children with CF were recruited to perform MBW.

In one study, 29 healthy controls and 31 children with asthma were recruited. Healthy controls performed 1 set of washouts, establishing a normative range. Children with asthma performed measurements before and after bronchodilator. Results showed increased LCI in children with asthma even though they were clinically stable as defined by symptoms. LCI stayed high even following bronchodilator suggesting evidence of residual airway disease in well controlled asthmatics despite adequate symptom control.

To investigate short term variability of MBW measurements, two other studies recruited 18 children with CF in each. They performed measurements before and after standard physiotherapy manoeuvres and during sitting and lying posture. LCI did not change significantly after airway clearance physiotherapy, compared with children who did no intervention. Variability was high in both groups however suggesting CF lung disease is a complex interaction of changing ventilation in adjacent lung units. Lying posture induced greater changes in lung function in children with CF than controls. LCI appears to be more sensitive to this change than standard lung function measurements (spirometry).

In another study 32 children with CF were recruited to perform serial lung function measurements over 18 months. These were data collected as part of the UK Cystic Fibrosis Gene Therapy Consortium (CFGTC) clinical studies in preparation for planned gene therapy trials. LCI appears comparable to FEV_1 and may be able to detect another aspect of airway disease.

All initial studies were performed in older children (>5yrs). The basic Innocor device is unsuitable for testing of younger patients with low breath volume and high respiratory rate. In-house adaptations following detailed lung model experimentation led to a faster analyser response, potentially capable of MBW in younger children.

The second part of this thesis concerns lab experiments and an in-vivo comparison with the current gold-standard MBW device, a respiratory mass spectrometer. 16 healthy volunteers and 9 children with CF were recruited. Ages ranged from 0.4 yrs to 49 yrs. Innocor values for lung volume estimation compared favourably with the mass spectrometer. No evidence of bias caused by Innocor error was seen, however intra-test variability was rather high, reducing the precision of the results.

These studies indicate Innocor is a robust, simple to use device with potential as a commercial lung function system. Modifications were made to make it suitable for use in all ages. Further development will need to focus on the patient interface and software, which is the domain of the manufacturers. The experiments contained in this thesis are therefore of interest to the wider respiratory research community as well as manufacturers of MBW devices.

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Publications

Papers

Ventilation heterogeneity in children with well controlled asthma with normal spirometry indicates residual airways disease.
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Chapter 1 - Introduction to validation of Innocor for MBW in children

Lung function in respiratory disease

In the UK, respiratory disease is common, being the cause of death in 1 in 5 people overall. This carries a large cost to the NHS in terms of care provision and drug prescribing, with associated costs in terms of loss of workforce productivity . There have been improvements in the past 20 years, with reduced mortality. However, the relative burden of respiratory disease is unchanged due to greater advances in the treatment of other conditions such as heart disease and cancer(*The Burden of Lung Disease 2nd Edition* 2006).

In infants and children, respiratory disease is the most commonly reported illness, with a long-term diagnosis reported in 10-12%. This can be chronic or acute, with a highly variable clinical picture, meaning determining prognosis is difficult. Factors such as peri-natal history, co-morbidities, socioeconomic status and family history alter the mortality and morbidity of respiratory disease in children.

A major goal, following a respiratory diagnosis, is to minimise or eliminate symptoms. Long-term care in progressive conditions (e.g. Cystic Fibrosis) has the extra challenge of treating symptoms while endeavouring to preserve normal growth and development, balancing the need for intensive management with effects of treatments on development and quality of life. Given this complexity, accurate measurement of disease severity is crucial to minimise under- or over treatment.

Lung function in paediatric research

Lung function is universally used in adult clinical settings to measure disease and detect an improvement following institution of treatment. In children, lung function can currently only be widely used clinically in older co-operative children (generally >4 years). In research settings, lung function is commonly used as a "gold standard" with which to measure the sensitivity of novel assays. There are a variety of lung function measurements, including lung volume, flow, gas mixing, ventilation-perfusion relationships and lung mechanics. No single measurement provides complete information on the whole respiratory system. In conjunction with other

measurements, lung function is able to indicate the extent of disease and detect an acute or chronic change in severity following institution of a treatment or physiological challenge. For slowly progressing conditions, lung function is used as a surrogate measure of lung disease, representing longer term outcomes such as death or severe disability.

For these reasons, along with its simplicity and repeatability, lung function is commonly used as an outcome variable, but ongoing research is attempting to find improvements.

In paediatric research, there are a number of reasons why measurements used in adults are not suitable in younger age groups. These issues are discussed below:

1. sensitivity to disease
2. different basic physiology
3. different factors contributing to abnormalities in function
4. ability/cooperation with test
5. availability of normative data

1. Commonly used tests are not able to detect mild/early disease

Evidence of early infection, inflammation and structural changes in children with CF, prior to abnormalities in spirometry, have led to the opinion that standard lung function testing (spirometry), may not be suitable for detection and tracking of lung disease in children(Sly and Brennan 2004). There is also evidence in children with asthma that early structural changes occur in the airways of children who wheeze, and that they may progress, contributing to the faster decline in lung function seen in adults with a history of asthma, compared to those with no respiratory history(Edwards et al. 2003).

2. Lungs in childhood are not just smaller than adults

A comprehensive review of human lung growth by Merkus et al provides the basis for understanding the importance of lung function tests designed specifically for younger subjects(Merkus et al. 1996). It is understood that the acinus develops prior to 20 weeks gestation, but the alveolar phase does not begin until 24 weeks gestation.

Even at birth, only 15% of alveoli have developed (~55million). Therefore, both lung development, as well as increase in size (volume) occurs in the first 2 years following birth, possibly up to 8 years of age. Infant and child airways are clearly smaller than in adulthood. Post-mortem studies indicate that peripheral airway resistance contributes more to total airway resistance in childhood compared with adulthood. It appears that the airways mainly increase in size along with an increase in alveolar size, at around 4-5 yrs old.

Compliance and elastic lung recoil are increased in infancy compared with the adult lung(Crystal and West 1991). Combined with increased chest wall compliance, there is reduced respiratory reserve (resting lung volume). In addition, because of increased elastic recoil, a larger proportion of the lung volume is expired earlier in expiration (decreased expiratory time constant). Infants control this to some extent by increasing post-inspiratory activity of the chest wall muscles and adducting the vocal cords. All of these physiological and mechanical factors lead to increased energy expenditure - accounting for differences in lung volume - in infants at rest compared with adults. The presence of airway obstruction (disease) disproportionately increased respiratory effort (symptoms) in children – especially infants - compared with adults.

3. Multiple pre- and post- natal factors contribute to lung development

Lung development in children has multiple influences of pre- and post- natal factors(Merkus et al. 1996). The main factors are summarised in Table 1.

It is recognised that many respiratory conditions affecting children and adults have origins in infancy(Warke et al. 2002, Kiley et al. 2007, Bush 2005, Dezateux and Stocks 1997). Early life events may be highly relevant to disease severity in later years.

Pre-natal	Post-natal
Oligohydramnios	Mechanical stretch (muscle activity)
Chest wall anomalies and diaphragmatic hernia	Hypoxia/ hyperoxia
Foetal movement and lung liquid	Nutrition
Maternal hypoxia, nutrition and drug use	Prematurity
Maternal hormonal abnormalities (e.g. insulin, chatecholamines, growth factors)	Respiratory disease –infection, wheeze
	Pollution

Table 1 - Factors influencing pre- and post- natal lung development(Merkus et al. 1996).

4. Patient ability and cooperation strongly influence selection of appropriate lung function measurements

Testing large numbers of young children is recognised as a challenging task(Stocks 2004). This is due to test complexity as well as the lack of cooperation, sedation requirements, and availability of willing healthy control subjects with which to compare disease groups. To allow meaningful comparisons between populations, the test reproducibility and repeatability within and between patients for each individual lung function test is also required(Miller et al. 2005a), with multiple measurements performed over time in the same children.

In young children (<5 yrs) and infants, unable to cooperate with breath manoeuvres, lung function was, until recently, limited to sedated and artificially ventilated subjects. A large number of data were collected about infant lung volumes and tidal flow measurements but many of these studies did not contribute much to the clinical care(Schibler and Frey 2002), and were prone to inconsistencies in equipment and analysis methods(Stocks 2004). The recent increase in lung function studies, including MBW, in non-ventilated infants and young children has been driven by efforts from a small number of groups eager to improve standards. Published safety, reproducibility and applicability standards intend to encourage multi-centre studies in large numbers of patients(Beydon et al. 2002, Beydon et al. 2007, Stocks 1996). These standards are based around use of equipment appropriate to the size of the

subject and an understanding of paediatric respiratory physiology with enhanced safety requirements(Gaultier et al. 1995, Lum 2006, Frey et al. 2000a, Beardsmore et al. 2007).

5. Reference ranges are only recently available in children

Lung function tests are readily performed in healthy populations to compare with disease states. Such reference ranges also take into account major factors that influence healthy populations, including age, height, ethnicity and sex. The most widely used adult spirometry reference values in Europe were published by the European Coal and Steel Community in 1983('Standardized lung function testing. Report working party' 1983). Standards are less clear in children as difficulties arise from using reference equations out-with the age range of the original population. Subbarao et. al. studied a healthy population of 70 children aged 6-18 years and compared their results against a selection of reference equations with both wide paediatric and adult age ranges, as well as those specifically limited to children(Subbarao et al. 2004). They found that those with specific paediatric age ranges were more accurate than those from wider age-ranges. Another study by Rosenthal et al sought to incorporate pubertal growth changes into the equations for adolescents as it is recognised that lung growth is not necessarily linear in this age range(Rosenthal et al. 1993). These studies highlight the importance of the use of correct reference ranges in the age range to be tested. Over the past 20 years, a greater emphasis has been placed on developing ranges in children of all ages, resulting in a large body of experience(Beydon et al. 2007, Stocks et al. 2001, Sly et al. 2000, Quanjer et al. 1995, Quanjer et al. 1989, Morris et al. 2001, Frey et al. 2000a, Frey et al. 2000b, Stocks and Quanjer 1995, Quanjer et al. 1997, Gustafsson 2005, Gustafsson 2002, Gappa et al. 2006, Gappa et al. 1993b, Gappa et al. 2001, Dundas et al. 1995, Castellsague et al. 1998, Brody et al. 2004, Beydon et al. 2002, Beardsmore et al. 2007, Bates et al. 2000). This now allows comparisons between studies, something that limited the applicability of early infant lung function studies(Stocks 2004). To enable a single set of reference equations to be used in all ages for spirometry, Stanojevic et. al. have released look-up tables assembled from 4 adult and paediatric population surveys. This mainly utilises data from a North

American population, with 2 smaller European populations. This publication is a valuable new tool(Stanojevic et al. 2008).

Cystic fibrosis

Cystic Fibrosis (CF) is an example of a progressive paediatric condition requiring early detection of mild disease and life-long follow up. Most recent paediatric respiratory studies have involved CF populations. A large proportion of this thesis concerns studies in children with CF, therefore I will provide a brief explanation of the condition with a summary of recent advances.

Cystic Fibrosis (CF) is a multi-system disease caused by a single gene defect. It has an autosomal recessive inheritance, affecting approximately 1 in 2500 live births in Caucasian populations. Disease is caused by a defect in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. This protein is expressed in multiple organs and has a role in ion transport across epithelial linings.

CF is a fatal disease with a current life expectancy of 30-35. The lungs are thought to be nearly pristine at birth(Sturgess and Imrie 1982) but are quickly affected in early childhood by progressive infection and inflammation of the airways leading to permanent and irreversible lung damage(Hoo et al. 2012). This is characterised by decline in lung function and loss of airway architecture (bronchiectasis)(Gustafsson et al. 2008, Dakin et al. 2002a, de Jong et al. 2006, Ranganathan et al. 2004, Mall et al. 2006, Bush and Davies 2005, Rosenfeld et al. 2001, Armstrong et al. 1997). There have been a number of advances in care of CF in the past 20 years leading to an improvement in survival. The widespread introduction of newborn screening for CF is designed to institute early respiratory and nutritional treatment to prevent progression of disease(Wagener et al. 1997). The advantages of this programme, in terms of improved lung function and increased survival however, have still to be convincingly shown(McKay et al. 2005, Farrell et al. 2005). The following aspects of CF lung disease are commonly measured disease surrogates used in investigation and management.

- lung function – e.g. spirometry, multiple breath washout
- inflammation and infection – e.g. sputum analysis
- airway structure - e.g. CT scanning

Lung function

Spirometry (FEV_1) is the most commonly used lung function measurement in CF research and the gold standard for assessing short- and long-term changes in older patients. Despite being highly reproducible and repeatable with affordable and widely available equipment, use in children is limited. The forced expiratory manoeuvre requires cooperation and an understanding of the procedure. It is therefore not suitable for infants. In addition, it may not be sensitive enough to detect early, subtle CF lung disease manifested in inflammatory and structural changes. This period of progressive lung disease manifested by clinical symptoms with little or no information from lung function testing have been termed the "silent years" of CF. The use of infant forced expiratory measurements and multiple breath washout have gained attention in the research community. Both are still highly specialised techniques and limited to a few centres. Multiple breath washout is the central technique in this thesis and will be explained in detail with particular attention to its use with a novel device in children. The main measure derived from multiple breath washout is lung clearance index (LCI). This will be fully explained in future sections.

Inflammation and infection

Recent discoveries have increased interest in the understanding of the genetic and molecular basis behind CF lung disease. Because of this, research groups have developed highly specific assays to measure inflammation severity and assess how these aspects of lung disease relate to other important measurements.

CF lung disease originates, conventionally, from a lack of functional CFTR protein, leading to abnormal electrolyte balance across airway epithelial cells. There is thought to be a reduced airway surface liquid height, leading to impaired ciliary function and therefore muco-ciliary clearance(Bush 2006). Trapping of debris and bacteria leads to a heightened inflammatory response. Until recently, it was not clear at what point this process started, and whether inflammation was directly related to bacterial infection, or whether absence of CFTR in itself is a trigger for inflammatory changes.

Evidence of early inflammation in the absence of infective organisms(Konstan and Berger 1997), by sampling Broncho-alveolar lavage fluid (BAL), has led

investigations into the primary role of dysfunctional CFTR in establishing airway inflammation.

Armstrong(Armstrong et al. 1997) sought to investigate inflammation in early CF by performing BAL in patients diagnosed by the newborn screening programme. In patients classed as infected (definitive infection at time of BAL) there was evidence of increased cell count, neutrophil elastase activity and cytokine concentration. There was an association between level of bacteria and inflammatory response. Those classed as pristine (no evidence of infected organisms, no symptoms and no previous antibiotics) showed similar cytokine levels as healthy control patients with no previous respiratory disease and those patients diagnosed with CF with previous evidence of bacterial infection (by previous BAL) or who were given antibiotics previously.

Bacterial infection stimulates an inflammatory response that is excessive and prolonged compared to similar bacterial load in non-cf patients(Rosenfeld et al. 2001, Muhlebach and Noah 2002). Levels of IL-8 and neutrophil numbers were higher compared to control. IL-10 (anti-inflammatory cytokine involved in inflammation reduction after infection) is lower in adult CF compared to non-cf, leading to the belief that inflammation may be prolonged due to intrinsic effect of CFTR dysfunction, or that it is due to inflammation dysregulation as a consequence of chronic, unrelenting infective stimulation.

To investigate the correlation of CF airway inflammation and lung function (FEV1), Sagel et. al. investigated 20 CF patients (8-19yrs) who expectorated sputum either spontaneously or by induction(Sagel et al. 2001, Sagel et al. 2002). All showed increased inflammatory indices (cell count, absolute neutrophil count, IL-8 and neutrophil elastase activity) vs. healthy control patients (n=11). Airway infection (bacterial counts) was also higher in CF patients. When analysing markers of inflammation against spirometry (FEV1) in the same cohort of patients, Sagel found a significant negative correlation between FEV1 and cell and polymorphonuclear (PMN) counts, IL-8 and elastase. Bacterial count, however, did not correlate with lung function or indices of inflammation. Furthermore, sputum matrix metalloproteinase 9 (implicated in the process of degradation of airways and lung

parenchyma) levels showed significant negative correlation with FEV1 indicating the role of these proteolytic enzymes in ongoing lung disease(Sagel et al. 2005).

Airway structure

High Resolution Computed Tomography (HRCT) is suggested as a surrogate outcome measure for longitudinal studies of lung disease in children with CF due to the highly detailed information gained(Brody et al. 2005). Widespread use is limited due to concerns about the 20x increased radiation exposure compared to standard chest x-ray radiographs. The development of appropriate protocols that minimise radiation exposure and scoring systems that accurately quantify early disease have led to a number of units using regular CT at yearly or two-yearly intervals as part of routine clinical care(Gustafsson et al. 2008, de Jong et al. 2006, Tiddens and de Jong 2006, Tiddens 2006).

HRCT gives detailed information about structural airway and parenchymal changes. CT has been shown to detect evidence of air trapping and airway wall thickness in patients as young as 8 months(Martinez et al. 2005).

In 34 children with CF, Dakin et al performed HRCT along with lung function (FEV1, FVC) and sputum sample analysis for inflammatory markers (IL-8 and cytology). While lung function correlated with most elements of the CT score, sputum inflammatory markers only correlated weakly with overall Bhalla score(Dakin et al. 2002b).

CT score therefore appears useful in detecting early structural evidence of inflammatory change but does not seem to correlate with sputum inflammatory markers.

An important recent study by Gustafsson et al investigated the agreement between lung function measurements (LCI and FEV1) and measurements of structural lung disease in 44 children [5-19 yrs] with CF. LCI correlated with CT score (adapted Brody score) better than FEV1, indicating increased sensitivity to structural disease. LCI also detected abnormality in some patients with normal CT score. This indicates LCI may even be more sensitive than CT at detecting early CF lung disease.

Important data is now available to allow detailed evaluation of CF lung disease. Many of the most widely studied measures are invasive or involve high doses of

radiation. This is of concern to many clinicians caring for young CF patients. Regular HRCT may provide much need information about the progression of CF lung disease but regular CT, combined with longer life expectancy due to improved treatment, may result in increase in iatrogenic malignancies.

United Kingdom Cystic Fibrosis Gene Therapy Consortium

CF is amongst the few conditions with a single gene defect, theoretically ideal for correction by transfection of the correct gene code. The UK CF Gene Therapy Consortium was established to develop and evaluate a clinically relevant gene therapy product[30].

Evaluation of the success of gene therapy depends on measures which detect a change in the primary defect (CFTR function, mucociliary clearance) and clinical assays that detect an improvement or slowing of deterioration in lung disease (structure, function, inflammation, infection and symptoms).

It is recognised that lung disease slowly progresses from birth throughout life. This decline can be measured over time and used as a surrogate for disease severity or as a predictor of death. In designing a clinically relevant gene therapy, it is thought that those with less advanced disease are likely to benefit most; however, measuring clinical benefit following treatment is difficult as lung disease is mild and decline in function progresses relatively slowly. FEV_1 as a sole measure may be insensitive to change after treatment, and a major task for the consortium is to assess relevant surrogate markers of lung disease that will be able to detect change following treatment in patients with mild to moderate lung disease. A further task is to decide which patients have disease that is advanced enough to detect a change with treatment but that is not so advanced as to render gene therapy ineffective due to physical barriers to inhaled gene transfection (e.g. mucous and inflammation)[32]

Currently planned therapeutic studies will involve patients aged 12 years and over, however future trials may recruit younger patients. Surrogate markers of lung disease developed for the first wave of therapeutic trials therefore also need to be relevant to younger patients. The current "run-in" study aims to assess the longitudinal repeatability and variability of markers of disease progression in individual patients as well as the suitability of these assays in future therapeutic trials. One important

assay is lung clearance index (LCI). Using the Innocor device, studies conducted by the UKCFGTC have been able to perform MBW as a standard assay. Therefore, development and validation of MBW equipment (Innocor) and measurement standards has immediate as well as future relevance. Validation of Innocor for measurement of LCI is central to this thesis.

Despite advances in understanding of the pathophysiology of CF, it remains a fatal condition. It is now better understood how early structural and inflammatory changes relate to functional deficits and with emphasis on early, mild disease the hope is that decline in health over time will be minimal, leading to prolonged life expectancy and decreased overall morbidity. Despite improvements, there are few non-invasive assays widely available that are able to detect and track lung disease. LCI, derived from MBW, is able to detect early, mild lung disease better than other lung function measurements, however there is still only limited use due to lack of standardisation and poor availability of equipment. Given the great need for improved lung function measurements, development of MBW and validation of equipment is central to progress in this field.

Available lung function assays for use in children and infants

Spirometry

Spirometry, a forced expiratory manoeuvre, is the most widely used measure of respiratory function. It assesses flow and lung volume at maximum forced expiration (airway flow limitation). In disease states, particularly inflammatory conditions (e.g. asthma), maximum flow is reduced because of decreased airway calibre (airway wall thickening, mucous and bronchospasm). In other conditions (e.g. emphysema), expired volume may be reduced because of loss of lung elastic recoil (disrupted lung architecture). Mainly because of its simplicity and relative independence of effort, spirometry has become a widely used test, able to detect acute and chronic deterioration. It is also able to distinguish between obstructive and restrictive causes of functional deficit.

While spirometry is useful in advanced disease states and can predict terminal decline in CF, it is not sensitive enough to detect early disease-related changes in patients with mild lung disease(de Jong et al. 2004). It generally cannot be

successfully performed in children <5yrs old, however modification of the technique and coaching has allowed reproducible results with evidence of flow limitation in children as young as 3 years(Eigen et al. 2001). For further advances to be made with treatment strategies, rigorously evaluated in double-blind placebo controlled trials, there must be alternative repeatable measures even in the youngest patients(Weiser and Kerem 2007).

In response to the above limitations of forced expiratory volume measurements in young children and infants, standardisation of the Raised Volume Rapid Thoraco-abdominal Compression Technique (RVRTC) has been reviewed(Lum et al. 2006). This technique obtains forced expiratory flow measurements ($FEV_{0.5}$) at flow limitation, similar to spirometry, in sedated infants and young children. In those with clinically diagnosed CF, with and without other evidence of lower airway disease, $FEV_{0.5}$ is reduced compared with controls(Ranganathan et al. 2004). The obvious advantage of this technique is high sensitivity to the presence of disease. It is, however, difficult to perform without expert support, requiring sedation in all but the youngest infants.

This thesis commonly uses standard spirometry as a comparison measurement for validation of LCI. This is because most similar published articles do the same, and spirometry is the most widely available measurement with recently updated reference ranges(Cole et al. 2009, Stanojevic et al. 2007a, Stanojevic et al. 2007b, Stanojevic et al. 2009, Stanojevic et al. 2008). It is also the technique most familiar to the patients taking part in these studies.

Multiple breath washout

Multiple Breath Washout (MBW) of inert gas is of particular interest for this thesis and will be discussed in detail in the following chapters. Lung function indices, derived from MBW, e.g. Lung Clearance Index (LCI), have been evaluated for use in children with mild, early CF. MBW studies are not new(Larsson et al. 1988, de Vries et al. 1981), however studies in children have only been conducted recently(Stromberg and Gustafsson 2000). Aurora et al showed that LCI in infants and children with CF was raised, showing a distinct separation from controls(Aurora et al. 2005b). This phenomenon is unusual in lung function testing and generated a

great deal of interest. Furthermore, the LCI upper limit of normal does not change from pre-school age to adulthood in health, allowing accurate comparisons to be made over time independent of height or age. Comparing LCI with standard spirometry in children aged 3-18 years with CF, Gustafsson et al showed ventilation heterogeneity in all patients with abnormal FEV_1, and in more than half of those in whom spirometry was normal(Gustafsson et al. 2003a). Aurora et al also demonstrated that LCI, along with specific airways resistance and forced expiratory volume in 0.5 seconds ($FEV_{0.5}$), were raised in children with CF aged 2-5 yrs(Aurora et al. 2005a). In his study, LCI was higher in patients infected with Pseudomonas Aeruginosa, whereas other measures were normal, suggesting LCI is more able to detect abnormalities in this age-group than other lung function measures.

It is assumed that lung function in children with CF declines over time along with an increase in chronic inflammation, bacterial colonisation and structural airway damage. As a highly repeatable technique, studies have yet to confirm LCI as a useful clinical tool to detect and track this decline over time, with the ability to detect improvements following initiation of treatment. Early indications are that such studies will bear fruit(Kieninger et al. 2011, Robinson et al. 2009a, Aurora et al. 2011, Amin et al. 2010, Amin et al. 2011). There has been an increase in studies performing MBW in infants and children with CF, but without rigorous standardisation of equipment, method and data analysis, results cannot currently be confidently compared between centres. Commercially available devices are only now being marketed. An ERS/ATS "state of the art" article is currently being prepared to document up-to-date experience with MBW [in press]. This will allow comparison of results across centres, and encourage new users, as well as further commercial development of suitable equipment.

Multiple breath washout in Edinburgh

Innocor

In 2006, when this project began, only a few centres had published data from multiple breath washout studies. Devices available either utilised mass spectrometry for direct measurement of expired gas concentration, or molar mass calculations for indirect concentration estimates.

Innocor (Innovision, Denmark), a device marketed for non-invasive measurement of cardiac output, was initially purchased by the UKCFGTC as an alternative to mass spectrometer and molar mass technology for MBW. Its relative affordability and simple design made it ideal for planned multi-site clinical studies. It is not designed or sold for MBW testing, therefore a series of technical adaptations, with clinical studies, were conducted by Dr Alex Horsley, Clinical Research Fellow based at the Western General Hospital, within our academic group. Importantly, no previous MBW experiments had been conducted using this device. These initial validation experiments are reviewed in a later chapter.

Prior to my thesis project, Innocor was untested in paediatric subjects (<16yrs old). The following chapters therefore discuss the use of Innocor, specifically in children, and the process of MBW equipment validation. Given the need for suitable equipment for MBW in young children, it was felt that limiting Innocor's use to older children and adults would severely hamper the interest in a specific commercial MBW device. While no funding or impetus for these experiments came from Innovision, the device manufacturer, it was hoped they would use the validation work conducted in Edinburgh for a clinically applicable MBW device. Innovision have been supportive of this work and interested in further applications of their gas analyser technology, however they were not directly involved in the validation work in Edinburgh, and did not provide financial support for any of the studies.

Paediatric lung function standards

In 2000, a European Respiratory Society/ American Thoracic Society lung function task force was established, aiming to publish widely agreed standards for infant lung function testing. This series provides guidance in performing lung function in young children and infants, as well as technical details for device manufacturers.

Frey et al published two documents in 2000, which dealt specifically with technical requirements for lung function equipment(Frey et al. 2000a, Frey et al. 2000b). These highlight important differences in equipment used for paediatric populations compared with adults. Because infant volumes and flows are much smaller, errors considered negligible in adult equipment (e.g. equipment dead space), must be

reduced or eliminated. Signal quality and analysis of data must be examined carefully to minimise error from additional factors (e.g. faster respiratory rate).

Recognising differences in basic physiology, a number of subsequent papers have introduced standards for lung model devices and comparison experiments between lung function equipment. Again, Frey et. al. described the components and performance of a mechanical lung model to test infant respiratory function equipment(Frey et al. 2001) Fuchs et al and Pillow et al used task force guidelines to compare lung function equipment as a process of validation(Fuchs et al. 2006, Pillow et al. 2004). More recently Beydon et al, on behalf of the ERS/ATS taskforce, published a complete statement of standards required for all aspects of respiratory function testing in infants(Beydon et al. 2007). From these, clinicians, research groups and equipment manufacturers may begin to use these techniques.

Equipment validation

For any clinical measurement, it is important that new equipment is properly validated, taking into account potential sources of error to minimise systematic bias(Beardsmore et al. 2007). Also, for a novel device or technique to be compared with an established alternative, the two must be similar in terms of individual component performance criteria (Frey et al. 2000a). It may not be feasible to have identical equipment in all centres, but comparisons of normative populations, longitudinal observational studies and interventional studies will only have limited application unless all equipment is fully validated.

The validation process described in this thesis seeks to understand and document the factors that limit accurate multiple breath washout in children using Innocor. Specific hardware, methodology and analysis changes have been made to compensate for equipment limitations as well as adjusting for physiological differences between children and adults. Accepted standards are often based on good evidence; but where evidence does not exist the standards are examined and challenged where appropriate. In addition, as MBW is performed in all devices using the same basic technique, the findings of this thesis are potentially relevant to other groups developing or validating similar equipment.

This thesis also describes the clinical studies conducted as part of this validation process. These allow comparisons to be made with other studies in similar populations to confirm the suitability of the Innocor device, but also add to the understanding of gas mixing and its relevance to airways disease in children.

Thesis aims

1. Show that MBW indices are a suitable selection for detecting and tracking lung disease in CF and other paediatric airway diseases.
2. Describe the MBW technique and modified Innocor equipment in detail.
3. Demonstrate through a series of clinical studies the use of Innocor in health and disease.
4. Evaluate suitability of Innocor equipment in younger children (<5 years) and demonstrate validity.

Conclusion

Given the convenience and relative ease of performing repeated lung function, even in children, and the disadvantages and risks involved in more invasive measures (e.g. HRCT or bronchoscopy), there is a concerted research effort to find suitable lung function measurements to replace spirometry(Beydon et al. 2007). The ideal situation is to have a highly reproducible, non-invasive measurement, such that the same test is used in infants, children, adolescents and adults, allowing tracking of lung function over time. This is vital to understanding the natural history of chronic respiratory diseases (e.g. asthma or cystic fibrosis) and the long-term effects of interventions and the implications of early life events on adult lung function (e.g. prematurity). The above scientific advances suggest this is possible with MBW; therefore, this thesis explores the use of Innocor in children and a series of validation studies. Because of this work using Innocor, it is hoped that development of commercially available MBW equipment will be stimulated, fulfilling a pressing need in paediatric respiratory research. Despite marketing of newer MBW devices, including nitrogen washout modules and SF6 devices for older children, there is still not a device for use in all ages.

This thesis is presented in 10 chapters. There are 2 chapters concerned with methods, to provide the basis for the subsequent clinical studies. Each clinical study presents

data based on a hypothesis, with overall contribution to the main thesis aims presented after each discussion section. To complete the thesis there is a summary chapter describing how all the chapters contribute to the main thesis aims above and how future work should proceed.

Chapter 2 – methods I: multiple breath washout description and initial Innocor validation

Introduction

This chapter describes multiple breath washout (MBW) methods. The aim is to prepare the reader for clinical studies in subsequent chapters, as well as to provide basic principles for adaptations to the Innocor setup in later chapters.

MBW is a study of gas mixing. While the test itself and the principles behind it have been studied for some 50 years(Cantor and Evans 1970, de Vries et al. 1981, Gappa et al. 1993a, Jonmarker et al. 1985, Light et al. 1980, Saidel et al. 1975, Schibler et al. 2000, Wall 1985, Engel 1985), it is only recently that technology has enabled MBW studies with larger numbers of subjects(Gustafsson 2005, Fuchs et al. 2006, Gustafsson 2007, Gustafsson et al. 2003b, Lum et al. 2007, Aurora et al. 2005a, Aurora et al. 2004, Aurora et al. 2005b). One of the greatest advantages is its applicability to all age groups from newborn babies to adults, which will potentially enable tracking of lung disease in infancy through childhood, right up to adult age. This is especially important for inherited progressive conditions but is also important for other paediatric airway disease.

An understanding of basic lung anatomy and respiratory physiology is important in development of this technique. This chapter therefore reviews the physiological principles behind MBW.

Many of the methods described are only recently published. There is some disagreement on the details of MBW methodology, and at the time of writing, these have not been resolved. A "state of the art" document, to be published in late 2012, seeks to resolve many of these issues, encouraging development of suitable commercially available equipment and wider clinical use of MBW. Some of the method development described in this thesis has contributed to this international document.

This chapter also contains detailed description of the MBW technique along with data analysis. Alex Horsley, who initially led the development of the Innocor device in Edinburgh, has recently published a thesis describing much of this. However, given the need for measurements to accurately detect and track lung disease from

infancy, there is particular interest in applying MBW specifically to early years. There is also a discussion of the main difficulties encountered during the experiments and clinical studies.

Background to MBW

- Gas Mixing
- Conduction, Convection and Diffusion
- Quantifying Gas Mixing
- Multiple Breath Washout Basics

Gas mixing

Simple anatomical studies of the airways reveal central branches repeatedly dividing into smaller and smaller diameters (Figure 1). These branches serve simply to conduct inspired gas into the smaller airways and alveoli, the region involved in gas exchange with the pulmonary circulation.

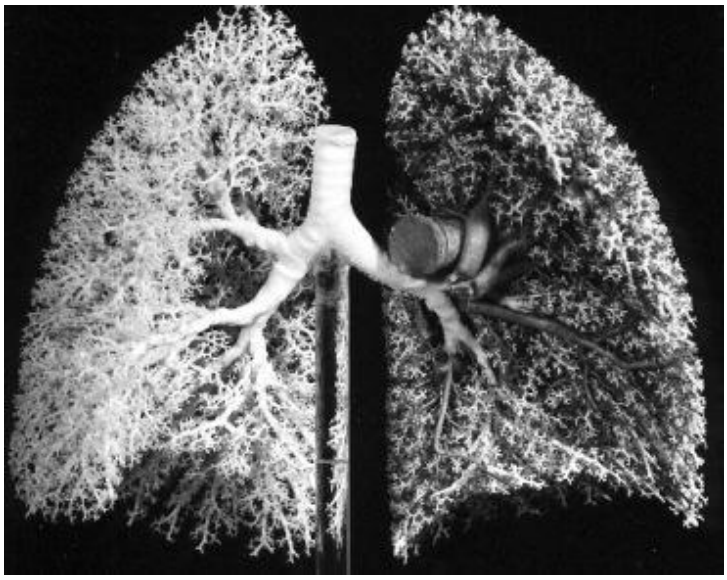


Figure 1 - anatomy of airway tree

This system of branching airways increases the efficiency of the system conducting inspired air to the gas exchange areas with minimal effort at rest. Oxygen is brought to the gas exchange membrane during inspiration, with carbon dioxide removal during expiration. This process is remarkably efficient because of an elegant airway bifurcation structure and the large cross-sectional surface area of the alveolar gas

exchange zone. At each bifurcation, the successive cumulative area of the daughter airways is larger than the parent. This has the advantage of reducing total resistance and slowing convective flow, enabling optimal gas mixing. Weibel's symmetrical model illustrates this, describing exponentially increasing total cross-sectional area with successive airway generations (Figure 2). The respiratory zone starts at approximately division 17, where gas exchange begins to take place. Prior to that, the airways simple conduct gas with no respiratory exchange. Initial research interest was in how gas behaves in the smaller airways, and how the lungs achieve such a degree of gas exchange so efficiently.

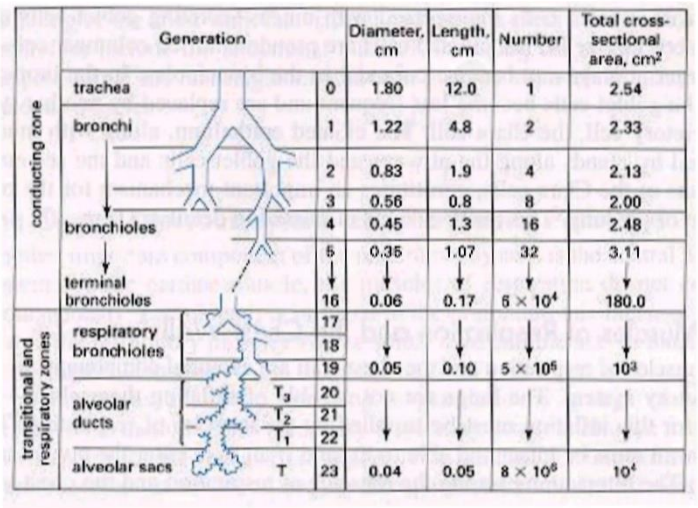


Figure 2 - Weibel's schematic description of airway levels

Conduction, convection and diffusion

Early gas mixing studies have attempted to measure physiological processes, building on prior knowledge of lung anatomy.

Initial results showed that the lungs do not completely empty with each breath, therefore inspired oxygen in the air must mix with the residual airway gas to reach the alveoli. A method to measure the volume of this functional residual capacity (FRC), using Fick's first law of diffusion and the Bohr equation, was published by Fowler(Engel 1985). This states that gas diffuses from areas of high concentration to low, and assumes that all gas mixing occurs in the relatively large alveolar compartment, as opposed to the conducting airways. Recent gas dynamics studies have shown that the conducting airways do not only feed gas to the alveoli, but are

also involved in gas mixing by longitudinal and axial gas mixing. Whilst the Bohr equation, as applied by Fowler, is easily applied to physiological tests, it is probably an oversimplification of multiple influences. Conclusions about the anatomical location of abnormalities, based on changing gas washout patterns, are balanced by the assumption that other regions simultaneously contribute to gas mixing to a lesser extent.

Fowler made another important contribution to gas washout studies in disease when he showed that, along with being able to measure lung volumes with gas washout, the expired N2 concentration following a breath of 100% oxygen followed predictable phases (Figure 3).

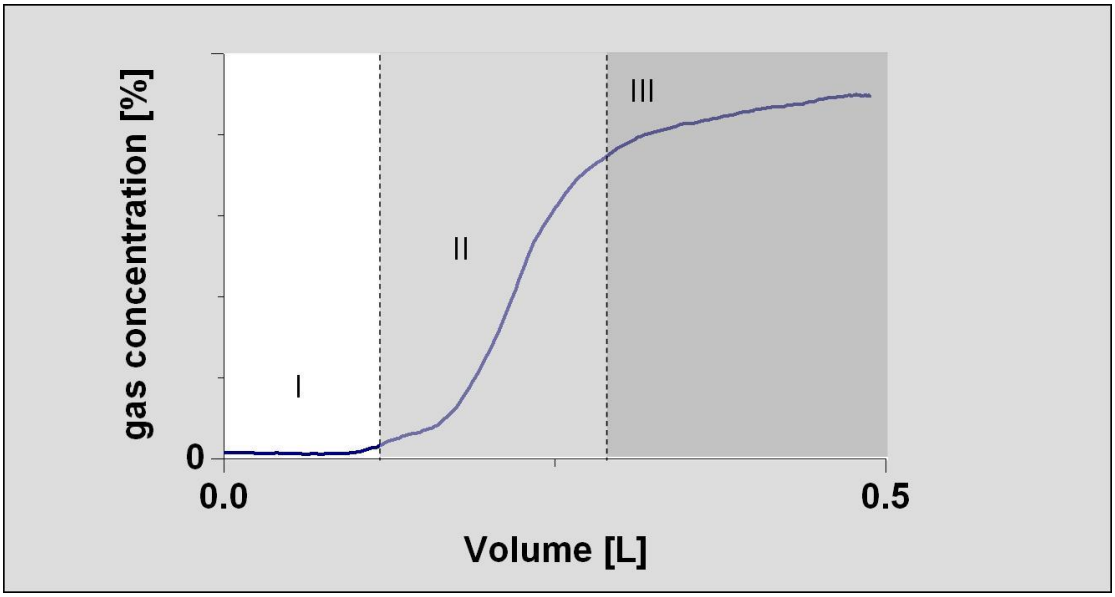


Figure 3 - typical pattern of single breath expired gas concentration. Phases of breath are shown in shaded areas.

- Phase I corresponds to the dead space within the conducting airways. In the case of N2 washout with 100% O2, the phase I concentration is near to zero, representing largely unmixed inspired oxygen.
- Phase II corresponds to the rapid rise in concentration associated with switching from the airway dead space with no nitrogen to the alveolar compartments with high N2 concentration. There is a diffusion front such that the concentration rise is sigmoid rather than instantaneous. This demonstrates the degree of axial and longitudinal mixing of gas within the conducting zone.

- Phase III (alveolar slope) represents the gas concentration in the alveolar compartment. The alveolar slope is such because rather than all lung units emptying at the same rate, there is a degree of sequencing, generating a gentle rise in concentration to the end of the breath. This rise in concentration during phase III steepens in disease.

Single breaths of nitrogen have subsequently been extensively studied after inhaling 100% oxygen(Engel 1985). Much information is now known about the effect of disease processes (e.g. obstruction) and physiological changes (e.g. increased flow) on the pattern of expired tracer gas concentration. In disease states, an increasing alveolar slope indicates greater sequencing of parallel lung units. The concept of ventilation heterogeneity and sequential emptying of parallel lung units therefore began with single breath washout analysis(Engel 1985).

Initial work with Nitrogen Washout techniques was conducted to investigate the effect of different physiological parameters (e.g. tidal volume, breath holding, lung volume) on gas mixing. In addition, Paiva and Engel pioneered lung modelling studies to describe the relative contributions of convection (movement of gas) and diffusion (mixing of molecules from high concentration to low) to gas mixing(Engel 1985). They developed early single breath washout tests to use exogenous tracer gases of different diffusivities. The hypothesis was that differences in the washout pattern between these gases would indicate to what extent diffusion of gas contributes to gas mixing. Differences in concentration at different airway levels indicated the anatomical location of the diffusion front. After multiple detailed studies analysing gas concentrations at various airway divisions, it appears that the convection diffusion interaction - where bulk flow (convection) stops and molecular diffusion takes over - exists within the acinus. This initial work was initially important for physiological studies in health, but is now important in studying disease states.

Single breath tests give comprehensive information only in very heterogeneous lungs. In situations with patchy mild lung disease, where some regions are not emptied until later on in a multiple breath washout, abnormalities may not be detected. Multiple breath washout (MBW), initially measuring washout of resident

nitrogen with 100% O2, was developed as a way of assessing sequential emptying of heterogeneously ventilated lung units because gas concentration is followed until it is almost completely washed out. Indices derived from MBW are now discussed.

Work by Verbanck and Gustafsson in separate institutions has widened the use of MBW techniques to various disease states such as COPD, CF and asthma(Verbanck et al. 1998, Verbanck et al. 2010). Importantly, its use is now extended to young children and infants, who are unable to cooperate with standard lung function measurements(Morris et al. 2001, Beydon et al. 2007).

Quantifying gas mixing

As described above, the lungs readily mix gas by conduction, convection and diffusion. With each breath, air is directed down 16 airway branches to reach the gas exchange zone in the terminal bronchioles and alveoli. At this level, the single trachea has divided into 60,000 small airways. There are multiple, self-contained lung units that extend from each airway bifurcation. It is useful to consider the lungs as a series of distinct parallel lung units, whatever the level, to understand how disease affects gas mixing. Parallel units in this situation could be lungs, lobes, lobules or acini.

With each breath, a complex balance of airway surface tension and tissue tethering forces in the lung parenchyma ensure even distribution of air(Engel 1985). There are physiological regional differences in ventilation between the apex and base, but between parallel alveolar units, the difference is negligible in health. Disease (e.g. inflammation) causes patchy obstruction in airways, distributing gas unequally throughout the airways.

To expand on this, consider two hypothetical parallel lung units in disease. Obstruction is different between units, therefore air is preferentially distributed to the unit of lower resistance. This increases the volume of this unit, unbalancing the tethering forces. The degree of filling between these units is imbalanced, affecting surrounding units. Even differences in small airways can lead to large differences in air distribution through imbalanced lung expansion.

This principle is important in gas mixing measurements, as the pattern of gas washout during multiple breath tests can be explained by interactions between parallel lung units, sampling the washout of a tracer gas over time. During both large

vital capacity breaths and simple tidal breaths, the lungs do not completely empty at the end of expiration. Therefore, gas mixing takes place to dilute the residual gas in the inspired portion. In health, where gas is distributed to parallel units evenly, a certain number of breaths are required to wash a tracer gas out of the lungs. Again, considering two single parallel units, if gas is not distributed evenly due to areas of obstruction causing imbalances in ventilation, one unit will wash out quicker than the other. These units could therefore be considered heterogeneous. Over the whole lung, a proportion of lung units will wash gas out at the same rate as a healthy lung, or even faster as they are preferentially ventilated. Only later in the washout, once the concentration gradient between inspired and residual gas is greater, will poorly ventilated units wash out gas. Depending on the degree of heterogeneity between parallel units, the difference in washout rates may be great. There are 2 phases to gas washout in this highly simplified situation - a fast phase and a slow phase, as illustrated in Figure 4. The whole lung is more complex than two parallel units, but principles of heterogeneity of ventilation between multiple lung units over the whole airway tree are derived from this model.

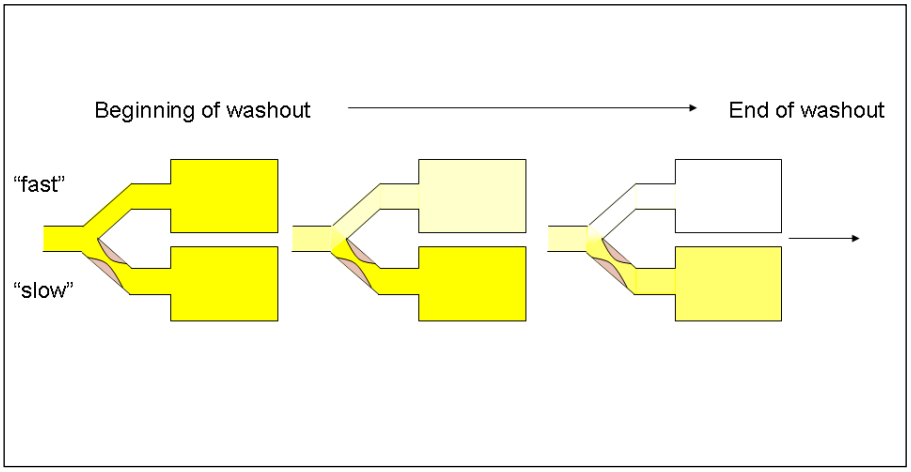


Figure 4 - this shows that in parallel units within the airway tree, a partial obstruction increasing resistance allows preferential ventilation of the unaffected unit, washing out the gas more quickly than the affected unit. Before the washout is over, the well-ventilated unit is empty of gas, while the concentration in the affected arm is still high. This leads to a prolonged washout with greater breaths required to empty the affected portion, and two phases to the washout.

Tracer gases

To measure ventilation heterogeneity, a suitable tracer is used to follow gas washout. This gas is measured on a breath-by-breath basis, therefore a relatively fast gas analyser is needed, responding accurately to sudden changes during inspiration and expiration. Concentration, followed as it is washed out, is plotted against volume of expired gas to calculate washout indices. The tracer gas selected should be inert, with no effect on airway patency, no endogenous production and no airway absorption.

The choice of gas is normally determined by available technology. Fast Nitrogen analysers have been available for many years, leading to the development of nitrogen washout tests, where inhaled 100% Oxygen displaces resident nitrogen. Other gases have been used more recently as suitable analysers have become available. Often a non-resident gas such as helium or sulphur hexafluoride is chosen in low concentrations as this minimally disrupts inspired viscosity and natural gas mixing. Details of the three main gases used are given below. Additional gases, such as methane or argon, are occasionally used to compare the effect of gas density on washout pattern.

Nitrogen

As early as 1941, multiple breath nitrogen washout (MBNW) tests were designed to assess gas mixing in the lungs(Cournand et al. 1941). This technique was simple, requiring subjects to washout residual nitrogen with tidal breath inhalations of 100% oxygen. The advantage to this system was the ready availability of accurate fast nitrogen analysers and 100% oxygen. This principle of nitrogen washout is still used today although the technique has been significantly refined since its early conception(Downie et al. 2007, Verbanck et al. 1998, Verbanck et al. 1999, Verbanck et al. 2001, Verbanck et al. 2003, Verbanck et al. 1997). Nitrogen washout is simple and requires no washin period. However, nitrogen is absorbed and produced in the lung and must be corrected for during washout calculations. Also, switching between air and 100% oxygen greatly alters gas viscosity, leading to difficulties in flow measurement. Additionally, there is a documented decrease in FRC in infants breathing 100% O2, which is not seen in older children and adults(Newth et al. 1997).

Helium

As an additional method for obtaining information about lung volumes, helium has been used since 1948 to calculate functional residual capacity in human subjects(Meneely and Kaltrieder 1949). The advantage of using a non-resident gas is the lack of requirement to calculate consumed or endogenously produced gas during the washout Helium is readily available as a test gas and this technique is well established for calculating functional residual capacity today in lung function laboratories(Cotes et al. 2006). Helium has a very low molecular weight. This makes it very prone to leaks during testing. During prolonged washouts leaks may be great and unpredictable, leading to inaccuracies in volume and gas mixing calculations.

Sulphur hexafluoride

Sulphur hexafluoride (SF6) is a synthetic, non-polar and insoluble non-organic compound. Importantly, it is inert and therefore is used in many industrial and medical applications. It was discovered in 1901, but was first used for lung function measurements in the 1970's(Paiva et al. 1976, Lacquet et al. 1975). With its high molecular weight, it was used in contrast to helium to measure gas mixing, particularly assessing differences in convection and diffusion of gas in the lung periphery. Adequate gas analysers for SF_6 are less readily available. Mass spectrometry is most commonly used, with other technologies only recently being developed for breath-by-breath measurement. This has hampered its use in centres without mass spectrometer technology and has limited clinical washout studies to very few centres. The utilisation of the Innocor gas analyser has enabled our department to develop SF6 washout techniques without purchasing mass spectrometer equipment.

Innocor

With a view to development of multiple breath washout techniques, the UK CF Gene Therapy Consortium investigated the use of an appropriate device to measure gas mixing in clinical studies. The mass spectrometer is the most commonly used device, however cost and complexity were prohibitive. Given its high SF_6 signal quality and

resolution, this department investigated the Innocor device as a more affordable novel alternative for MBW.

Innocor is manufactured by Innovision, Denmark (www.innovision.dk). The gas analyser technology is central to this company's research and development, with a spin-off company in designing techniques for the space industry. Importantly, the Innocor device is not designed or marketed as an MBW device, as the internal construction and software is solely intended for exercise testing and cardiac output measurement. Because of safety regulations (CE0543, ref aur5a0711v141f282, cardiopulmonary function test equipment class IIa) and commercial reasons, the company are not able to alter the software to optimise it for MBW testing. For this reason, the software was adapted to include a simple protocol, allowing constant gas analysis without interruption. Programmes to perform daily flow meter and gas analyser calibration are present in the software package, however, MBW analysis is performed offline on a separate computer using a custom-built programme.

Basic system components

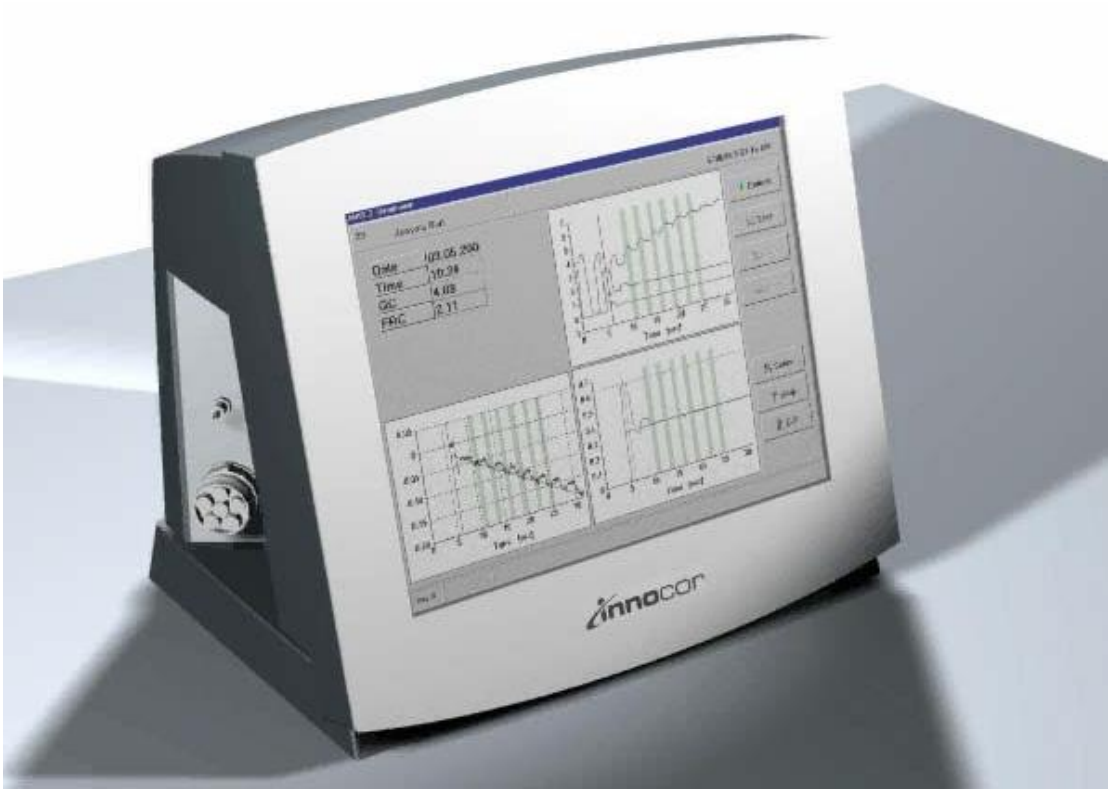


Figure 5 - Innocor gas analyser and touchscreen computer.

Innocor (Figure 5) consists of a computer, gas analysers, gas pumps, switches and a touch screen in one compact and portable unit. The detachable Respiratory Valve Unit (RVU, Figure 6) consists of the patient interface (filter and mouthpiece), flow meter and valve unit which automatically switches between a re-breathing circuit of inert gases and air. Re-breathing is a method of washing in gases from a closed large-volume bag to minimise gas wastage. The gas mix is stored in an integral small compressed gas cylinder (18L uncompressed volume, 5% N_2O, 1% SF_6, 94% O_2). This volume and method of delivery is not appropriate for prolonged MBW testing, therefore the first adaptation is a separate gas supply delivered in an open, bias flow fashion. A separate large gas cylinder containing SF_6 0.2% in medically balanced air is used. This is manufactured and delivered by BOC ltd. (www.boc.com, material code 159499-L-PC).

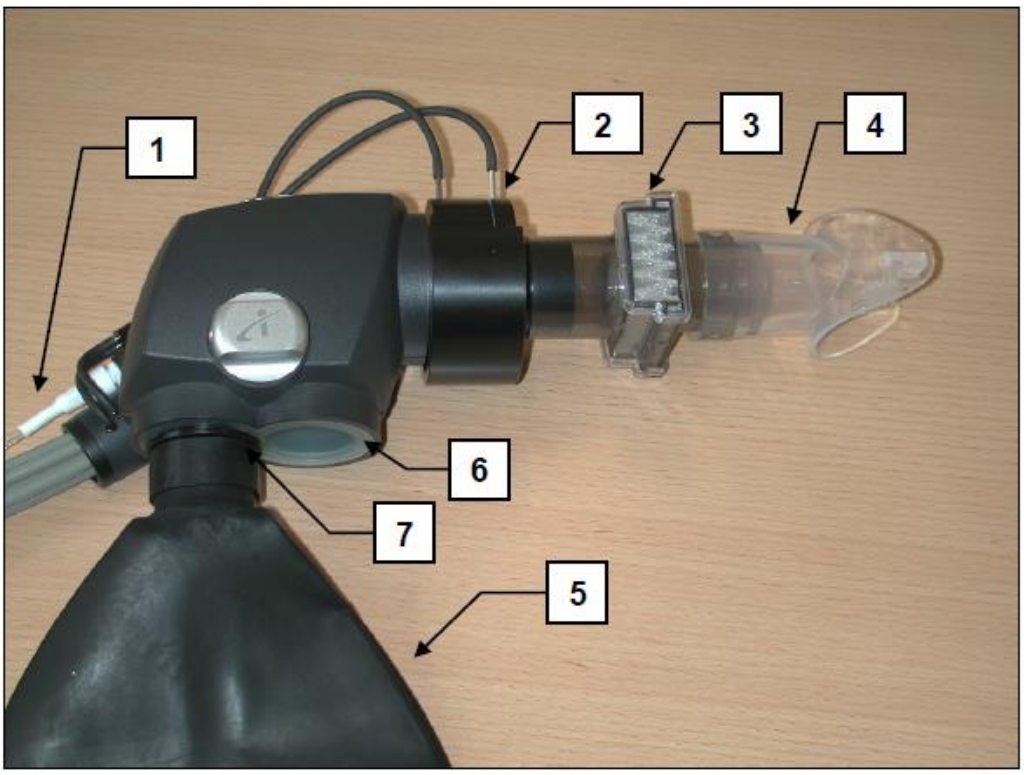


Figure 6 - Rebreathing valve unit with connections. 1- gas sampling tube, 2- flow meter, 3- bacterial/viral filter, 4- mouthpiece, 5- rebreathing bag, 6- exhaust port, 7- rebreathe port

There is no need for constant online visual display of gas concentration in cardiac output testing. Because of interest in this new MBW application, the company were willing to add a software module. This allows a "live" view of flow and gas concentration, with a constantly updated numerical display of gas concentration to ensure high quality washouts with minimal leaks and disruptions to flow.

There were very few barriers to development of Innocor as a MBW device. There are, however, a number of inconvenient aspects remaining. Future developments have improved some of these but full development requires investment by Innovision.

One problem with the current software is the regular timed flow meter reset. These reset intervals can be altered prior to, but not during, a washout. While signal resets are important for volume calculations as flow meters have a natural drift over time, this generates a sudden large flow signal for 1 second during the washout (100L/s). Therefore, during testing, a flow reset needs to be avoided to prevent data errors. Furthermore, different subjects take variable lengths of time to complete a washout. It is desirable for the subject to limit total testing time for reasons of comfort and tolerability. If the flow reset is set at too short an interval some subjects may not be washed in by the reset time.

Photo-acoustic gas analyser

The core technology within Innocor is the photo-acoustic gas analyser (PGA). The principle of photoacoustic spectroscopy is that gas molecules absorb light in proportion to their concentration. An infra-red light, split by specific frequencies by a rotating wheel, is shone on sampled gas. Light absorbed by sampled gas results in temperature/pressure changes, which are heard as sound by a sensitive microphone. Change in amplitude is directly proportional to gas concentration. The photoacoustic method can only measure di-atomic molecules, and the PGA is specifically set to measure SF_6, N_2O and CO_2. A schematic is shown in Figure 7.

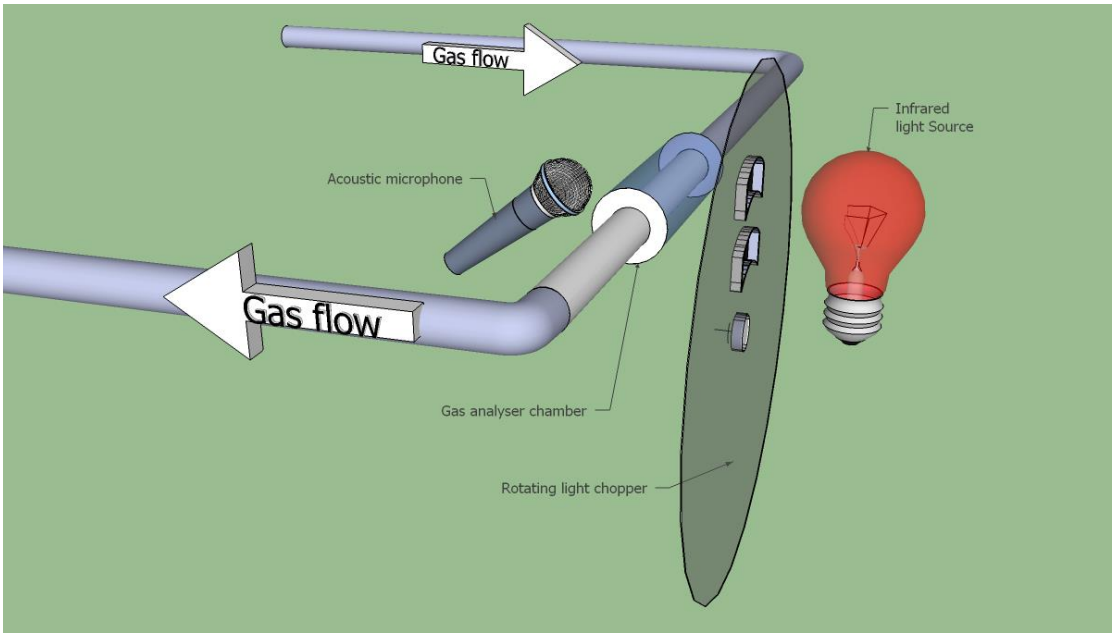


Figure 7 - schematic of photo-acoustic gas analyser (PGA). Light, chopped at specific frequencies, is shone on sampled gas which emits an acoustic response. The amplitude of this response is directly proportional to the concentration.

The advantages of this technology are the compact design and high signal-to-noise ratio. The disadvantages are the relatively large volume of sampled gas required and relatively slow response to changes in gas concentration. This will be discussed in detail in subsequent chapters.

Comparison with most commonly used MBW device.

Even with the above limitations, there is an incentive to develop Innocor for MBW. Table 2 compares Innocor with a mass spectrometer to illustrate the benefits and disadvantages of each device against the other.

	Mass spectrometer	Innocor
Size	Large	Small and portable
Durability	Sensitive to any moving. Requires frequent servicing and technical support.	Can be readily moved. Requires only annual analyser calibration.
Cost	£65,000	£30,000
Gas Analyser	Highly specific, multigas analyser. Very fast response time	Very limited range of gases analysed. Moderate response time.
Gas Signal quality	Very high	Very high
Software	Requires offline MBW software. Excellent on-line visual signals	Requires export and offline analysis. Limited on-line visual signal
Potential for further physiological research	Very fast, specific analyser with further research potential. Multiple gas capacity.	Limited further research potential due to limited range of measurable gases.
Potential for clinical application	Requires external flow meter and calibration equipment. Unlikely to be available in clinical setting due to high costs and servicing requirements.	Compact, self-contained device. Potential for clinical use only after commercial development.

Table 2 - comparison of Innocor with gold standard MBW device.

Environmental considerations

SF_6 is a known greenhouse gas, with a 24000x greater potency than the same weight of CO_2. Before MBW becomes more widespread, many ethics committees and device manufacturers may wish to consider the environmental impact of washout tests.

SF_6 consumption varies with age and extent of heterogeneity. An older patient with advanced CF may take 8 minutes to wash in, whereas an infant may take less than 2 minutes. Larger subjects also require a higher gas flow rate.

Conventionally 3 washins are performed, with MBW indices calculated from washout, although this is not universal. Calculating "MBW" indices from the washin potentially reduces gas consumption(Bell et al. 2008).

Using basic figures, the consumption of SF_6 was calculated for various ages of patient, with the equivalent weight of CO_2 (Table 3). Figures quoted are based on average uses of SF_6 during washins of 0.2%, rather than 4%. These numbers are also illustrated in Figure 8. With increasing age of subject, SF_6 consumption increases exponentially because of greater time taken to washin, accompanied by increased gas flow.

Age (yrs)	length of washin (mins)	gas flow (L/min)	consumption of gas mix (L)	consumption of pure sf6 (L)	consumption of pure sf6 for 3 washins (L)	equivalent vol of CO2 (L) (x 24000)	equivalent weight of CO2 (kg)
1	2	3	6	0.012	0.036	864	1.554
3	2	4	8	0.016	0.048	1152	2.071
5	2	6	12	0.024	0.072	1728	3.107
8	3	8	24	0.048	0.144	3456	6.213
12	4	10	40	0.080	0.240	5760	10.357
16	4	12	48	0.096	0.288	6912	12.428
20	5	15	75	0.150	0.450	10800	19.418

Table 3 - comparison of estimated SF6 consumption based on age of subject

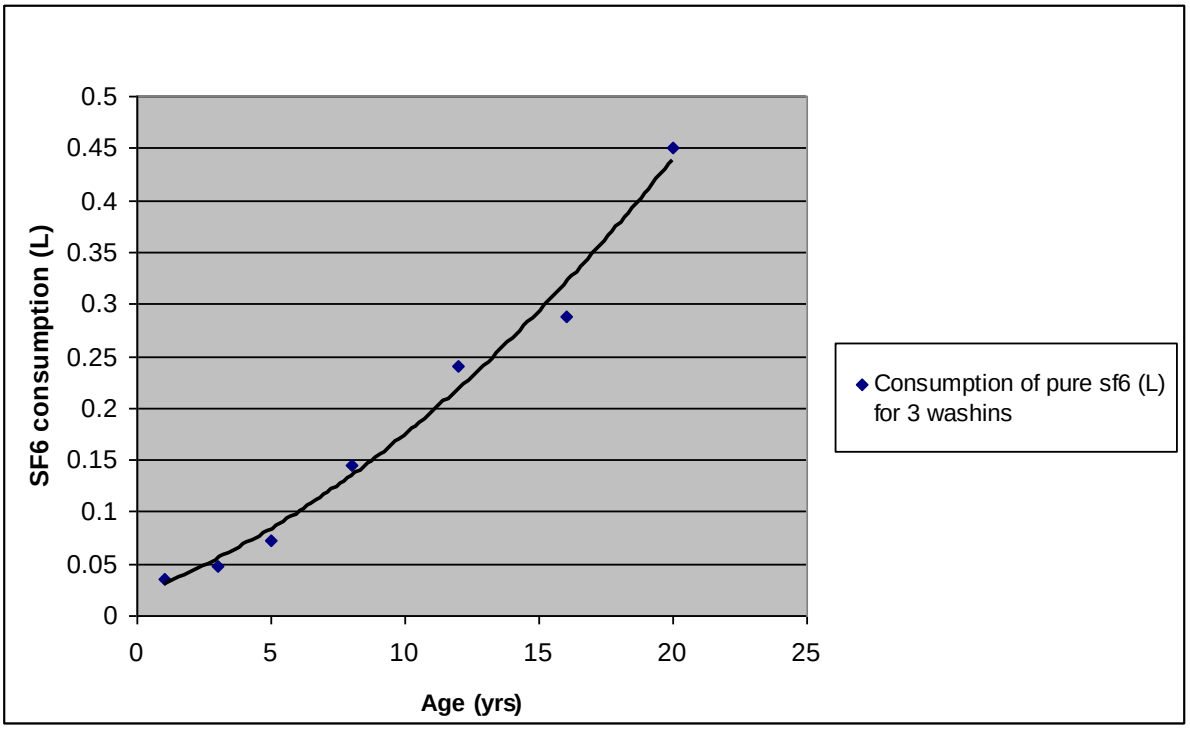


Figure 8 - illustration of exponential increase in SF_6 consumption because of increased bias flow requirement and prolonged washin duration

CO_2 equivalence

According to the Intergovernmental Panel on Climate Change (IPCC, 2001) SF6 is 24,000 times more potent than CO2 in terms of greenhouse potential(Intergovernmental Panel on Climate Change. et al. 2001). Providing equivalent CO2 consumption is important to ease environmental concerns about widespread MBW testing.

One flight from London to New York (~33400 L (0.6 tons) CO2 per person) is equivalent to MBW in about 46 average people each performing 3 washouts (Innocor, 0.2% SF6). If the alternative 4% SF_6 concentration is used, as in other systems, 1 flight to New York is be equivalent to only ~2.5 average people performing 3 washouts each. Also, 1 adult with CF (greatest SF_6 consumption) would use the equivalent of 20kg CO2 per 3 washouts (0.2% sf6) (This is the same as driving 116km (72 miles) in a 1.8L car). With 4% sf6, 3 washouts would be the equivalent of 1440 miles.

It is important to stress that a child with CF (e.g. 5 yrs old), would use approximately 6 times less SF_6 than an adult with CF (2 minute washin at 6l/min vs. 5 minute washin at 15L/min).

MBW technique

Equipment

Equipment for performing multiple breath washout is much the same as that used in previous studies using nitrogen washout with oxygen. The subject breathes through a pneumotach to measure flow, while delivering gas via a bias flow system. This delivery system allows the patient to breath normally with no valves or switches. On inspiration, the flow to the subject is biased towards gas coming from the flow of SF6. On expiration, the bias is towards expired gas leaving the system via the exhaust end (Figure 9 below).

SF6

The SF_6 concentration used for measuring lung volume and gas washout should be high enough to provide high signal to noise ratio right to the end of the washout. High concentrations of gas, however, may interfere with normal gas mixing due to changes in viscosity and density. Concentrations of 0.5 % were used in early SF_6 washout studies using an infrared analyser(Larsson et al. 1987). Studies using mass spectrometry commonly use 4% SF6 due to poor signal to noise ratio at lower concentrations. The Innocor device uses 0.2% SF_6, as there is high signal to noise ratio with this analyser throughout the washout. This is an advantage, as any changes in viscosity that may alter flow readings with higher SF_6 concentrations are not a concern at 0.2%. There is also less gas consumption, which may be an ecological advantage as discussed above.

Gas analyser

The lack of fully developed gas analysers, suitable for breath-by-breath analysis, has hampered the wider use of MBW of SF6. According to published guidelines, the ideal gas analyser must:

- be capable of detecting breath-by-breath changes in gas concentration, with high update frequency and high signal to noise ratio(Beydon et al. 2007)
- ensure accurate estimation of end-tidal gas concentration, expired gas volume and alveolar slope analysis(Beydon et al. 2007)

- be able to respond quickly to sudden changes in flow. The speed of response is often quoted as the 10-90% rise time(Brunner and Westenskow 1988). This issue is discussed in detail in a later chapter (8).

In a side-stream system such as the Innocor, gas is sampled from the subject's expired flow. A metal capillary is positioned in the stream of the subject's breath, usually within the Pneumotach flow meter (Figure 10).

Pneumotach requirements

Pneumotach (PNT) flow meters are highly standardised devices that estimate flow over a pre-defined range. Any device employed to measure flow in small children should be a balance of low resistance against low dead space(Frey et al. 2000a). The most commonly used flow meters are: screen pneumotachs, hot wire anemometers or ultrasonic devices. The most commonly used screen PNT for MBW testing is the Fleisch-type, which is durable and easy to clean but expensive. Hans-Rudolph flow heads are cheaper but required dismantling before sterilisation. A number of Hans Rudolph sizes are available, operating within set recommended flow ranges and minimising equipment dead space. Importantly, PNTs do not measure volume directly. They estimate flow by utilising the phenomenon of a linear increasing pressure differential with increased flow across a partial obstruction. In the case of a Hans-Rudolph PNT, the partial obstruction is a mesh within the flow head. Pressure difference is converted to flow by an electronic transducer, and integrated with time to calculate volume. Care is taken to ensure that a PNT is calibrated accurately. A number of variables affect the pressure differential, e.g. humidity, gas composition, temperature, size of breath and magnitude of flow.

Published guidelines recommend PNT specifications for use in patients of all ages(Miller et al. 2005a, Wanger et al. 2005). Of particular interest to this thesis are guidelines relating to children and infants(Frey et al. 2000a, Beydon et al. 2007). Expected infant tidal volume (2-12kg) is 5-8 ml/kg, and total PNT dead space should be no more than 2ml/kg. Heating is also required in small low-flow devices to prevent condensation build-up on the PNT screen, especially when used within humidified circuits (e.g. mechanical ventilation). Temperature within a heated PNT should be 30-37^{o}C. The PNT frequency response should be measured using all the

pieces of equipment in place and should be within ± 5dB at up to 10Hz. The frequency response is defined as ability of the equipment to accurately convert a physiological signal to an electrical signal, faithful to the magnitude (attenuation) and temporal relationship (phase lag) over a defined frequency range(Frey et al. 2000b).

Linearisation and daily calibration of flow meters is essential(Frey et al. 2000a). This involves using a calibration syringe to deliver known volumes of air over the flow ranges expected during testing, tuning the electrical voltage response. Daily calibration measurements should be within ±5% of each other.

Bias flow gas supply

The bias flow system is a simple way to deliver tracer gas while encouraging tidal breathing. Without valves, there is also low dead space. A stream of gas is passed via elephant tubing over the end of the pneumotach, with a longer distal exhaust arm, to create greater resistance than the proximal section during inspiration to minimise re-inspiration of expired gas (Figure 9). The gas flow rate should be higher than the subject's maximal tidal inspiratory flow. This setup ensures that during inspiration, the subject receives only fresh gas, not expirate. During expiration, because of fresh gas flow, there is minimal back flow of expirate up the proximal tubing.

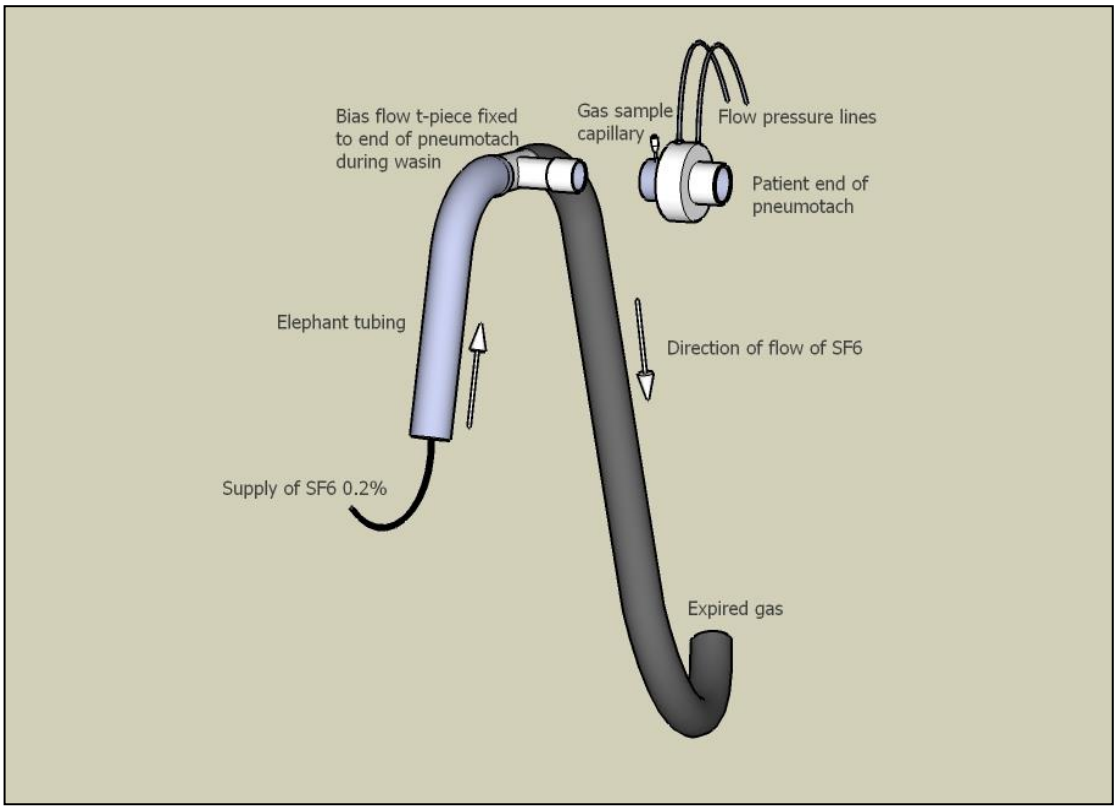


Figure 9 - Pneumotach with gas sample line and bias flow gas delivery

Mouthpiece and noseclip

Estimated gas quantity should be accurately measured throughout the test, and leaks in the system increase error. In older children and adults, a mouthpiece and noseclip is used. This can be of any type but must be able to achieve a good seal with minimal discomfort. The mouthpieces used in Innocor experiments were obtained from nspire (www.nspirehealth.com). To avoid dismantling and disinfecting the Pneumotach after each patient, a single use bacterial/viral filter (Air Safety ltd.) is used in all subjects where deadspace restrictions did not prevent it. After each use, the mouthpiece and nose clip were cleaned and disinfected.

An image of the standard setup for use in older children and adults is shown in Figure 10.

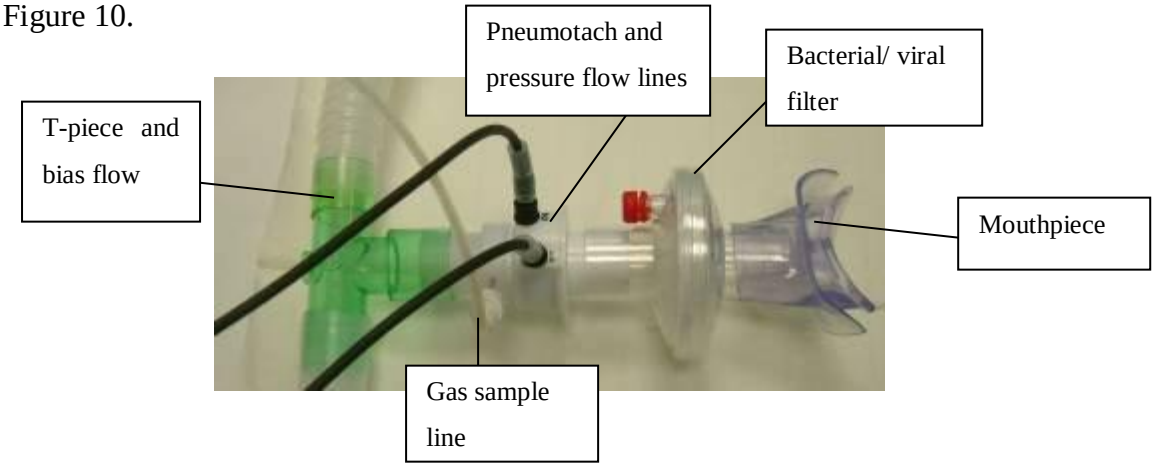


Figure 10 - Image of standard patient interface - mouthpiece, filter, pneumotach and bias flow circuit

Facemask

In children younger than 5 years, a facemask is used, as noseclips do not fit easily and infants are obligate nose-breathers. Again, a good seal must be achieved with minimal equipment dead-space.

Current recommendations for infant lung function testing state that equipment dead-space be minimised to less than 2ml/kg body weight(Frey et al. 2000a). This is primarily for safety reasons, as proportionally large volumes of re-breathed air compared with tidal volume can cause CO_2 retention. In addition, a large narrow dead-space increases respiratory effort and may change tidal breathing pattern.

Rendell-baker masks are low dead-space and fit easily with the addition of malleable putty(Frey et al. 2000a), which also minimises dead-space(Beydon et al. 2007). The volume of the facemask is measured by water displacement, subtracting 50% of this volume to take into account the approximate volume occupied by the subject's face(Morris 1999).

Standard multiple breath washout procedure

Multiple breath washouts estimate functional residual capacity (FRC) by gas dilution and ventilation heterogeneity indices, by analysing the pattern and rate of gas washout.

To properly analyse inert gas washout, the gas is first washed in, via the bias flow system, so the concentration is uniform throughout the lungs – so called equilibrium.

Over time, the concentration in the lungs becomes evenly distributed, therefore the expired concentration approximately represents the concentration throughout all the airways. If equilibration is not reached, conclusions cannot be reached about washout of gas.

At equilibrium, the gas supply is detached during expiration, leaving the subject to wash out the gas with relaxed tidal breathing. With each breath, air is inspired, diluting the gas within the lungs and over time the gas is washed out completely. To reduce the time taken to complete the test, and with a recognition that gas analysers lose signal resolution at very low concentrations, the end point of the test is not complete washout of gas. Once the concentration in the breath (measured at the end of each breath, end tidal concentration [C_{et}]) reaches $1/40^{th}$ of the starting concentration the test is ended. $1/40^{th}$ is chosen by convention rather than particular sensitivity or specificity. This is the end-point for all MBW devices, whatever the starting concentration(Beydon et al. 2007).

In the Innocor system the starting concentration is 0.2%, therefore the final end-tidal washout concentration is 0.005%.

The operating procedure below has been developed using published guidelines(Beydon et al. 2007, Gustafsson 2005), refined with local department experience. While the basic technique applies to all age groups, different approaches are required with children and infants, as shown below.

Wash in phase

1. The subject performs the test seated, in an upright chair. Slouching should be avoided so that uniform lung volumes are maintained.
2. The mouthpiece and noseclip are attached, with the head positioned so the neck is neither flexed nor extended.
3. Gas is delivered with the flowpast T-piece connected to the end of the pneumotach. The flow-past rate should be set at a sufficient rate to ensure that no expired air is re-inspired. For an adult this will be 10-15 L/min, but is increased if the patient breathes more deeply than expected. Insufficient flow-past can be viewed on the Innocor screen as a dip in SF_6 concentration during inspiration.

4. The subject breathes in a relaxed fashion throughout, expiring to FRC, or relaxed lung volume, avoiding long pauses between breaths.
5. Subjects should be distracted with television or a DVD. This ensures the tidal breathing is as relaxed as possible. Concentrating too much on breathing pattern can disrupt the normal regular rhythm.
6. The online gas concentration should be observed until the constant online concentration reaches 0.2%.
7. Time taken to achieve equilibrium varies depending on lung volume and extent of disease. Infants take less than 30 seconds; adults with advanced disease can take many minutes.
8. In practice, some patients may take a long time to reach equilibrium. In advanced disease, this may take 10 minutes. In addition, small leaks may prevent reaching complete equilibrium. As a compromise, the washin is judged complete once the maximum and minimum concentrations differ by no more than 0.004% (absolute concentration)
9. To ensure poorly ventilated regions are all equilibrated, the washin should continue for approximately 30 seconds after the equilibrium point is reached.

Figure 11 shows a typical washin of SF_6. The black tracing is flow (left axis, L/s) and the green is SF_6 concentration (%). With each breath (positive flow), the gas is progressively washed in to equilibrium. The regular flow meter reset is seen near the end of the washin (sudden extreme drop in flow).

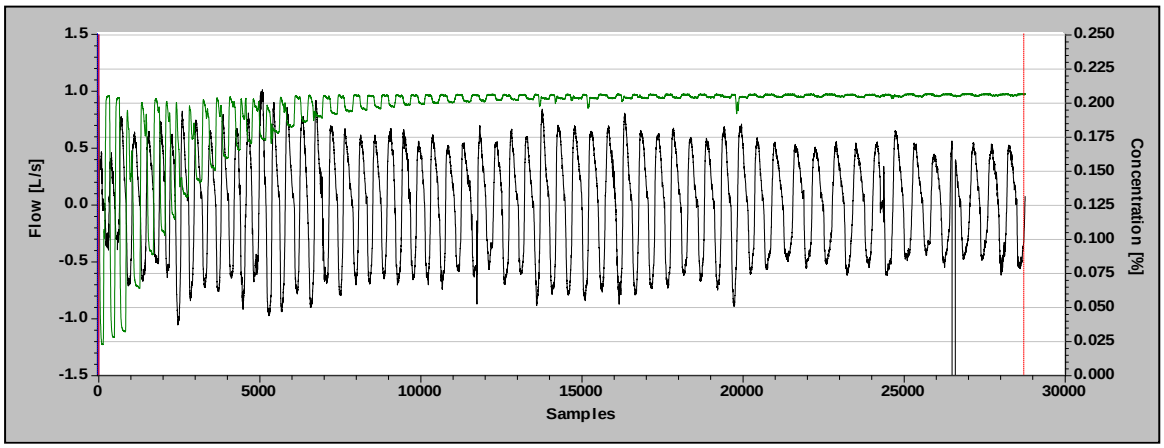


Figure 11 - Standard washout trace. Black line is flow. Green line is SF_6 concentration.

Wash out phase

1. Once the SF_6 has been fully washed in, the bias flow T-piece is disconnected rapidly and smoothly during expiration. A swift detachment means no gas is inspired in the first washout breath.
2. The patient now breathes room air until the end-expiratory SF_6 concentration has fallen to less than $1/40^{th}$ of the starting concentration, or 0.005%.
3. Careful attention must be paid to pauses in breathing or evidence of leaks (sudden drops in concentration). The subject may need some encouragement to breathe regularly.

Figure 12 shows a typical washout pattern. The first sudden drop in concentration is the first inspiration of room air after detachment. The washout continues with even, regular breaths and no pauses, indicating good quality. The red line indicates the approximate finishing point of the washout, where the end-tidal concentration is 0.005%. It is good practice to continue the washout beyond this point to ensure the washout is finished as incomplete washouts cannot be analysed.

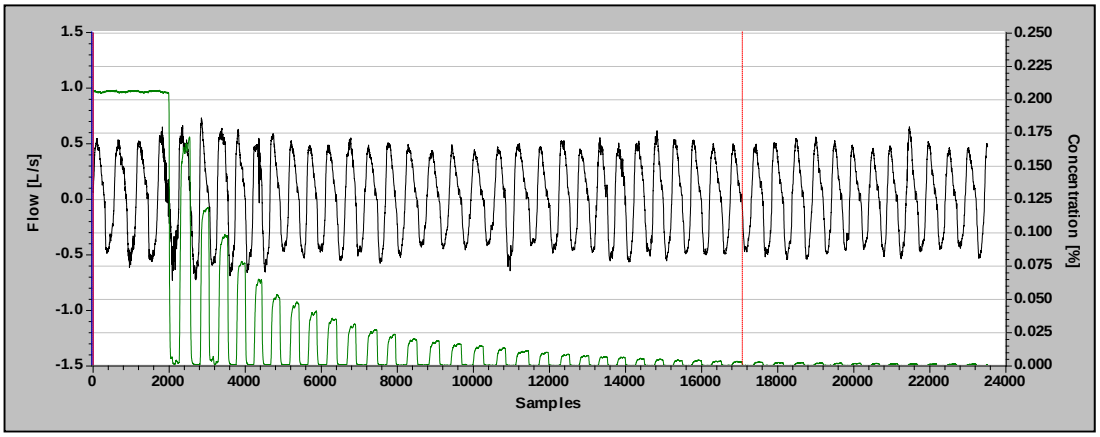


Figure 12 - Typical multiple breath washout pattern. SF_6concentration reduces with each breath

Washout standardisation

Standardised washouts must be conducted to enable quantitative analysis between measurements and between research units. Explained in the following paragraphs are important standardisation requirements.

Standard concentration of SF6

The measured concentration of SF_6 should always be within the calibration range of the analyser. 0.2% is optimal for Innocor washouts. While it does not matter greatly what the exact concentration is, large differences in concentration between subjects may alter gas viscosity. Each tank of gas supplied by BOC is guaranteed to be within 5% of the documented concentration.

Documented equipment dead-space

The dead space of the equipment is removed from the reported estimated lung volume. Mouthpieces and filters have documented volumes that can be checked by water displacement. The Pneumotach dead space in front of the gas sample capillary is also subtracted from the final volume, as all calculations are made at the point the gas is sampled.

Post-capillary dead-space volume, if included in the washout analysis, is subtracted from each breath.

Daily flow meter and flow gas delay calibration

The Innocor flow meter should have calibration documented daily. This ensures there are no leaks or other malfunctions in the system. Small errors in flow measurements may lead to large errors in volume estimation over many breaths. Daily flow calibration delivers known volumes (calibration syringe) over varying flow rates.

There is a discussion of flow gas delay calibration in chapter 8. This process aligns flow and gas concentration signals, to take into account time taken for sampled gas to reach the analyser. Small errors in signal alignment lead to large volume estimation errors(Arieli and Van Liew 1981). Flow gas delay should also be recorded daily to detect problems in the gas analyser if the value drifts over time.

Mouthpiece and noseclip to minimise gas leak

The mouthpiece and noseclip ensure minimal leak. They can be uncomfortable initially but get more tolerable as the test continues. A nose-clip should always be worn, as absence invalidates the measurement due to leaking of unquantifiable volumes.

Sitting posture

As discussed in detail in a later chapter, posture affects gas volumes and gas mixing(Gronkvist et al. 2002). In terms of method development, I noticed that tidal volume dropped if subjects slumped or slouched forward. A strict upright sitting posture is therefore encouraged in all subjects.

The method of supporting the Pneumotach is also important. An electrode stand was adapted to hold the PNT, with the subject sitting at a table it is easy to maintain optimal position. The stand is multidirectional and easily manipulated, making it suitable for all sizes of older children.

In younger subjects, the investigator should encourage sitting posture and support the pneumotach and facemask to make sure there is no leak. This picture was published by the Institute for Child Health, Great Ormond Street Hospital, London(Aurora et al. 2005b).

Figure 13 - example of performing MBW on a young child. Extracted from Aurora et al(Aurora et al. 2005b).

Tidal breathing

An important aspect of multiple breath washout is maintaining a consistent tidal volume. When observing washouts in individuals with more variable tidal volumes between tests, it was clear that increased tidal volume decreases FRC. As ventilation heterogeneity is calculated using FRC, increased tidal volume may increase ventilation heterogeneity.

In initial published MBW studies, researchers recognised the influence of tidal volume on gas mixing. If breaths are not large enough to reach beyond the respiratory dead space, alveolar gas mixing will not be measured(Engel 1985). Most early studies were conducted in adults, developed from single breath vital capacity or restricted volume tests. All subjects in these studies were asked to perform 1 Litre breaths at all times to reduce variability between subjects and aid alveolar slope analysis. This large volume is not practical in children and in those with severe respiratory disease. As an alternative, relaxed tidal breathing is encouraged, incorporating adapted protocols to take into account variable tidal volume(Aurora et al. 2005b) between subjects. A simple visual tidal volume feedback was designed in our department to investigate the benefits and disadvantages of such a protocol in terms of washout consistency and acceptability. I found this not to be useful in most children, as washouts became complicated by nervousness and difficulty breathing as some children found the feedback difficult to use. Therefore, volume feedback was not used in any of the paediatric studies.

Detachment of flow-past circuit

The washout test requires switching from SF_6 to air smoothly. Mixing of gas and air during inspiration confuses analysis, as mixed re-inspired gas cannot be distinguished from equilibrated airway gas at the beginning of the washout.

Clear switching from flow-past gas to air is achieved by detaching the flow past circuit during expiration, when only expired gas is passing over the gas analyser. This is difficult to achieve when chest movement cannot easily be seen and in those with fast respiratory rates. This is an important skill to achieve when performing washouts, especially in children.

Washout endpoint

The Innocor shows a real-time SF_6 concentration, however the end-tidal display is not given as the value only updates every second. Therefore, the displayed concentration taken early in inspiration may be below $1/40^{th}$ whereas the end-tidal concentration is still higher. In addition washout becomes prolonged in advanced disease states and the end-tidal concentration may fluctuate around $1/40^{th}$ for a number of breaths. Ending the test early usually invalidates the whole measurement. For these reasons, the protocol encourages finishing the washout only when the on-line display does not rise above 0.002%.

Experience of MBW in children

MBW has most recently been developed with children in mind. Despite this, what is simple for older children and adults, can be challenging in younger children. Those younger than 10 years, in general, have a short attention span and may have difficulty in understanding, leading to non-compliance with requests.

As with all techniques there is an associated learning curve in performing MBW. The procedures described above are based on experience with patients attending for clinical studies. Prior to this, washouts were performed on colleagues to help practice the sequence. My experience has shown that the following important factors contribute to a good quality tests in children:

- Testing environment – the room used for testing should be bright and non-threatening. Familiar posters were put up to encourage conversations and rapport.
- Careful explanation – children may have no prior understanding and can be nervous of new experiences. A clear explanation of what is going to happen avoids surprises. Attention must be paid to the use of some words (e.g. gas) as this may have a particular meaning to them. One 5 year old boy refused to perform the test when gas was mentioned.

Practice with the mouthpiece and noseclip before starting the test was helpful in some nervous children.

- Posture – slouching or changing posture alters lung volumes. MBW should be conducted in a sitting position. A large soft chair may be suitable for adults and older children but can be uncomfortable for younger ones (<10 years). I finally chose a simple wooden chair designed for classrooms with some height adjustability (q-learn, CS60281 senior chair, www.morleys.co.uk). This encourages children to sit upright and offers no ability to slouch. Other chairs (e.g. an adjustable cushioned office chair) were too high, deep and moveable so that children were uncomfortable, wriggling and swinging during testing. The classroom chair also offers the advantage of wipe-able surfaces to comply with infection control guidelines.
- Distraction – this is an important part of MBW testing. TV or DVDs should always be used, no matter the age, as too much attention to breathing rate can interrupt tidal flow patterns. This distraction also serves to make the test more enjoyable and will divert from what may be frightening pieces of equipment. Younger children are especially distractible, and perform tidal breathing very well with an engaging cartoon. Obviously, the distraction should be age-appropriate without encouraging laughing or singing. The most successful distraction I use is "The Simpsons", which has appeal across a wide age-range, but does not tend to cause laughing due to minimal visual humour.
- Regular tidal breathing – simple commands can be given during testing to encourage even breathing and maintaining a seal around the mouthpiece. I observed that too many commands could disrupt tidal breathing by causing confusion.
- Poor flow past detachment - this invalidated many early washouts I conducted. When teaching others to perform MBW, this has always been the area requiring most attention. Initial confidence eroded when washouts had to be discarded. The most common error was detaching the flow-past late in expiration, so that re-inspiration of SF_6 was seen.

- Preparing children too much for the detachment by taking too long or talking, can cause deep breaths or pauses as they expect the change. I found that a swift timed detachment with no verbal instruction was best. Occasionally, children came off the machine at this point thinking the test was over. This could be prevented by repeating a command to stay on device until told to come off.

Data analysis

Washout measurements

Multiple Breath Washout is designed to generate data for analysis of airway gas mixing. Key components of the technique – tidal breathing and inert gas washout from equilibrium to pre-defined end-point – are carefully standardised so analysis reveals predictable outcomes, comparable with other studies in other research centres.

This section describes the most common measurements obtained from this technique. There is also a description of the analysis process, and alterations made to this in this department to improve reproducibility.

Functional residual capacity

Functional Residual Capacity (FRC) is a lung volume measurement, obtained from inert gas washout analysis. While this is not a measure of ventilation heterogeneity, it can be used clinically and is the basis for calculating gas mixing indices.

In physiological measurements, the lungs are divided into functional compartments. This reflects normal circumstances, where full lung capacity is reserved for increased respiratory demand, and the lungs do not completely empty with each breath, leaving a Functional Residual Capacity (FRC). The FRC is therefore defined as the volume left in the lungs at the end of a normal tidal breath (Figure 14).

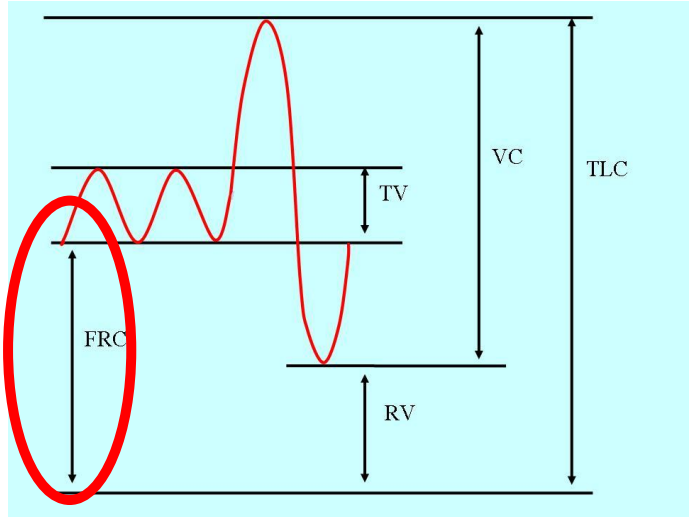


Figure 14 - standard function lung volumes

FRC is important, as a standardised measure of lung volume. The gas dilution method of assessing FRC may differ from plethysmographic measurements due to complete obstruction in some regions will prevent any gas filling in that area. Nevertheless, this measurement is important as a basic consistent volume on which to base gas-mixing measurements.

FRC is calculated from the Fick diffusion principle, that (concentration x volume) in one compartment is equal to (concentration x volume) when moved to another compartment.

The total amount of gas within the FRC at equilibrium is equal to the (FRC x concentration) at the start. The total amount of gas at the end of the washout is equal to total amount breathed out, plus the (FRC x concentration) at the end. Concentration is continually measured, but the end-tidal concentration of each breath represents the concentration within the FRC.

This can be expressed, with FRC derived from:

$$\text{total amount } SF_6 \text{ (L) at start} = FRC \times C_{init\%}$$

$$\text{total amount } SF_6 \text{ (L) at end} = V_{exp} + (C_{et\%} \times FRC)$$

$$FRC \times C_{init\%} = V_{exp} + (C_{et\%} \times FRC)$$

$$V_{exp} = (FRC \times C_{init\%}) - (FRC \times C_{et\%})$$

$$V_{exp} = FRC\,(C_{init\%} - C_{et\%})$$

$$FRC\ (L) = \frac{V_{exp}}{C_{init\%} - C_{et\%}}$$

Assumptions and limitations

Functional Residual Capacity (FRC), Concentration at start of washout ($C_{init\%}$), Concentration at end of washout ($C_{et\%}$), Cumulative volume SF6 expired (V_{exp}). All volumes in litres (L)

Figure 15 - Derived calculation of multiple breath washout FRC

This is a simplified equation used to measure a complex airway tree. Especially in disease, it is unlikely that gas concentration is uniform throughout the lungs. The end-tidal concentration, measured at the lips, is used as an appropriate measure of alveolar gas concentration.

This calculation also assumes the FRC is constant throughout the test. Calculated FRC rises initially with each breath, reaching an asymptote as volumes involved increase, reducing error. It is therefore assumed the FRC is more accurate with each successive breath. If tidal volume increased, for example, during the test, this may reduce the estimated FRC, introducing error into the equation that would not easily be detected. Changes within the airways (e.g. mucous clearance due to cough) may also alter FRC during the measurement.

Other techniques use Nitrogen and Helium to calculate FRC. Nitrogen is produced from the bloodstream and must be accounted for in the equation. Helium is extremely prone to leaks and can result in under-estimation of FRC in prolonged washouts. SF_6 is completely inert, synthetic and not so prone to leaks, making it ideal for prolonged washouts.

Finally, FRC calculated by gas dilution only measures volume connected with the airways. Any volume completely obstructed during tidal breathing is not measured. There is a documented disparity between gas dilution and plethysmographic FRCs in all age groups(Stocks and Quanjer 1995).

Lung clearance index

Lung clearance index (LCI) is the most widely used measure of ventilation heterogeneity derived from MBW, mainly due to the relative intuitiveness to researchers and subjects.
LCI is defined as:

The number of resting FRC turnovers (TO) required to wash out SF_6, with tidal breathing, from equilibrium to the pre-defined end-point.

LCI is therefore calculated from the total volume of air breathed out (cumulative expired volume, CEV) during the washout, divided by the FRC, measured at the same time by gas dilution. The end of the test is conventionally when the end-tidal ("alveolar") concentration reaches $1/40^{th}$ of the initial concentration(Beydon et al. 2007). In the case of washouts using Innocor, 0.2% SF_6 is washed out until the end-tidal concentration has fallen to 0.005%.

$$\text{Lung Clearance Index (LCI)} = \frac{\text{Cumulative Expired Volume (CEV)}}{\text{Functional Residual Capacity (FRC)}}$$

If a normal lung uses tidal breathing to wash out a gas such as SF_6, it will take a certain number of breaths as the lungs do not completely empty with each breath and mixing of gas takes place. What can also be seen from studies in healthy volunteers is that it takes roughly the same number of FRC turnovers (TO) to wash the gas out whatever the age or size of the subject(Aurora et al. 2005b). Therefore, there is a very similar range of normal in all published studies using the same gas. The measurement is automatically standardised for lung size, because the denominator in the equation is FRC. In disease, LCI increases as a larger number of lung turnovers

are required to wash out the gas. As discussed above, this is thought to relate to the degree of heterogeneity between parallel lung units.

LCI is therefore an intuitive, simple measure of overall heterogeneity. A number of groups have shown a narrow range of normal that does not change with age(Gustafsson et al. 2003a). This is unusual for most physiological tests and may increase its application in tracking disease states (e.g. CF) throughout life. The concept of the measurement is simple to explain to children and adults, making it potentially suitable as a repeatable clinical measurement.

Multiple cross-section clinical studies, published in the past 10 years, have demonstrated increased LCI in patients with cystic fibrosis compared with controls in infants from 10 weeks of age up to adulthood(Gustafsson et al. 2003c, Gustafsson et al. 2003a, Aurora et al. 2004, A. R. Horsley et al. 2008). Furthermore, these studies demonstrated that LCI appears to be more able to detect CF lung disease in children and adults than standard lung function (FEV_1).

Only one large study has performed longitudinal LCI measurements(Kraemer et al. 2005). This showed that LCI deteriorated earlier than FEV_1 and other standard measures in children aged as young as 6 years old. LCI was also able to discriminate between those with and without lower airway Pseudomonas Aeruginosa colonisation. Further longitudinal studies are required in children with CF, especially infants newly diagnosed by newborn screening. It remains to be seen whether LCI, an alternative measure of CF lung disease, is able to track lung disease to allow assessment of therapeutic interventions.

LCI has also been measured in children with asthma. Gustafsson demonstrated that LCI was raised in a group of moderately severe asthmatic children, compared with healthy controls. LCI in children with asthma is discussed in detail in chapter 4.

Assumptions and limitations

LCI is considered to be a measure of global ventilation heterogeneity. However, it is not possible from LCI alone to determine at which functional level the heterogeneity occurs. LCI is often assumed to be able to detect disease in the small, as opposed to medium-sized or large, airways, however this has not been proven. As gas mixing takes place in small airways, LCI may be affected by heterogeneity at this level, however it could also be that small differences between large units of lung (medium

airway branch-points) induce the same pattern of prolonged washout. It is therefore inaccurate to state that LCI is a measure of small airway disease.

LCI measurements assume a constant FRC during testing. Increasing tidal volume, for example, will reduce the FRC. As the denominator in the equation is FRC, LCI may be artificially raised. Alternatively, larger breaths may actually improve gas mixing. It is therefore ideal to maintain as consistent a tidal volume as possible, while encouraging relaxed breathing. Average tidal volume and number of breaths required to washout are important additional data used to detect reasons for high variability or changes between measurements.

As stated for FRC, LCI does not measure lung regions with little or no gas exchange. It is feasible that advanced disease or acute deterioration may cause loss of a section of the airway tree. If this region was previously poorly ventilated, there may be a paradoxical reduction (improvement) in LCI. Similarly, if acute treatment to improve lung function and airway recruitment opens a previously unventilated region, LCI may increase (worsen) as this previously unused are is now involved in gas mixing. This is an important limitation in our understanding. More clinical studies are needed to understand how acute interventions alter gas mixing efficiency. Clinical studies in children with asthma and cystic fibrosis, conducted for this thesis, are discussed in later chapters.

There is a lack of published longitudinal LCI data in large cohorts. This would enable correlation with other measures of disease to understand better the clinical significance of increased ventilation heterogeneity. This paucity of data is mainly due to a lack of commercially available equipment. There is currently one device licensed to perform MBW in all ages. Other devices (including Innocor) have advantages and disadvantages but are not widely available without experienced technical support. The main aim of this thesis is to validate Innocor for use in younger age groups, thus increasing its appeal for large, longitudinal cohort studies.

Phase III slope analysis

Analysis of Phase III (alveolar portion) of each breath has been studied for the past 50 years(Verbanck et al. 1998, Crawford et al. 1985, Engel 1985). Interest stemmed from observations in single breath tests of the sloping alveolar plateau in lung disease compared with a stable alveolar concentration in healthy controls. During tidal multiple breath washout, the Phase III slope can be seen to steepen in those with prolonged washouts, especially cystic fibrosis. Phase III slope analysis seeks to quantify this phenomenon and, based on detailed lung model studies, assign changes in alveolar slope to distinct functional lung regions.

Each stage of phase III slope analysis is described, followed by an explanation of the resulting ventilation heterogeneity indices.

1. Phase III slope - SIII

The output of a washout test consists of a gas concentration signal and flow meter readings. During each breath, the numerical change in concentration over the alveolar phase against volume expired is the Phase III slope. This slope (SIII) is measured for every expired breath. An example of numerical slopes plotted against time (breath number), taken from a 5 year old healthy control, is shown in Figure 16. It is clear from the graph that the phase III slope (absolute change in concentration) of each breath falls as the gas is washed out, due to the falling gas concentration.

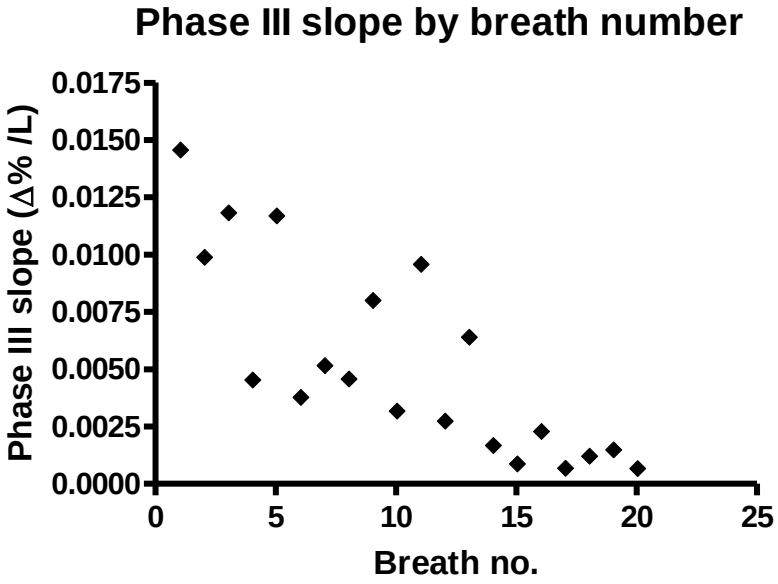


Figure 16 - Plot of breath number vs. phase III slope value. With each breath, phase III slope naturally decreases with falling gas concentration

2. Normalised Phase III slope - S_nIII against lung turnover

To allow comparison of slopes over the whole washout, the concentration is "normalised" against the mean concentration of the slope of that breath. This normalised slope (S_nIII) can be compared against breath number, but is normally compared against cumulative number of "FRCs" expired - lung turnover. This modification allows comparison of the concentration normalised slope against different sized subjects with naturally different breath number and washout time based on their lung size. The graph below illustrates the plot of the same normal washout with the above refinements. The plot of the normalised slope in this patient allows comparison of slope values throughout the washout. There is a trend towards increasing normalised slope in this patient as the washout progresses.

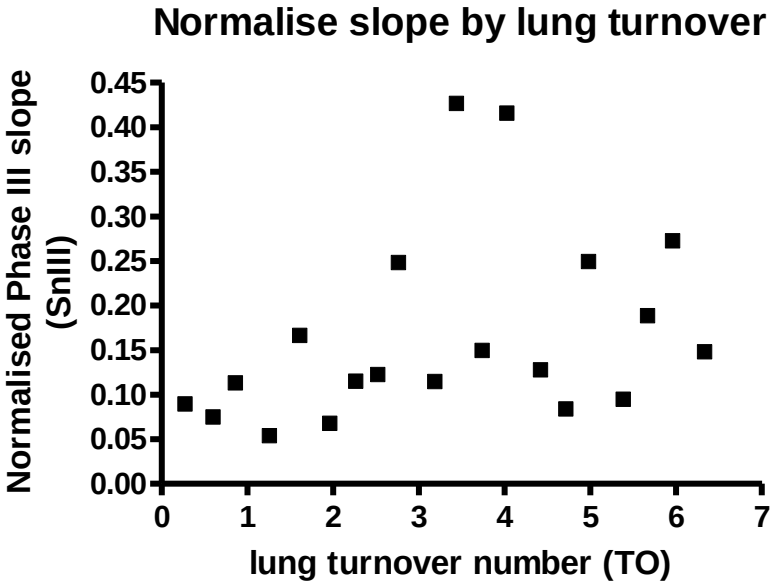


Figure 17 - Lung volume turnover (expired volume /FRC) vs. normalised phase III slope. Effect of normalisation of phase III slope shows upwards progression as the washout proceeds.

3. S_nIII normalised for tidal volume

Early clinical studies in phase III slope analyses were conducted with adult subjects. To simplify comparisons between subjects they were carefully limited to washing out gas with 1-litre breaths. This is a large but comfortable volume for most adults even with moderate lung disease. The advantage in performing washouts to this standard is that a litre is a constant proportion of the FRC and is plotted more easily. Also, change in concentration against change in volume does not vary and therefore is automatically normalised.

While a litre is a comfortable volume in most adults, this standard is not possible in children and infants. Aurora described the alteration of SnIII analysis to account for this(Aurora et al. 2005b). He described a non-linear relationship between expired volume and S_nIII of the first breath. Multiplying the SnIII of the first breath by the tidal volume of that breath removed the relationship. Following tidal volume correction calculations became age-independent from 2 to16 yrs. The explanation for this may be that different time constants exist in different sized lungs i.e. in smaller lungs, a larger proportion of alveolar gas is expired earlier in the breath as lung volume increases. The article does not make this clear.

Performing the 3-stage method above, the overall tidal volume-normalised SnIII is plotted against lung turnover over the washout. This, in itself, does not quantify ventilation heterogeneity. Further analysis allows washout abnormalities (rising phase III slope) to be localised to functional and anatomical regions.

Diffusive- and Convective-Dependent Inhomogeneity

Based on experimental models, Engel and Paiva described a series of processes that interact within the small airways(Engel 1985). As previously described, gas is conducted down airways to the gas exchange zone, at which point convection of gas (bulk flow) diminishes, to be replaced by molecular diffusion. There is a zone of overlap where both processes occur simultaneously, the so-called convection/diffusion front, which occurs at or around the entrance to the acinus.

To attempt to explain the phenomenon of the sloping alveolar plateau originating from gas within the acinus, as seen in cadaveric modelling studies, Paiva then conducted theoretical mathematical modelling studies to simulate gas mixing in a convenient single-branch model. This model shows two trumpets, representing the vastly widening overall airway cross-sectional area beyond any one branch at this level(Engel 1985). Disease creates asymmetry between parallel units, represented by unequal volume in Figure 18.

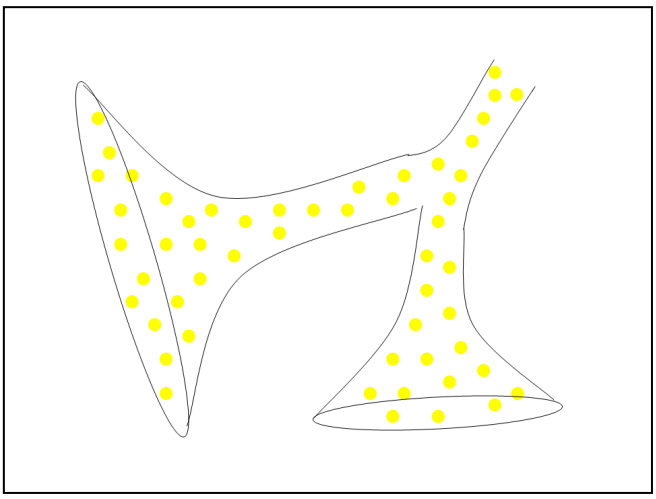


Figure 18 - diagram of 2-trumpet model (adapted from Engel et al[95]), representing a single airway branch point, and exponential increase of overall cross-sectional area in subsequent branches in the gas exchange zone18. This model demonstrates asymmetry of volume in disease.

When considering gas exchange during multiple breath washout of gas, Paiva simulated the relative contributions of convection, diffusion, relative unit volume and concentration gradients. At the convection-diffusion front, the interaction of these factors explains the rising alveolar slope in asymmetrical lung units. This is termed diffusion- and convection-dependent inhomogeneity (DCDI).

During a multiple breath washout test, there is theoretically complete equilibration of gas within all units. As the washout commences, the following processes take place in the 2-trumpet model according to Paiva's modelling. The points below refer to Figure 19.

1. Fresh air is inspired and passes by convection to the convection-diffusion front. In units of asymmetrical volume, these fronts are at different levels indicated by areas of blue shading.
2. The smaller unit is better ventilated, and gas dilutes more readily, because fresh gas has less distance to diffuse. This creates a concentration gradient between the parallel units at the end of inspiration.
3. As expiration commences, the concentration expired is lower than the mean concentration because initially gas molecules travel from the larger unit to the smaller down the concentration gradient. Only at the end of the breath does the concentration rise. This generates a 2-phase concentration change (indicated by the volume concentration graph at breath 1). With contributions from multiple units there is an alveolar slope at the first breath.
4. This back-diffusion of gas from larger to smaller unit is opposed by relatively larger convective flow out of the larger unit so that the concentration gradient remains between the units.
5. As the next inspiration commences the better ventilation of the smaller unit again results in an amplified concentration gradient at the start of expiration, leading to a larger change in concentration over the breath. This is seen as a steeper alveolar slope at breath 2, represented by the respective volume concentration graph.
6. In subsequent breaths the process continues, but any increased concentration gradient is balanced by the decrease in concentration difference between

inspired and resident gas as the alveoli are washed out. This is eventually balanced by breath 3 or 4 so that the concentration slope still exists but does not increase further. A "steady state" exists between inter-unit interactions and progressive dilution of gas by inspired breaths(Crawford et al. 1985).

To summarise, the increasing alveolar slope with subsequent breaths, seen during multiple breath washout of gas, is contributed to by interactions between parallel lung units at the convection-diffusion front (DCDI). After the steady state is reached, no further rise is seen and the slope remains constant with subsequent breaths.

Figure 19 summarises this process. The small volume concentration curves for each breath indicate the result from only 2 parallel units. This step change is not seen when multiple units of different heterogeneities contribute to the expired breath. At the lips, multiple units generate the characteristic alveolar slope.

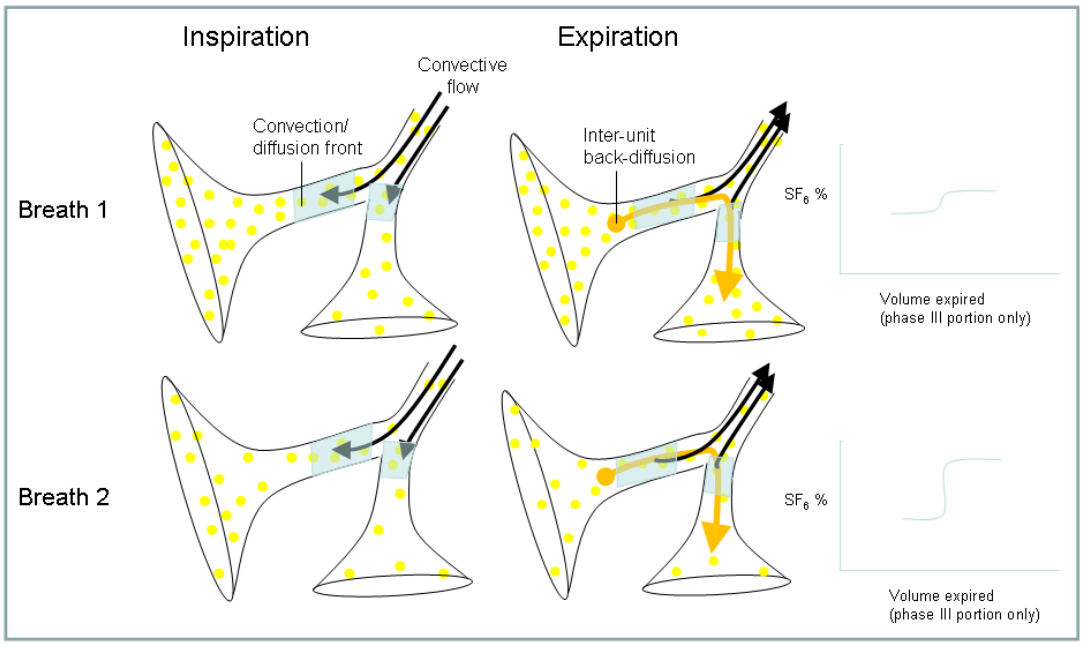


Figure 19 - Illustration of origin of intra-acinar alveolar slope in heterogeneous lung ventilation (see text).

Convection-Dependent Inhomogeneity

The rising alveolar plateau seen in disease states during multiple breath washout also has a contribution from asymmetry of ventilation between parallel units within convection dependent lung regions. Rather than being due to interactions between units, as in DCDI, this is due to sequencing of emptying.

Engel described the behaviour of gas as it is washes out of a simplified 2-compartment model(Engel 1985). As in the trumpet model above, this represents 2 parallel units, which could be at any level within the conducting airway region. This model is illustrated below (Figure 20), and indicates the simplified concentration change seen at the bifurcation of a single airway. The step-change shown is not seen at the mouth as multiple units contribute to an overall sloping concentration change over each breath.

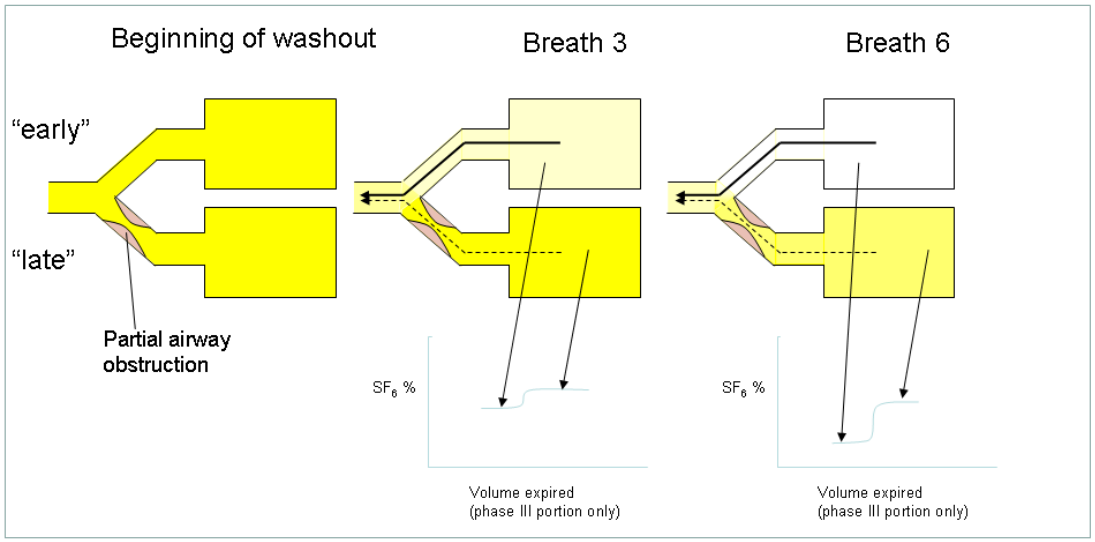


Figure 20 - Convection dependent inhomogeneity shown by sequential emptying of "fast" and "slow" lung units

In a completely homogeneous system, gas is distributed and washed out evenly between two parallel units as the washout commences. In that situation, the alveolar portion will be flat with each breath. If there is a poorly ventilated unit, the concentration will remain higher than the well-ventilated one. This poorly ventilated unit, which empties later in the breath, will increase the concentration at the end of the alveolar slope. This sequencing of parallel units progresses throughout the

washout depending on the degree of asymmetry. The conductive contribution to the alveolar slope becomes greater as the concentration difference between well and poorly ventilated units increases. This progression of the alveolar slope is termed convection dependent inhomogeneity (CDI).

S_{acin} and S_{cond}

Verbanck et al applied DCDI and CDI concepts to measurement of disease(Verbanck et al. 1998, Verbanck et al. 1997). These authors hypothesised that DCDI and CDI measurements could be considered independent measurements corresponding to airway abnormalities within specific anatomical lung regions. Because the convection-diffusion front is thought to be situated at, or near, to the entry to the acinus, DCDI was renamed S_{acin}, or slope progression corresponding to inhomogeneity in the acinar region. Similarly, CDI is a measure of inhomogeneity in the conductive airways, where convection primarily takes place. This was therefore renamed S_{cond}.

S_{cond} and S_{acin} are calculated in the following way(Verbanck et al. 1997):

- Conductive zone inhomogeneity (S_{cond}) contributes to progression of the phase III slope throughout the washout.
- Acinar zone inhomogeneity (S_{acin}) contributes to progression of the phase III slope only in the first 3-4 breaths.
- The progression of the normalised slope between lung turnover 1.5 – 6 is considered to be purely Scond.
- Sacin is standardised as being the slope of the first breath minus the back-extrapolated S_{cond} contribution (i.e. Scond x the turnover number of the first breath).

Performing multiple breath washout of nitrogen with 100% oxygen, Verbanck et al demonstrated a significant increase in CDI (Scond) following histamine provocation in both those who had a significant drop in FEV_1 and in those who did not. DCDI (Sacin), however, did not increase(Verbanck et al. 1997). In another study, Phase III slope analysis was performed in patients with Chronic Obstructive Pulmonary Disease (COPD). Increased Sacin was linked to measurement of acinar function (e.g.

diffusion capacity) and Scond was linked to measurement of conductive airway function (specific airway conductance, SGaw)(Verbanck et al. 1998).

The following example from a key publication(Verbanck et al. 1999) illustrates the effect of disease on S_{cond} and S_{acin} (Figure 21).

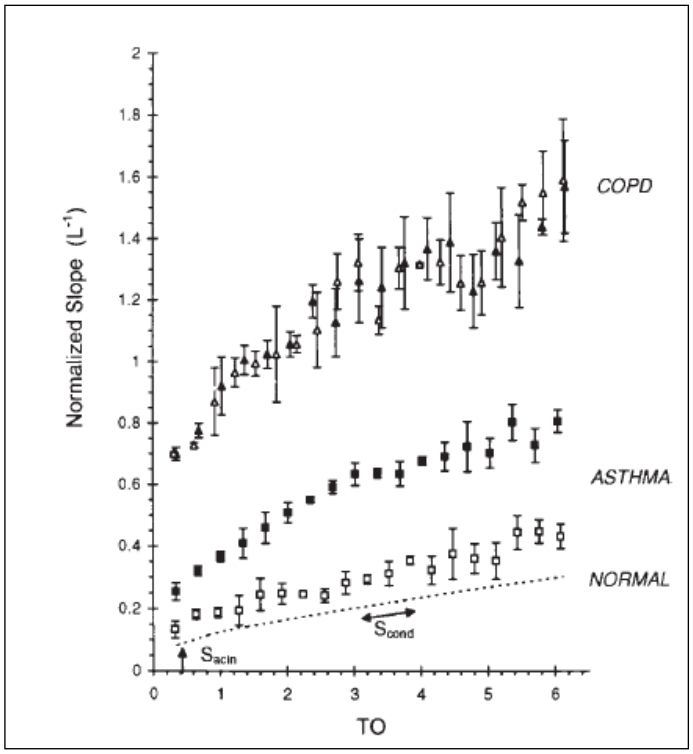
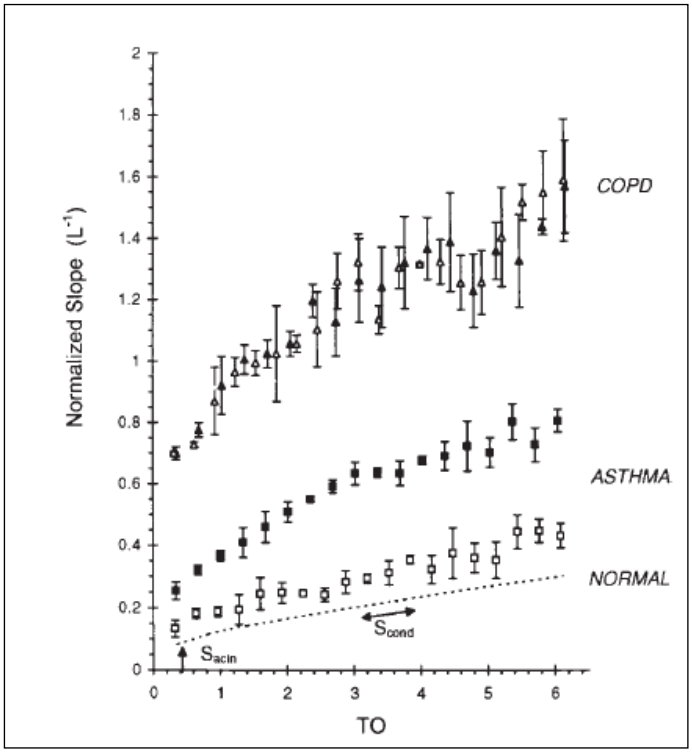


Figure 21 - This shows previously published normalised phase III slope values by Verbanck et al, averaged over three washouts, from three patients with different respiratory status. The dotted line indicates how Scond and Sacin¬ are derived. There is some progression of the slope in the healthy control patient, but the asthmatic patient shows an increased progression over turnover 1.5 -6 (increased Scond) but a similar Sacin (minus the Scond component of the first breath). The patient with COPD, however, shows both a greatly increased offset of the first breath (Sacin) and increased progression over TO1.5-6 (Scond).



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Scond and Sacin have also been measured in cystic fibrosis. It is thought that cystic fibrosis originates in the small conducting airways, rather than the alveoli or proximal airways(Mall et al. 2006). Gustafsson measured Scond and Sacin in children (>6 years old) and adults with cystic fibrosis. He showed they were both increased compared with healthy controls, indicating involvement throughout the airways(Gustafsson 2007). Similarly, Horsley et al published Scond and Sacin measurements, performed in our research department, in children (aged >4 years) and adults with CF(Alex R. Horsley et al. 2008). Both Scond and Sacin were significantly higher in CF patients compared with an age-matched healthy control population.

These studies may indicate that phase III slope indices are able to discriminate between inhomogeneity in the distal gas exchange zone and the more proximal conducting airways.

Limitations to S_{acin} and S_{cond}.

There are a number of published limitations to the use of S_{acin} and S_{cond}. These measurements originate from experimental inhomogeneous models, indicating that the behaviour of gas during washout can be applied to humans in health and disease, with abnormalities in measurements linked to specific anatomical regions.

Original studies conducted measurements in adults breathing 1-litre volumes with strict tidal volume feedback. As previously stated, this is not possible in children, leading to the development of tidal volume correction of Phase III slopes(Aurora et al. 2005b). Horsley et al also found that 1-litre breaths were difficult for adults with moderately to severe CF, inducing bouts of coughing(Alex R. Horsley et al. 2008). Tidal volume correction may eliminate the relationship between breath volume and Phase III slope over the whole washout, however, the breathing pattern in infants and young children can be highly variable(Aurora et al. 2005b). It was highlighted by Aurora et al that it has not been shown how Phase III slope indices are affected by changes of tidal volume and FRC during the measurement. There is a need for future research in this area(Aurora et al. 2005b).

The assumptions of SnIII indices are that they relate to the stated anatomical regions in all situations. It has not been shown whether abnormalities in S_{acin} seen in patients with CF are truly due to acinar inhomogeneity or whether widespread severe airway obstruction and structural changes alters the location of the convection-diffusion front.

S_{cond} is calculated from the progression of the Phase III slope, normalised for concentration through the early-mid portion of the washout. The mathematical alterations to the measurement mean the volume-concentration plot always rises to the equivalent of 1 over phase III. The alveolar slope is always preceded by phase I and II, meaning there is a maximum possible numerical rise over Phase III. This was demonstrated previously by Paiva when describing convection-dependent inhomogeneity(Paiva 1975), and confirmed by Horsley et al, who also showed this in

adults with moderate to severe CF. The value of Scond reached an asymptote of approximately 0.15 with increasing ventilation heterogeneity (LCI)(Alex R. Horsley et al. 2008). This had not been clinically demonstrated previously, presumably because patients with such severe ventilation heterogeneity had never been tested before. There was no linear rise in Scond with advancing lung disease, preventing significant correlations being made with other lung function measurements and reducing the applicability of Scond in advanced CF lung disease.

Finally, early experiments compared diffusion of gas in the small airways with gases of different densities and diffusivities. It is clear that the values of Scond and Sacin may be affected by the different gases and concentrations used between centres,. Early studies with nitrogen washouts can not necessarily be compared with SF6. Furthermore, different washin concentrations of Sf6 are used by different devices (0.2% vs. 4%). The effect of gas diffusivity and concentration may prevent wider use of Phase III slope analysis.

Phase III measurements are technically more complicated and less intuitive than LCI. They rely more on controlled even breathing; changes in respiratory rate or volume may invalidate results. As described below, data analysis is not standardised, making comparisons between research departments difficult. However, the underlying principles are potentially important for determining the effect of disease and management on specific anatomical regions.

Washout analysis procedure

Initial analysis of MBW performed with Innocor is done using custom-built offline software. This was provided by Professor Per Gustafsson (Goteborg, Sweden). The programme is adapted from that used for the mass spectrometer device. Prof Gustafsson devises the breath-capture algorithms, with programming done in collaboration with Eddie Bergsten, software engineer. This software is available to us, with occasional updates to improve performance and ease of use. This section describes the software, the analysis procedure and a reciprocal analysis experiment performed amongst three researchers. This was to confirm the suitability of our data processing standard operating procedure used in a multi-site study. There is also discussion of problems encountered during analysis that affect washout accuracy.

The Innocor device performs no internal analysis. Therefore raw data must all be analysed offline. The two elements of breath-by-breath washout analysis are flow signal, from which volume is derived, and concentration signal, from which gas washout can be followed.

Analysis software

The washout analysis software was written with testpoint programming software (Measurement Computing Corporation, www.measurementcomputing.com). The screenshot below shows the layout of the analysis screen.

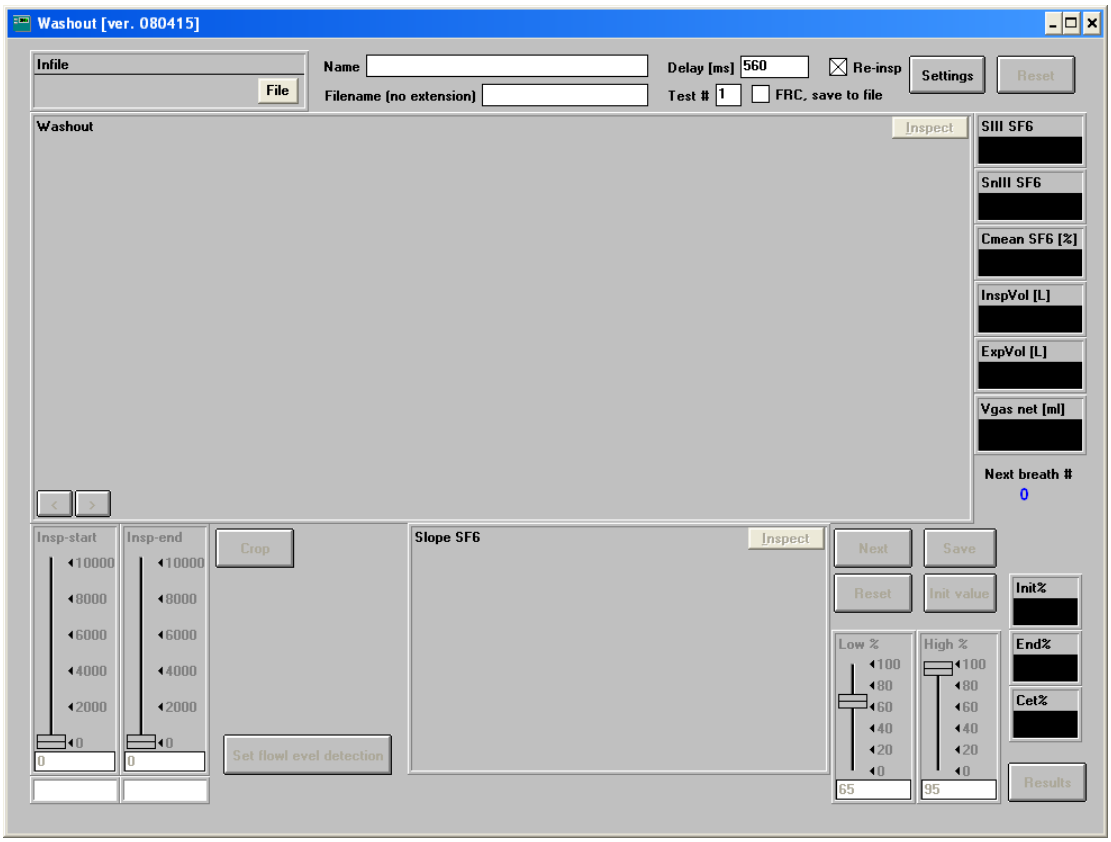


Figure 22 - Screenshot of washout software

A standard operating procedure (v1.2) has been developed by me with Alex Horsley and Clare Saunders (respiratory physiologist, UKCFGTC, London). To ensure results generated for a multi-site clinical study were comparable, this SOP deals with basic data analysis but also provides guidance for difficult washouts:

1. The exported Innocor raw data files (.ino) must be altered to select only time, flow and gas concentration. A programme produced by Innovision (Innofile converter) allows selection of these data, and conversion to a basic text file (tab separated values).
2. This text file is selected using the file button on the Washout screen, loading the flow and concentration traces.
3. The subject name and results filename is entered.

4. The flow gas delay value is measured and recorded at calibration, to align the flow and concentration signals.
5. Re-inspired gas can be incorporated into the analysis if required by selecting the relevant box (see technical adaptations chapter).

A sample test is shown here, with the obvious switch to washout (Figure 23).

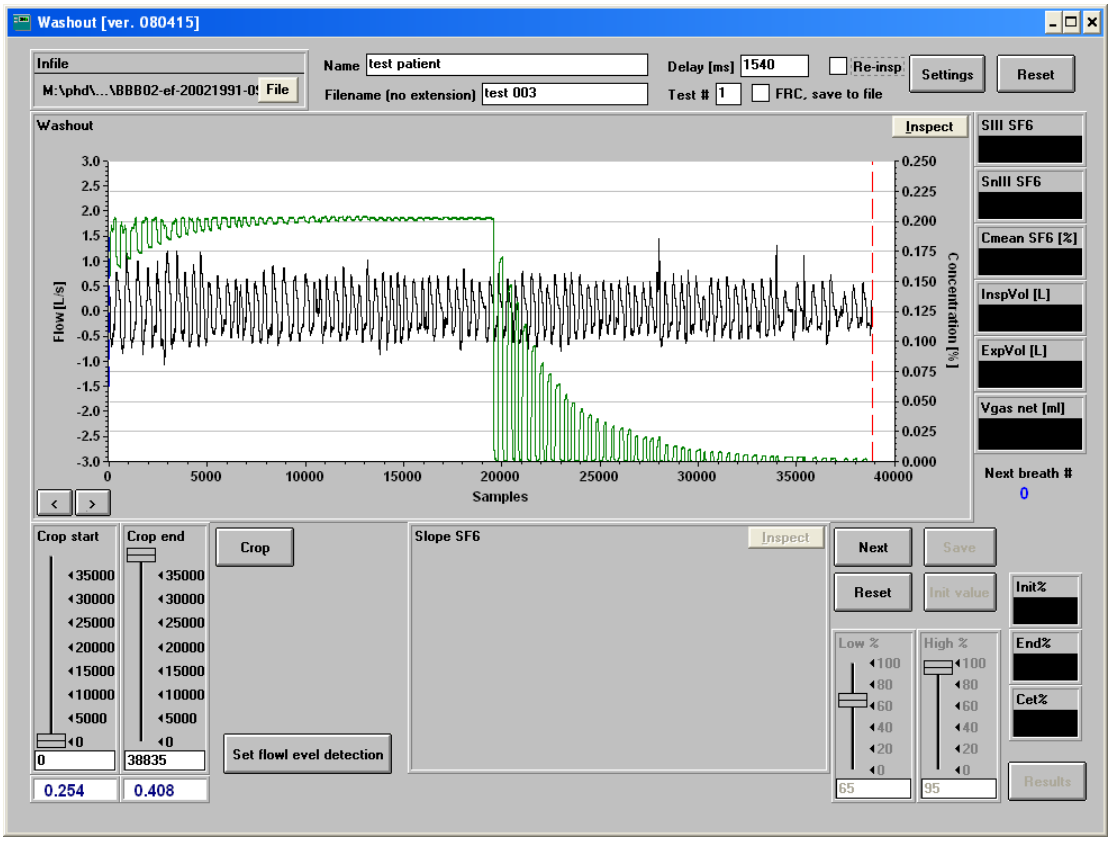


Figure 23 - end of washin, with switch to washout

6. The portion of the file of interest is the washout. Crop start and end are adjusted to focus on this portion.
7. The flow level to trigger a breath detection can be altered to take into account low flows (e.g. infants) or washouts with long breath pauses and small flow fluctuations that are not breaths. This adjustment is visual, rather than numerical, introducing a degree of subjectivity.
8. In "settings" the point which the end-tidal concentration is determined can be set. Conventionally, we measure from 15 sample points (150ms) back from the end of the breath, over an average of 15 sample points. This is reduced in younger subjects with shorter breaths. Counting 15 breaths back as also only

necessary in the Innocor system as the rising gas signal is speeded. This speeding alteration means the end of the breath includes part of the falling gas concentration. Counting back prevents underestimation of the end tidal concentration.

9. Once the trace is cropped to include only the washout, the "next" button advances one breath at a time. The first breath to be included in the analysis is the first expiration after the detachment of SF_6, circled below, and magnified in the following screen.

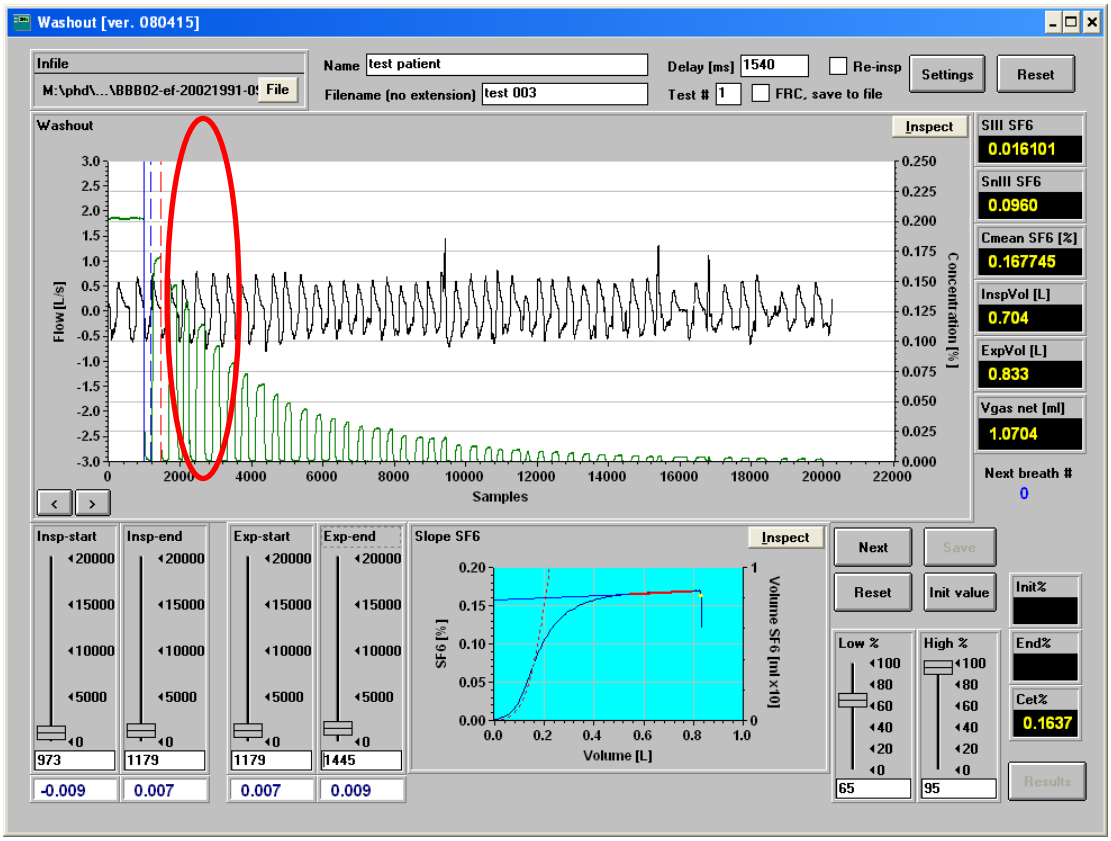


Figure 24 – complete washout shown with first breath captured

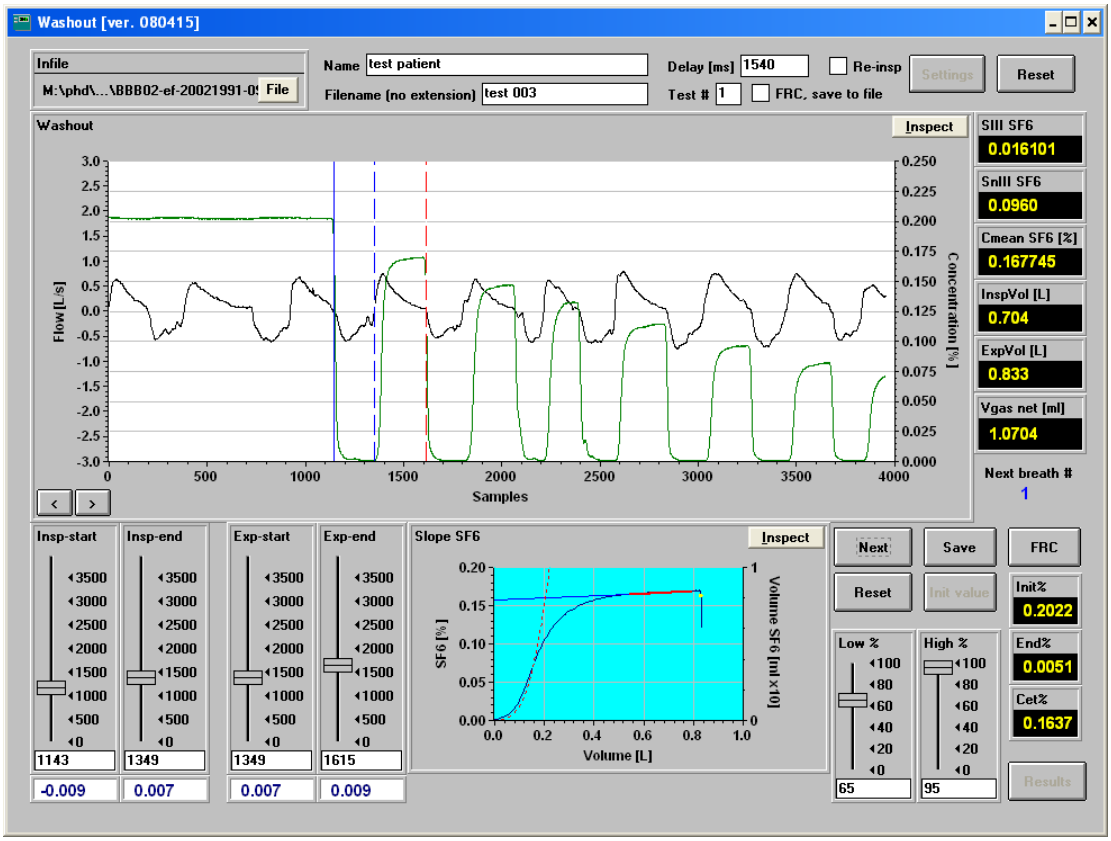
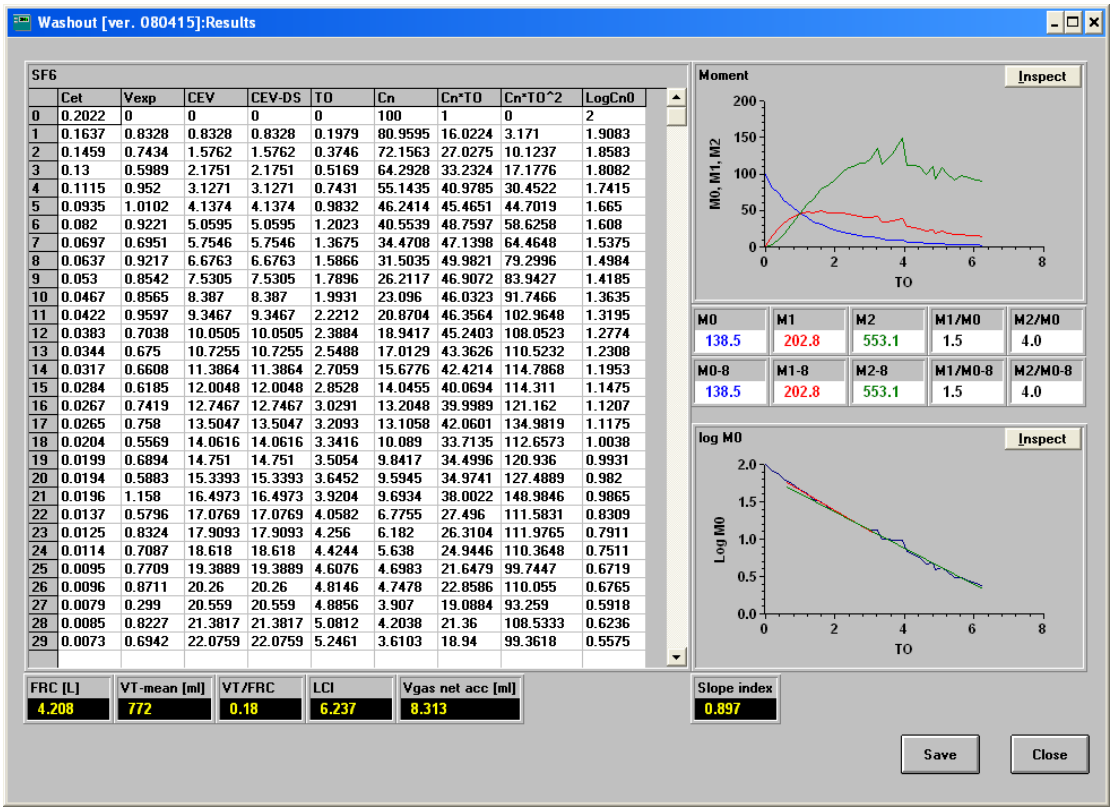


Figure 25 – analysis screen showing semi-automated breath capture.

10. It can be seen that the software is capturing inspiratory and expiratory phases with 4 points (4 sliders, “Insp-end” and “Exp-start” overlapped). These points are defined by a change in flow beyond a pre-determined threshold. As there are frequent small fluctuations in flow at the end of breaths, the software must determine a minimum flow rate that generates the start or end of a breath phase. It is important that the full breath is captured therefore manual adjustment of the threshold points is achieved by adjusting the sliders. The two numbers underneath each slider are the sample number and flow value.
11. Once the first breath is identified, the “init value” button is pressed to instruct the software to store values and calculate results.
12. “Next” is pressed repeatedly to capture each breath of the washout. The end point (End%) is calculated from 1/40th of the initial value (Init%). Cet% refers to the end-tidal concentration.
13. Each breath is shown in a volume concentration graph. This is used to ensure only a single complete breath has been captured, and to aid accurate Phase III

slope measurement. It is conventional that the alveolar slope should be measured from 65-95% of the maximum concentration of that breath. At low concentrations and smaller breaths this may need to be adjusted to visually cover the phase III portion.

14. Once the end tidal concentration is reached, the washout is complete. The "results" button is pressed and values are displayed (Figure 26).



	Cet	Vexp	CEV	CEV-DS	TO	Cn	Cn*TO	Cn*TO^2	LogCn0
0	0.2022	0	0	0	0	100	1	0	2
1	0.1637	0.8328	0.8328	0.8328	0.1979	80.9595	16.0224	3.171	1.9083
2	0.1459	0.7434	1.5762	1.5762	0.3746	72.1563	27.0275	10.1237	1.8583
3	0.13	0.5989	2.1751	2.1751	0.5169	64.2928	33.2324	17.1776	1.8082
4	0.1115	0.952	3.1271	3.1271	0.7431	55.1435	40.9785	30.4522	1.7415
5	0.0935	1.0102	4.1374	4.1374	0.9832	46.2414	45.4651	44.7019	1.665
6	0.082	0.9221	5.0595	5.0595	1.2023	40.5539	48.7597	58.6258	1.608
7	0.0697	0.6951	5.7546	5.7546	1.3675	34.4708	47.1398	64.4648	1.5375
8	0.0637	0.9217	6.6763	6.6763	1.5866	31.5035	49.9821	79.2996	1.4984
9	0.053	0.8542	7.5305	7.5305	1.7896	26.2117	46.9072	83.9427	1.4185
10	0.0467	0.8565	8.387	8.387	1.9931	23.096	46.0323	91.7466	1.3635
11	0.0422	0.9597	9.3467	9.3467	2.2212	20.8704	46.3564	102.9648	1.3195
12	0.0383	0.7038	10.0505	10.0505	2.3884	18.9417	45.2403	108.0523	1.2774
13	0.0344	0.675	10.7255	10.7255	2.5488	17.0129	43.3626	110.5232	1.2308
14	0.0317	0.6608	11.3864	11.3864	2.7059	15.6776	42.4214	114.7868	1.1953
15	0.0284	0.6185	12.0048	12.0048	2.8528	14.0455	40.0694	114.311	1.1475
16	0.0267	0.7419	12.7467	12.7467	3.0291	13.2048	39.9989	121.162	1.1207
17	0.0265	0.758	13.5047	13.5047	3.2093	13.1058	42.0601	134.9819	1.1175
18	0.0204	0.5569	14.0616	14.0616	3.3416	10.089	33.7135	112.6573	1.0038
19	0.0199	0.6894	14.751	14.751	3.5054	9.8417	34.4996	120.936	0.9931
20	0.0194	0.5883	15.3393	15.3393	3.6452	9.5945	34.9741	127.4889	0.982
21	0.0196	1.158	16.4973	16.4973	3.9204	9.6934	38.0022	148.9846	0.9865
22	0.0137	0.5796	17.0769	17.0769	4.0582	6.7755	27.496	111.5831	0.8309
23	0.0125	0.8324	17.9093	17.9093	4.256	6.182	26.3104	111.9765	0.7911
24	0.0114	0.7087	18.618	18.618	4.4244	5.638	24.9446	110.3648	0.7511
25	0.0095	0.7709	19.3889	19.3889	4.6076	4.6983	21.6479	99.7447	0.6719
26	0.0096	0.8711	20.26	20.26	4.8146	4.7478	22.8586	110.055	0.6765
27	0.0079	0.299	20.559	20.559	4.8856	3.907	19.0884	93.259	0.5918
28	0.0085	0.8227	21.3817	21.3817	5.0812	4.2038	21.36	108.5333	0.6236
29	0.0073	0.6942	22.0759	22.0759	5.2461	3.6103	18.94	99.3618	0.5575

Figure 26 – "results" screen after washout analysis.

15. The results of interest are FRC(L) and LCI. These are stored in a results file but can be viewed here to ensure consistent results are achieved over three washouts.

This procedure is semi-automated, in that breaths are identified according to an internal algorithm and washout values calculated and stored. However, because of frequent difficulties in washout analysis it is not appropriate to have a completely automated programme. The difficulties encountered during washout analysis are described later.

Once analysis is completed, raw washout measurements require correction for equipment dead space and Body Temperature, Pressure, Saturated (BTPS) conditions. This is done by adjusting the calculated volumes from the results in a spreadsheet. A full description of BTPS correction is given below.

Analysis development

Software updates

Washout analysis software is custom designed and alterations are made by Per Gustafsson and Eddie Bergsten, based on our recommendations. Each update has allowed greater ease of use in washout analysis, especially in difficult cases. Version 080415 was specifically developed for infant washouts, allowing adjustment of breath detection thresholds. Previous versions detected a breath phase with a fixed change in flow beyond zero. As infant flow rates are much lower, often no breaths were detected. Ver. 080415 is also able to magnify the flow display. This is a major improvement allowing closer infant and adult washout inspection.

Protocol development

When analysing washouts it became clear to us (those developing analysis SOP) that certain important issues must be addressed for studies in large numbers of subjects and those with prolonged washout periods (e.g. well-established CF lung disease). Due to the impending CF Gene Therapy Consortium clinical studies, a protocol was developed as follows.

1. SF_6 washin concentration and starting alveolar gas concentration.

FRC calculation depends on the difference between starting- and end- alveolar SF_6 concentrations. It can be difficult to completely wash in SF_6 to very inhomogeneous lungs and minor fluctuations are expected at equilibrium. The difference between inspired and expired concentration at equilibrium should be less than 2% (absolute change of +/- 0.004%). Even when achieving this, the concentration can drop slightly just before detaching so it is important that the true alveolar starting concentration (Cinit) is chosen, which may mean adjusting the marker.

2. End of Washout

The end of the washout is defined as the breath where the end-tidal SF_6 concentration (Cet%) is washed out to $1/40^{th}$ of the starting concentration. Clearly, this point will probably occur between two breaths. The agreed protocol was to save the 1^{st} breath where the Cet% was below $1/40^{th}$ and end the analysis if the next one is also below this value. It was important to realise that in prolonged washouts, the concentration can fluctuate around the $1/40^{th}$ point meaning a number of borderline Cet breaths may need to be saved before the end of the washout. Furthermore, small "breaths", caused by swallows or coughs near the end of the washout may prematurely end the analysis. Therefore, a minimum breath volume was chosen to end the washout, based on calculations by Aurora et al(Aurora et al. 2005b). Those less than 0.4L (8ml/kg in children) was chosen as the minimum volume required to count the end-tidal concentration at the end of a washout, based on the expected airway and equipment dead space.

3. Interruptions to flow

Volume of expired gas, calculated by integrating flow and gas concentration, can be affected by interruptions to flow.

a. Innocor flow meter re-zeroing.

The Innocor internal flow meter (as with all Pneumotach pressure transducers) converts a pressure signal, converting this to flow based on calibration. Small calibration inaccuracies lead to drift in flow over time. Innocor overcomes this problem by having a timed reset at regular intervals. This interval can be altered prior to testing, but if it occurs during the washout, 1 second of 100ml/sec is inserted in the data. The SOP deals with this by ignoring the reset portion, and including as much of the breath as possible. The re-zero can also be "flattened" by inserting an average flow reading in the raw data file following testing. Either solution is a compromise, as the true breath volume is compromised.

b. Subject flow interruptions

Relaxed regular tidal breathing is encouraged throughout the washout, however, subjects of all ages frequently exhibit pauses in the middle of, and between, breaths that confuse analysis.

Brief flow pauses trigger the end tidal marker, whereas examination of the gas concentration reveals a rise in gas concentration as the breath continues. Prior to the beginning and after the end of a breath, brief spikes in flow are often seen. These do not represent breaths as there is no rise in gas concentration but the software may measure a small expired volume. If the breath is of very small volume (<50ml) or if there is no rise in concentration the breath should be skipped. An example of a washout with flow interruptions is shown towards the end of this chapter (Figure 30 Figure 32).

c. Mis-identification of expiration

Pauses at the end of inspiration cause mild fluctuations in flow that may be interpreted as the start of a new breath. A feature of the software is that between breath phases, negative and positive flow is all counted as positive leading to a large measured expired volume prior to the true start of expiration. The expiratory start marker in this case must be moved to the true breath start.

BTPS correction

All reported lung volumes should be reported taking into account the changes that temperature, humidity and pressure make to gas volumes(Frey et al. 2000b). The principle behind this is that inspired gas at ambient pressure, temperature and humidity occupies a different volume in the lungs. Hence all volumes are corrected and reported at body temperature, pressure saturated (BTPS) conditions, rather than ambient conditions (ATP). Expired gas, while being almost at BTPS, tends to have a temperature of 30-35^{o}C, and is 98% saturated.

Correction of expired and inspired gas to BTPS is recognised as complicated and difficult to standardise. For the expired portion, the correction is small (2.6%, see below for equation). For the inspired portion during MBW, only the volume of gas re-inspired is of interest, not the full inspired breath. Gas is detected at the beginning

of inspiration, then the concentration is zero, as there is no SF6 in the ambient air. As inspired volumes are not of interest, only re-inspired gas, the correction would be for gas residing in the pneumotach at the end of expiration, gas that is still warmer than ambient, and still saturated. As time passes this gas within the pneumotach at the end of expiration is cooling and drying, therefore it is difficult to measure the humidity. It cannot be assumed to be ambient conditions.
In addition, there is no need to correct volumes to BTPS when calculating LCI, as this is a ratio, and errors in volume are assumed to be proportionally equal on either side of the equation. Therefore, only the reported FRC should be corrected.

The initial protocol in our department was derived by Alex Horsley, after discussion with other members of the research team.

$$conversion\ factor\ for\ FRC\ = \frac{ATP\ \rightarrow BTPS}{Expirate\ \rightarrow BTPS}$$

$$\approx 1.1(dependent\ on\ ambient\ conditions)$$

This equation multiplies FRC proportional to the conversion of ambient air to expirate conditions, rather than BTPS. The equation derived was part of the official analysis protocol, but was found to have a flaw when looked at again by Dr Nick Bell, clinical research fellow at the Western General Hospital, Edinburgh. Dr Bell and I discussed a better way of deriving the correction, only correcting expired gas to BTPS conditions, ignoring ambient conditions as they do not contribute to the condition of expired gas. Nick Bell derived a new equation, converting expirate volume to BTPS:

$$conversion\ factor\ for\ FRC\ = Expirate\ \rightarrow\ BTPS$$

$$= 1.026\ (fixed, as\ not\ affected\ by\ ambient\ conditions)$$

BTPS volume correction is derived from the following equation:

$$V2 = V1\ \times \left(\frac{T2}{T1}\right) \times \left(\frac{Pb - Pwv1}{Pb - Pwv2}\right)$$

Where V2 is BTPS volume and V1 is the expirate volume. T2 is BTPS temperature (K) and T1 is expirate temperature (K). Pb is the ambient air pressure. Pwv1 is the partial pressure of water vapour, derived from a lookup table and dependent on the relative humidity of the expired gas. Pwv is the partial pressure of water of BTPS gas, at 100% humidity.

This change in department protocol posed a difficult problem. As the clinical studies in chapters 4 and 5 had been completed, I decided not to repeat the analysis, bearing in mind the only affected measurement is FRC. LCI is not affected by BTPS corrections. Within each study, including others where older data was used, the same conversion equation was used.

This represents an error in analysis, but not affecting the primary outcome of any of the studies.

Intra-test repeatability

Washout tests are performed 3 times, the mean value of acceptable washouts generating the result. The above analysis issues, as well as leaks and true physiological variability, can produce outlying unacceptable results. Published guidelines state that an FRC value should be discarded if it varies by more than 10% of both other values(Beydon et al. 2007). This standard is repeated in a later consensus paper (ERJ, in press). 10% is a figure agreed by experts in the field, not based on experimental evidence. There was some discussion when preparing the consensus statement about the appropriateness of performing 3 washouts when 2 may suffice, given the time-consuming nature of the test. For the purposes of uniformity we performed 3 washouts in all subjects.

In addition to control on FRC limits, if an LCI differs by more than 20% of both others values that washout should be discarded. This was agreed for our SOP, recognising that some washouts can be greatly prolonged because of a change in breathing pattern or physiological variability in advanced disease. Accidental deviation from washin or washout protocol can cause variability if washin is incomplete or washout is ended prematurely. This usually means the washout is discarded.

Finally, there may high variability between all 3 washouts despite visible technical accuracy. This exceptional situation may allow an average of all 3 still to be accepted.

As will be seen in the subsequent chapters, the above variability rules result in discarding approximately 30% of washouts. In reality, while 3 washouts are performed, 2 is the minimum for analysis. There is no clear evidence base behind discarding washouts based purely on variability. In the absence of this evidence the current agreed standards are the only way to compare data across different centres. Future studies may provide a better way to decide on washout acceptability.

Reciprocal data analysis

The results of the following study were presented in the form of an electronic poster at the European Respiratory Society International Conference 2007(Macleod et al. 2007). The first 3 authors (Macleod, Horsley, Saunders) were involved in conception and execution of the study.

Background

The explanation of data analysis procedure and standardisation protocols is important if results performed between sites, by different researchers, can be compared(Stocks et al. 2000). In preparation for planned multi-site clinical studies (CFGTC) using the Innocor device to perform MBW measurements in patients with Cystic Fibrosis, and standard operating procedure was developed and tested to ensure inter-site consistency.

To validate the above standards, this experiment compared analysis of washouts across the 3 sites in question. Subjects with moderate to severe respiratory disease were selected as most likely to have prolonged and complex washouts and multiple examples of flow disruptions from patients.

Aims

To assess agreement of the standardised data analysis protocol, minimising variance during multi-site studies.

Methods

To compare results using the standardised analysis protocol, data were selected from washouts performed by 3 different researchers in a selection of subjects with CF from 3 respective study sites (Royal Brompton Hospital, London; Western General Hospital, Edinburgh; Royal Hospital for Sick Children, Edinburgh). Three washouts were selected from each subject, attending on one occasion as part of a separate clinical study; one which involved patients with CF attending on three separate occasions at the time of a respiratory exacerbation. All patients attended prior to starting and immediately following antibiotic therapy, with one more visit 2-5 weeks after completing antibiotics.

All washouts, performed in triplicate at each visit, were analysed independently by three researchers. Researchers are numbered 1,2 and 3. Mean results were calculated from three acceptable washouts independently by each researcher, with acceptability defined as in the above detailed methods explanation. Washouts were discarded where FRC differed by more than 10%, or the LCI differed by more than 20%, of both other washouts.

Washout results were analysed by calculating mean differences and 95% limits of agreement of FRC and LCI measurements between centres in pairs(Bland and Altman 1986). Bland Altman Plots were constructed to identify error between researchers in pairs. Analysis of variation compared mean values between three centres together. A p-value less than 0.05 is considered statistically significant.

Analysis and graphical presentation was performed on Minitab statistical software (Minitab, USA).

Results

10 patients were selected, from 3 different sites. Nine completed all three visits and one completed only two visits. Twenty-nine washout triplicates were therefore available for analysis. One triplicate was deemed unsuitable for analysis as we were unable to select at least two washouts within 10% of each other.

Table 4 shows patient demographics and baseline data at the start of antibiotic treatment.

	Subjects
Number	10
Male/Female	6/4
Mean Age	24
[range]	[12 – 43]
Mean (SD)FEV_1 %predicted	52.99 (14.01)
[range]	[37.8 – 86.8]
Mean (SD) FRC	2.24 (0.50)
[range]	[1.66 – 3.35]
Mean (SD) LCI	14.17 (1.07)
[range]	[10.13 – 18.02]

Table 4 - Basic patient demographics

Boxplots below (Figure 27) show the similarity of FRC and LCI measurements across the 3 researchers.

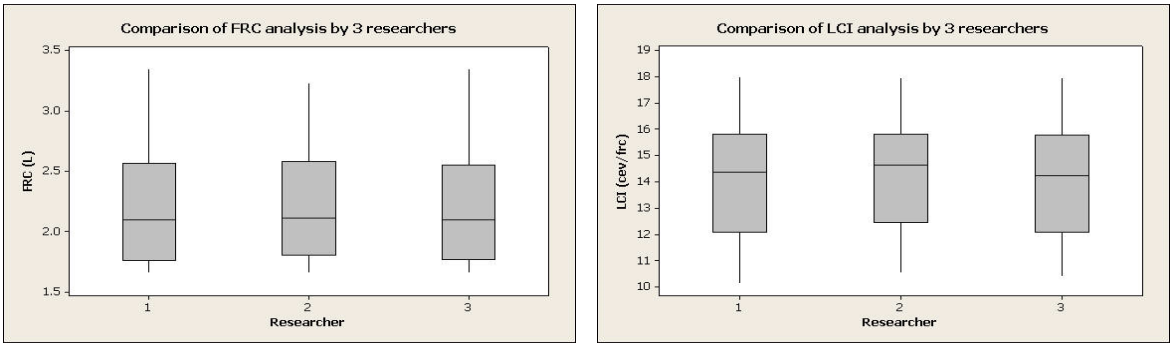


Figure 27 - comparison of mean FRC and LCI values calculated from 10 washout triplicates by 3 researchers. Box shows median line, with interquartile ranges.

Comparing pairs of researchers in turn, the mean differences in FRC and LCI are shown in

Table 5. The maximum difference in mean FRC was 330ml (12% of the mean value). The maximum difference in LCI was 0.99 (6.6% of the mean value).

	site 1 vs. site 2	
	mean difference	95% limits of agreement
FRC (L)	0.004	(-0.17 - 0.18)
LCI (cev/frc)	-0.005	(-0.61 - 0.60)
	site 1 vs. site 3	
	mean difference	95% limits of agreement
FRC (L)	0.001	(-0.08 - 0.08)
LCI (cev/frc)	-0.041	(-0.76 - 0.68)
	site 2 vs. site 3	
	mean difference	95% limits of agreement
FRC (L)	-0.003	(-0.15 - 0.15)
LCI (cev/frc)	-0.040	(-0.40 - 0.32)

Table 5 - Mean differences and limits of agreements. Sites compared against each other in turn.

To illustrate effect of magnitude of measurement (degree of abnormality) on difference between researchers, the following Bland-Altman plots were constructed (Figure 28Figure 29). These plot average measurement between 2 researchers for individual subjects against the mean of these 2 measurements. If the difference in measurements increases with higher or lower values, there may be a systematic bias. 95% limits of agreement (mean bias +/- pooled standard deviation) are shown on the following pages.

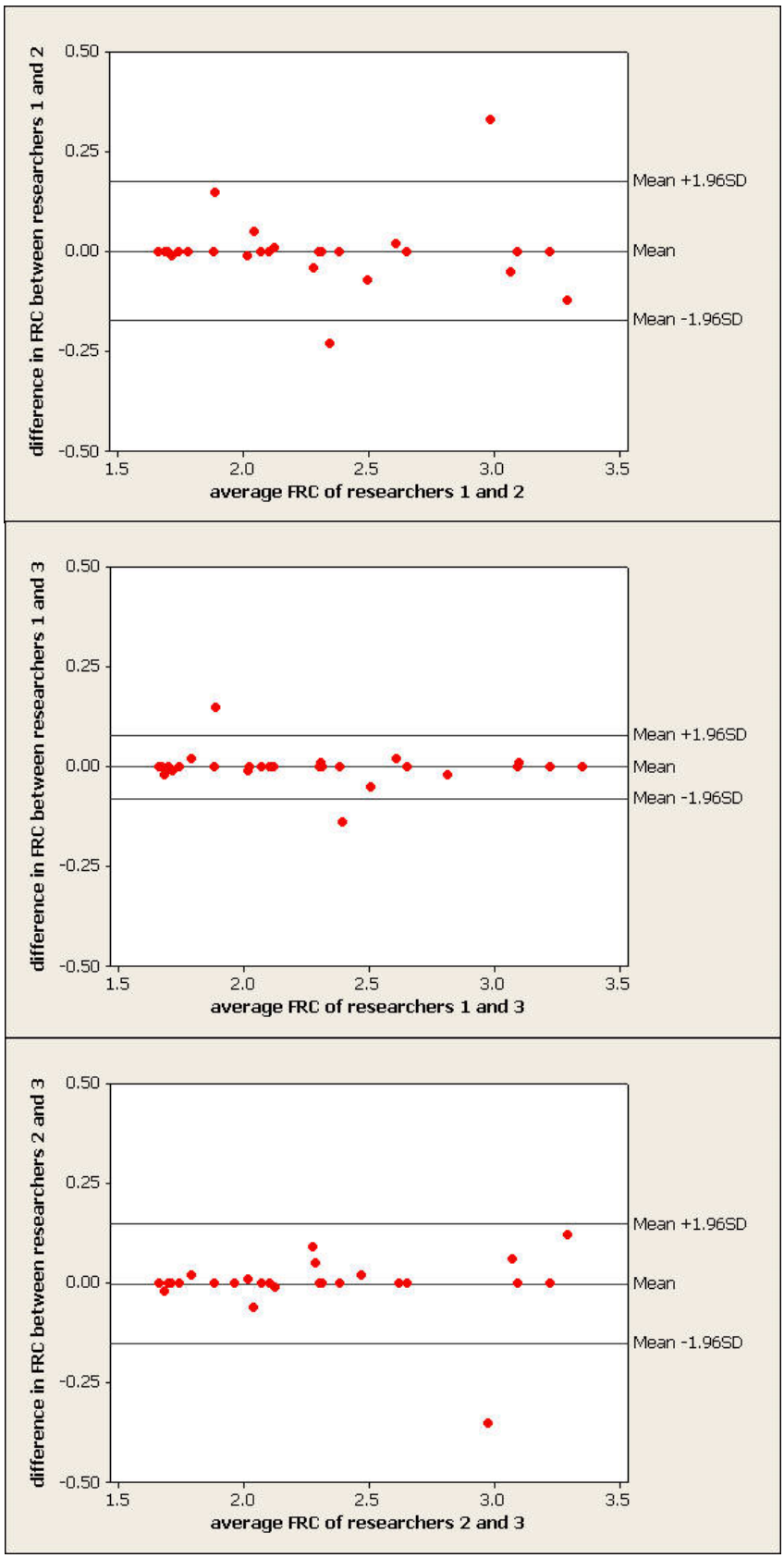


Figure 28 - comparison of mean FRC measurements between researcher (site) pairs. Individual plots are the average of paired measurements against the difference. Mean bias and 95% limits of agreement are shown by the horizontal lines

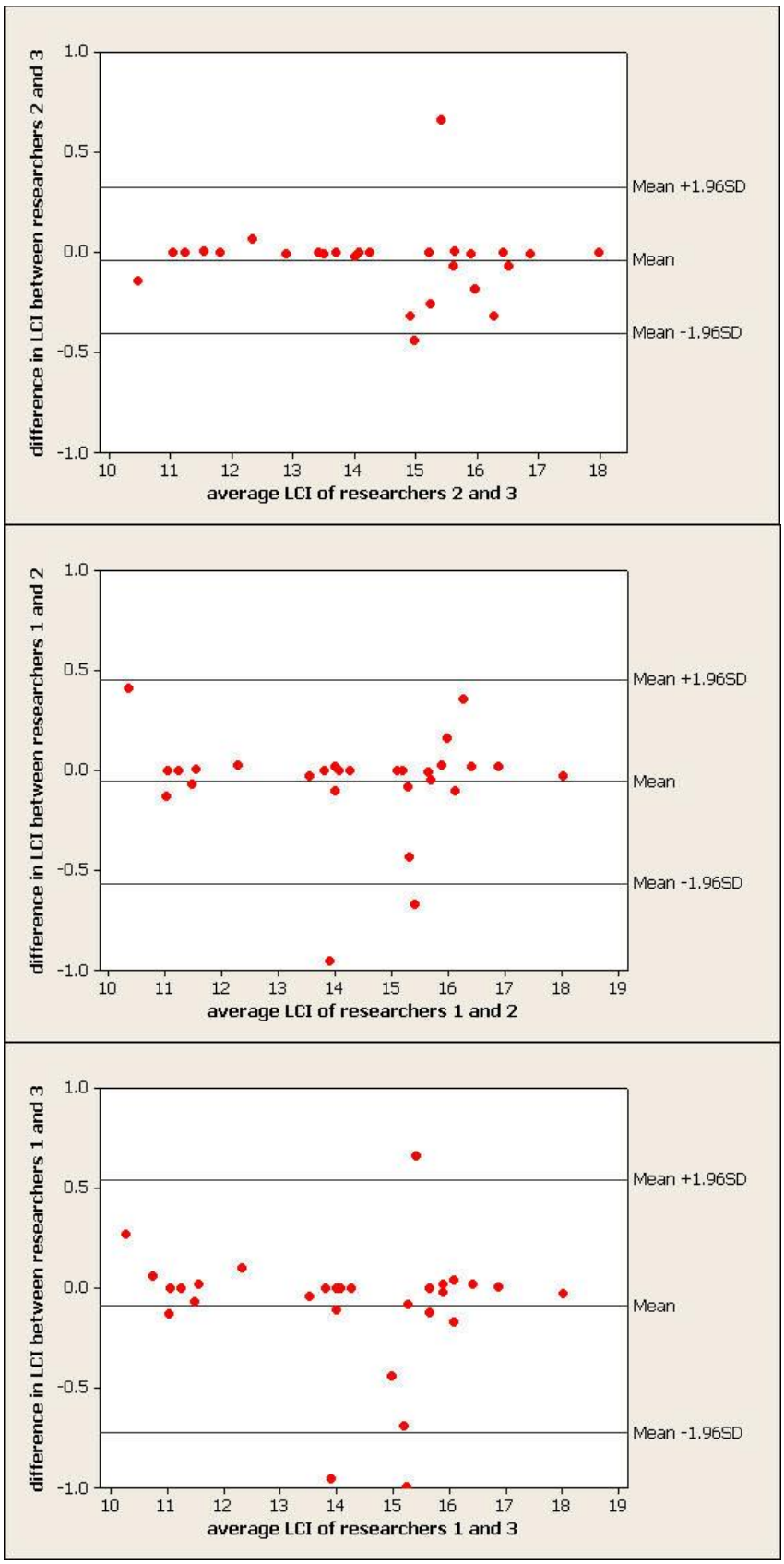


Figure 29 - comparison of mean LCI measurements between researcher (site) pairs. Individual plots are the average of paired measurements against the difference. Mean bias and 95% limits of agreement are shown by the horizontal lines

There was no evidence of overall systematic bias between researchers, although the 95% limits of agreement for FRC measurement pairs were higher when comparing researcher 2. This is not consistent, however, as when comparing LCI values, limits of agreement are higher when comparing researcher 1.

Comparing all 3 researchers by analysis of variation (ANOVA), there was no statistical difference ($p>0.05$). Importantly, this is not an adequate test for detecting differences between the same measurements performed by different researchers. It is included for completeness.

Conclusion

This small reciprocal study was designed to test a written MBW analysis protocol in preparation for a multi-centre observational study (see chapter 7). When comparing results of washouts in patients with CF, taken during a respiratory exacerbation, there was no evidence of systematic bias between researchers following the same protocol. While 95% limits of agreement were variable between researcher pairs, the differences in measurements were small. A maximum difference in FRC measurements of 300ml is rather high, but is an obvious outlier, looking at the Bland-Altman plot.

The patients in question were difficult to test. During an exacerbation LCI is likely to be raised from the baseline(Horsley et al. 2007) and therefore the washout period is longer. Also, patients are less well, and are likely to cough more or to feel effects of breathing through a device if slightly short of breath. This results in more episodes of shallow breathing, pauses and aborted washouts. These washouts are challenging to analyse as decisions must be made about which breaths to include and which to discard when very small. Very shallow breathing does not ventilate beyond the anatomical dead space and therefore does not contribute to LCI, although the CEV rises. Examples of irregular breathing patterns are shown in Figure 30-Figure 32.

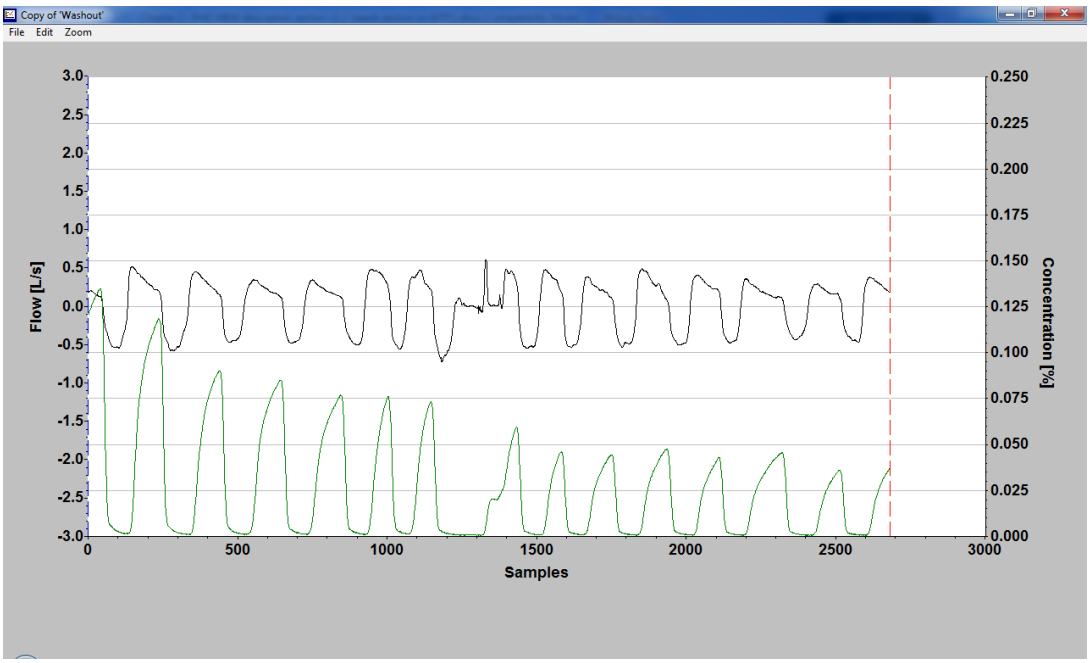


Figure 30 - Example of pause in breathing early in washout. This subject has paused during expiration, creating a spike with no gas washout.

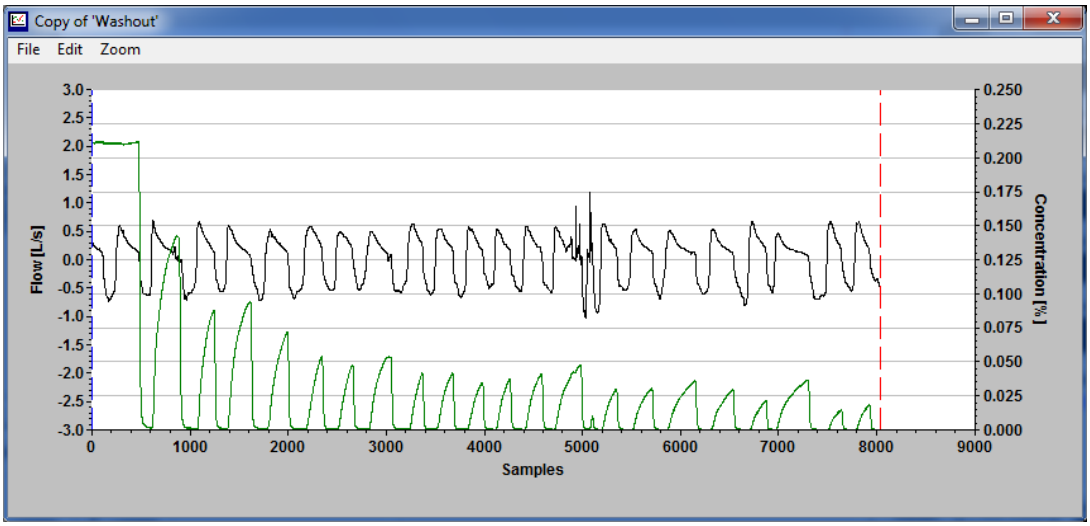


Figure 31 - Another example of erratic breathing shows a disrupted expiration and inspiration causing a small spike in gas concentration which is not a real breath

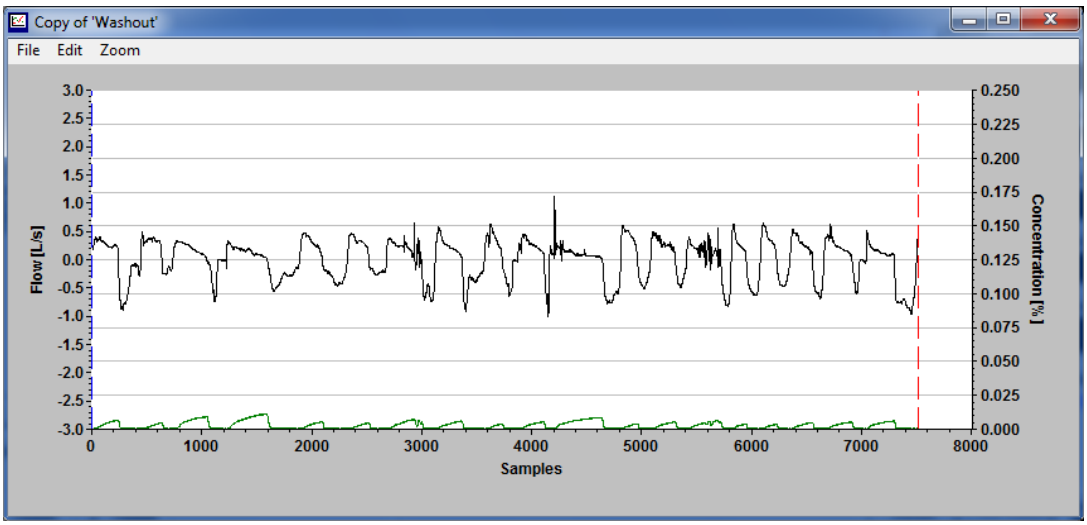


Figure 32 - This example shows very erratic breathing. Breaths are hard to capture accurately, leading to variability in defining the end of the washout.

This study was an attempt to validate a written analysis protocol, and results indicate close correlation when washouts are analysed by different researchers. The researchers were blind to the others' results until analysis was complete. In terms of equipment validation this is a strength of this study. It could be assumed that all researchers involved in writing a protocol will analyse the data in the same way, however this study shows that even with frequent erratic breathing and prolonged washouts the final results are very similar.

The patients were selected from an existing cohort and non-randomly. These subjects are therefore not representative of a wider population. This does not compare results before and after use of the protocol, and there is no attempt to test the protocol in a new set of researchers. A weakness of this study is that the protocol was written based on local experience and understanding of the test. There is no published article that explicitly details how washouts should be analysed. Indeed, many existing articles are in younger populations where the problems of advanced lung disease, exacerbations and prolonged washouts are not present. It is hoped the pending European Respiratory Journal "state of the art" document, due to be published in late 2012 will contain adequate detail to allow groups to analyse data in the same way. This will enable accurate comparisons between studies using different equipment and research personnel.

Learning points and contribution to thesis

This study is important in validating the Innocor system. In proposing Innocor as a suitable MBW device for multi-centre studies, data analysis between researchers is crucial. In preparing for the CFGTC "run-in study" (chapter 7), we used this data sharing and reciprocal analysis strategy to harmonise our analysis protocol. This helps to ensure everyone involved in analysis is fully taught and increases confidence in multi-centre data.

This study helped us as a research team to standardise the analysis protocol. As each step was discussed we were able to refine the technique and change parts that were not logical or sensible. In terms of this thesis, all the following chapters use the standardised protocol. The analysis protocol used by the CFGTC is under review, as automated software is now available. This changes many of the analysis steps, and allows swift comparison between different analysis methods. As this was only developed towards the end of this thesis, no automated software has been used in the thesis studies.

Chapter conclusion

This chapter has discussed the following topics:

- Physiological basis behind MBW measurement
- Differing method between devices
- Innocor specifications and comparison with different systems
- Innocor MBW equipment
- Data analysis and MBW indices of lung disease
- Standardisation of MBW analysis

This chapter has described in detail the multiple breath washout technique and derived ventilation heterogeneity indices. This builds upon previously published work in other institutions, as well as work completed in this department. As many of the concepts have been newly developed following experience in new populations (i.e. children in health and disease), there are no definitive evidence-based guidelines to follow. It is therefore important to understand the underlying pulmonary physiology and the resulting effects of disease prior to conducting measurements in a

novel device such as Innocor. Experience with MBW using Innocor has led to publication of local data analysis guidelines. These take into account the difficulties associated with MBW measurement in challenging populations – children and those with advanced lung disease. Clinical studies published in this thesis use the above methods and guidelines to validate Innocor in paediatric populations. As mentioned above, BTPS proved to be a problematic standardisation. The change in the official departmental method for BTPS correction was partly due to an oversight, and also due to poor application of scientific principles. It is important to recognise again that the primary outcome (LCI) was not affected by BTPS correction.

Development of a standardised method is important to be able to compare results between research sites. This method was successful in a comparison of washout analysis in adult CF patients between 3 researchers. Use of Innocor in a variety of clinical studies has highlighted areas of good performance, as well as limitations. Studies in novel population have also raised important questions about the physiological basis of standard washout indices in subjects with advanced lung disease.

Particularly in the area of phase III slope analysis, only limited populations have been studied previously. Analysis in children and adults with moderately severe CF lung disease has introduced important questions about the underlying assumptions behind Scond and Sacin. This is a challenge to the wider research community and may stimulate important discussions, leading to improvements. The MBW technique is simple and the basic measurements are intuitive to subjects and researchers. Development of standardised equipment and analysis methods is less simple. Consideration of equipment specifications, BTPS correction, effect of lung disease on washout accuracy and data analysis standardisation is an important part of Innocor validation. When aiming to adapt and validate Innocor for use in younger children and infants, all of these considerations have been re-visited and are discussed in the next chapters. This continued validation has the aim of demonstrating accuracy and suitability of Innocor for use in younger children and infants. This is important because the increasing interest in detecting and tracking lung disease from early life to adulthood, is limited due to lack of widely available equipment.

Chapter 3 - Introduction to clinical studies in older children

Introduction

The main aim of this thesis is validation of the Innocor device for use in children. At the beginning of this project, the Innocor device had already been adapted for use in adults and Alex Horsley, clinical research fellow for the UK CFGTC, was conducting some clinical studies. A small series of lung model experiments led by him showed that Innocor may be suitable for use in children as young as 5 years of age(A. R. Horsley et al. 2008).

At the time of starting my project, no children (<17yrs) had performed Innocor washouts. Approximately 5 children performed MBW to establish suitability of equipment prior to commencing the studies in this thesis, however the data were not used.

Clinical studies in age-specific healthy and disease populations allow comparison with other published data, and contribute to the understanding of lung physiology in airways disease. In terms of change in measureable lung function over time; many factors such as age, lung volume, disease progression, and physiological variability contribute to within-subject differences. LCI has the advantage of a stable reference range throughout life, and detecting change over time does not have to incorporate age and lung volume related factors.

As described in the main introduction, a growing volume of evidence supports the use of MBW in paediatric research(Robinson et al. 2009b). Clinical studies using Innocor are compared to published data, with the aim of validating Innocor for use in this age-group. In addition, examining the sensitivity of LCI to airway changes – induced short-term effects and longitudinal change – allows us to hypothesise on the suitability of Innocor LCI to wider research and clinical practice.

This introductory section aims to show how chapters 4 to 7 contribute to the larger topic of equipment validation. Whilst they are all clinical studies in their own rights, they also demonstrate a number of important validation principles, which will be summarised afterwards. The most basic over-arching question when considering equipment validation is: how accurately do Innocor MBW indices distinguish disease

from health, and how can this disease be measured over time on multiple occasions in large populations?

Validation aims

The validation principles to be answered in chapters 4 to 7 are listed in Table 6

		Key Validation Questions
General Issues		· Is the Innocor device acceptable to children? · Does the presence of lung disease affect test acceptability? · How well-suited is Innocor to larger studies with minimal expert guidance?
Healthy Controls		· Does Innocor use in healthy children demonstrate expected reference values? · Do individual values and calculated reference ranges change over time when measured in the same subjects? · Does supine posture affect LCI in healthy children?
Children with lung disease	Asthma	· In children with well-controlled asthma (mild disease), can LCI using Innocor demonstrate latent disease? · Can the use of short-term intervention in asthmatic demonstrate the sensitivity of LCI to airway changes?
	Cystic Fibrosis	· Can Innocor LCI detect airway disease in children with CF? · How variable are the results compared with healthy controls both within- and between- subjects? · Can active manoeuvres – physiotherapy and change in posture – induce LCI sensitive airway changes? · How repeatable are measurements in children with CF compared to healthy controls? · How sensitive is LCI to longitudinal change in disease as CF progresses?

Table 6 - aims of clinical studies

Contribution to thesis

The methods in the chapters that follow all refer to chapter 2, which describes the Innocor device and the multiple breath washout technique. Therefore, the methods section within each chapter is short and specific to that study. These chapters are also written as distinct articles, discussing contributions to the current scientific knowledge, as well as relevance to the thesis as a whole. The clinical chapters showing the contribution to thesis aims are show in Table 7.

Clinical Studies	Subjects	Contribution to thesis
MBW in children with asthma and healthy volunteers	Healthy Volunteers Children with Asthma	· Reference population · Detection of mild airways disease with LCI following acute intervention
Effects of physiotherapy on LCI in children with CF	Children with CF	· Detection of CF airways disease with LCI compared with healthy controls · Change in measurements following induced airway manoeuvre (physiotherapy)
Effects of posture on LCI and FEV_1 in health and disease	Healthy volunteers Children with CF	· Change in measurements after change in posture · Change in measurements after change in posture · Sensitivity of LCI to postural change and effect of disease on magnitude of change
Longitudinal change in LCI in children with progressive lung disease	Healthy volunteers Children with CF	· Long-term variability of LCI and effects of disease on long term measurements · Sensitivity of LCI to longitudinal change in disease severity – independent of underlying variability

Table 7 - contribution of thesis chapters to thesis aims

Chapter 4 - Multiple breath washout in children with well-controlled asthma and healthy volunteers

Introduction

Asthma is a common paediatric condition, with a variable course in childhood. Recently published evidence indicates the presence of clinical phenotypes, relating broadly to age at onset, presence or absence of atopy and length of time with symptoms(Stein et al. 1997, Goksor et al. 2006, Silvestri et al. 2003, Kiley et al. 2007, Sears et al. 2003). Whilst symptoms are controllable with treatment leading to minimal symptoms in many cases, there is evidence that some symptom-free individuals have ongoing evidence of airway obstruction and inflammation(van den Toorn et al. 2001, Warke et al. 2002).

Asthma severity is conventionally assessed by spirometry (FEV1) and symptom control. Recent evidence suggests FEV_1 may be insensitive to airway obstruction in the small airways, leading to some with normal lung function and well-controlled symptoms but ongoing airway inflammation. This may be associated with the development of airway remodelling, leading to the observed increased decline in lung function in symptom-free adults with a previous diagnosis of asthma compared with disease-free individuals(Edwards et al. 2003).

Current opinions suggest that spirometry is able to detect airway obstruction and reversibility in the medium to large airways, but may be insensitive to subtle changes in the distal airways (Macklem 1998, Gustafsson 2005). As previously stated in this thesis, young children also have difficulty in performing spirometry as it requires cooperation and learned techniques. Ventilation Heterogeneity, measured by Lung Clearance Index, is a sensitive and repeatable measurement suitable for use in children, and is increasingly recognised by research groups as a measure to detect and track lung disease in CF.

At the time of planning this study, LCI had not been similarly studied in children with asthma. Since then, a study in adults with asthma measured ventilation heterogeneity before and after treatment with inhaled corticosteroids, which demonstrated residual ventilation heterogeneity (measured by Scond and Sacin) independent of inflammation(Downie et al. 2007). It was postulated that this

abnormality represents airway remodelling, although there was no direct link made with bronchial biopsy results. This is important to those conducting MBW studies, as abnormalities in ventilation heterogeneity may be surrogate measures of subtle airway wall changes (remodelling). Downie et al suggested that ventilation heterogeneity in adult asthmatics is independent of airway inflammation(Downie et al. 2007), such that fraction of exhaled nitric oxide (FeNO), a commonly used measure of asthma control, may not be able to detect changes associated with airway remodelling.

Only one study has previously assessed ventilation heterogeneity in children with asthma(Gustafsson 2007). LCI and FEV_1 improved post bronchodilator, but remained significantly different from controls. Children in this cohort had a low baseline FEV_1 and hence would not be considered well controlled by current standards. No study had measured ventilation heterogeneity in children with well-controlled asthma to look at subtle abnormalities, possibly not detected by spirometry. The majority of children with asthma have well-controlled and stable disease. It is expected that children who have an improvement in FEV_1 following bronchodilator may have a similar change in LCI. In addition, given the increased sensitivity of LCI compared with FEV_1 in other disease states, LCI may be a more suitable measure of asthmatic airways disease, given its ability to detect changes in smaller airways, than FEV_1.

The aims of this study of LCI in asthma were:

- Measurement of ventilation heterogeneity in children with well-controlled asthma attending out-patient clinics, compared with FEV_1 and FeNO.
- Assessment of acute response after administration of inhaled bronchodilator.
- To obtain the first MBW measurements in a healthy control paediatric population using the Innocor device.

The study was designed in conjunction with Alex Horsley, who completed a similar study in adult patients. Abstracts of the data I collected were presented as a poster at the American Thoracic Society International Conference, 2007 and as an oral presentation at the British Thoracic Society Winter Meeting 2007. The data were also

published in Thorax in 2009(Macleod et al. 2009). Healthy volunteer data were also used in other publications for which I am an author(A. R. Horsley et al. 2008, Alex R. Horsley et al. 2008).

In addition to the clinical research applications, this study is an important part of the wider theme of Innocor validation. The research questions this chapter addresses are as follows:

- Do the measurements obtained in healthy control children and adolescents, as well as those with disease, correspond with previously published data?
- Is Innocor an acceptable system for studies in this age-group and may it have an advantage over other devices for measurement of ventilation heterogeneity?

Methods

Patients

Asthmatic patients, aged 5-16 years, were recruited from those attending the Royal Hospital for Sick Children specialist asthma clinic. Age-matched healthy volunteers were recruited from patients attending for follow-up of stable upper-limb fractures as well as children of hospital staff.

Inclusion criteria were:

Children with asthma

1. Known asthma diagnosed by a consultant respiratory paediatrician.
2. No previous history of severe asthma requiring admission to intensive care.
3. No increase in symptoms or any exacerbation requiring increased use of inhaled bronchodilator or systemic steroid treatment in the four weeks prior to testing.

Healthy volunteers

1. No history of recurrent wheeze, pertussis or tuberculosis.
2. No previous diagnosis of asthma or previous use of asthma medication.
3. No prematurity (<34 completed weeks gestation).
4. No neuromuscular weakness or bone disease likely to affect respiration.
5. No congenital cardiac disease requiring medication.

Study design

Patients with asthma attended on two separate occasions one week apart. All patients took normal preventer medication on the day of testing but were asked to withhold short-acting $beta_2$ agonists for six hours prior to testing.

Each visit consisted of completing multiple breath washout (MBW) and standard spirometry before, and 20 minutes after, inhaled salbutamol (200mcg metered dose inhaler (MDI), Allen and Hanburys, GlaxoSmithKline (GSK), UK) or placebo (propellant only MDI, Allen and Hanburys, GSK, UK). In addition, fraction of exhaled nitric oxide was measured at each visit after the first spirometry assessment (Figure 33).

Both salbutamol and placebo were delivered via standard spacer (Aerochamber plus, GSK, UK). MDIs were blinded by covering and labelling. An extra covering was used to disguise the difference in size and weight of placebo and active MDIs. Labelling was done in private by an independent person. Code was sealed in an envelope, with un-blinding only after washout analysis was complete.

Patients were administered salbutamol or placebo in a randomised, double blind manner, with the alternative intervention given at the second visit to ensure variability in tests was valid. Salbutamol and placebo MDIs were masked by an independent person. Order of intervention was determined by sealed, sequentially numbered, opaque envelopes, also prepared independently of the study team.

Healthy volunteer subjects did not receive any intervention and simply attended on one occasion to complete MBW, spirometry and FeNO.

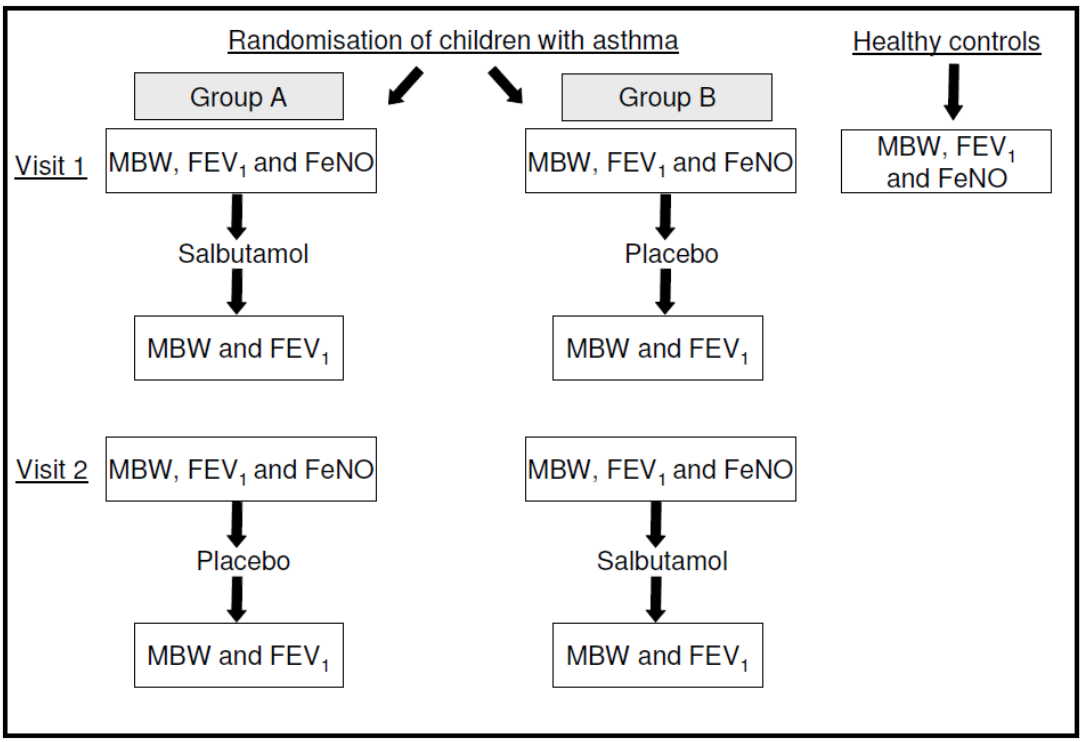


Figure 33 - Summary of study protocol

Multiple breath washout

MBW was conducted as per the method described in chapter 2. In children with asthma, three washouts were completed both before and after salbutamol or placebo. 6 washouts in total. Healthy volunteers completed 3 washouts in total.

A mouthpiece was used throughout. Most children were able to hold the whole mouthpiece inside their mouth. Occasionally, younger children could only complete washouts holding the inner part of the mouthpiece with their lips. This pragmatic solution for children with smaller mouths did not greatly alter the equipment dead space but occasionally caused visible leaks. Therefore careful observation was needed.

LCI, FRC, S_{cond} and S_{acin} are all quoted as the mean of at least two reproducible measurements out of three washouts. As a quality control measure, washouts were excluded if the measured FRC differed by more than 10% from both other repeats(Beydon et al. 2007).

Spirometry

Spirometry (Easyone, nnd, Sweden or Micromedical, UK) was performed according to ARTP guidelines('Guidelines for the measurement of respiratory function. Recommendations of the British Thoracic Society and the Association of Respiratory Technicians and Physiologists' 1994). Measurements were performed standing up, without a noseclip, to conform the protocol to that used in adults. A noseclip is recommended in more recent guidelines(Miller et al. 2005b). Three reproducible measures (defined according to ERS/ATS standards(Miller et al. 2005b)) were required for a satisfactory result, with the best of three recorded. This definition ensures results are reproducible, with each FEV_1 and FVC required to be within 150ml of one another (or 100ml if FVC< 1litre).

Standard deviation scores (z-scores) were calculated from the reference population residual standard deviation(Rosenthal et al. 1993).

The Easyone is now a widely used device even in children. In published studies it has performed well in comparison to standard respiratory lab devices in children aged 5 years and over(Mortimer et al. 2003, Coates 2005). It does not have a visual incentive display, as is common in paediatric devices, in the standard setup. This is an option with additional software but was not purchased for this study.

Exhaled nitric oxide

Fraction of expired Nitric Oxide (part per billion) was measured using the online NioxMino analyser (Aerocrine, Sweden). This device requires the child to breathe in to full vital capacity through a mouthpiece, then to breathe out steadily against resistance. A single acceptable measurement was performed with flow rate 50 +/- 5ml/s, exhalation time 6seconds, according to ATS standards('ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005' 2005). An upper limit of 20ppb is considered abnormal.

This manoeuvre can be difficult to perform for some children. No display is given to the child with the Niox Mino. A light is shown but regulation of expired flow, especially after performing forced expiratory manoeuvres, is difficult for some.

Statistical analysis

Data were analysed using Minitab version 14 statistical software (Minitab Inc., USA).

Data are presented as mean and standard deviation unless otherwise stated. Student's T-test for parametric data and Mann-Whitney Test for non-parametric data were used for comparison of groups and effect of treatment. Correlations between two measures were assessed with Pearson's correlation coefficient. Non-parametric paired comparisons were analysed using the 2-sample Wilcoxon Signed Rank Test.

P values less than 0.05 were considered significant.

Ethical approval

Prior approval was given by the Lothian Research Ethics Committee (REC: 06/S1104/29) and Lothian NHS Research & Development.

Summary of analysis

- Description of population demographics.
- Comparison of measurements in healthy volunteers and children with asthma.
- Correlation of LCI with other measurements.
- Comparison of change following salbutamol vs. placebo.
- Comparison of measurements following salbutamol vs. healthy control single measurements.

Results

Subjects

Thirty-one patients (mean age 10.6 years, range 5-15) and 29 healthy volunteers (mean age 11.1 years, range 5-16) were recruited. Five asthmatic patients withdrew after one visit (two suffered exacerbations and three were unable to find time to return).

All patients were taking regular inhaled corticosteroids at the time of testing. Treatment regimens according to BTS/SIGN guidelines for asthma management('British guideline on the management of asthma' 2003) are shown in

Table 8. 58% of patients had evidence of atopy (positive skin-prick testing to aeroallergen with a history of hay fever and/or eczema).

BTS/SIGN management step	Regimen	%
Step 2	regular inhaled corticosteroids 200-400mcg/day	6%
Step 3	regular inhaled corticosteroids 200-400mcg/day with long acting ß$_2$ agonist (LABA) or Montelukast	68%
Step 4	regular inhaled corticosteroids >400mcg /day +/- LABA or Montelukast	26%

Table 8 - Distribution of management regimes of children with asthma

Baseline data

Patient demographic characteristics and baseline measurements from the first attendance in the study are shown in Table 9. Groups were matched for age, sex and height. Mean (SD) baseline FEV_1 z-score at the first visit was not statistically lower in the asthmatic patients compared with controls (-1.09 (1.28) vs. -0.70 (0.88), p=0.16). Both groups were within the normal range (+/- 1.96SD). There was also no difference in median [range] FeNO (ppb) between asthmatics and controls (14 [5 – 300] vs. 18 [3 – 50], p=0.63).

Mean (SD) baseline LCI was higher in the asthmatic group than controls at visit 1 (6.69 (0.91) vs. 6.24 (0.47), p=0.02) indicating greater overall ventilation heterogeneity. There was a trend towards higher S_{cond} in the asthma group, though this was not significant (0.026 (0.02) vs. 0.017 (0.02), p=0.06). There was no difference in S_{acin} between the groups (0.14 (0.02) vs. 0.12, (0.06) p=0.23). There was no statistical difference in mean tidal volume, FRC or dead space (Fowler method) to tidal volume ratio (Vd/Vt).

There was no correlation between age and either LCI, S_{cond} or S_{acin} in healthy volunteers or asthmatics. Increased LCI did not correlate with increased steroid dose or BTS step of asthma treatment.

		Healthy Controls	Asthmatic Patients at first visit baseline	p
Number		29 (28 for S_{cond} and S_{acin})	31	
Mean age (yrs)		11.1	10.6	0.54
[range]		[5 - 16]	[5 - 15]	
Sex (male/female)		18/11	17/14	
Atopy	+ve skin prick testing to aeroallergen	-	58%	
	Eczema	-	16%	
	Hay fever	-	71%	
Mean Height (cm)		148	144	0.38
[range]		[118-180]	[110-186]	
Median FeNO (ppb)		18	14	0.63*
[range]		[3-50]	[5-3 00]	
Mean (SD) FEV_1 z-score		-0.69 (0.88)	-1.09 (1.28)	0.16
[range]		[-2.64 – 1.28]	[-4.00 – 1.82]	
Mean (SD) LCI (CEV/FRC)		6.24 (0.47)	6.69 (0.91)	0.02
[range]		[5.14 – 7.05]	[5.49 – 9.46]	
Mean (SD) S_{cond}, Vt corrected		0.017 (0.02)	0.026 (0.02)	0.06
[range]		[-0.03 – 0.06]	[-0.01 – 0.09]	
Mean (SD) S_{acin} Vt corrected		0.12 (0.06)	0.14 (0.02)	0.23
[range]		[0.02 – 0.29]	[0.05 – 0.40]	
Mean (SD) Tidal Volume (L)		0.55 (0.24)	0.45 (0.17)	0.07
[range]		[0.19 – 0.99]	[0.23 – 0.80]	
Mean (SD) FRC (L)		2.14 (1.02)	1.91 (0.78)	0.31
[range]		[0.80 – 4.51]	[0.85 – 3.82]	
Mean (SD) Dead Space to Tidal Volume Ratio (Vd/Vt)		0.27 (0.06)	0.27 (0.05)	0.89
[range]		[0.13 – 0.37]	[0.17 – 0.36]	

ICS, inhaled corticosteroid. LABA, long-acting beta$_2$ agonist

**Mann-Whitney non-parametric test.*

Table 9 - Subject characteristics and baseline measurements at first visit

At visit 1, pre-intervention, there was no correlation between LCI and FEV_1 z-score in asthmatic patients (r^2 = 0.11, p=0.08). Figure 34 shows relationship between baseline FEV_1z-score and LCI. While some subjects have a low FEV_1 and normal LCI, others have a raised LCI with normal FEV_1.

There was a significant correlation between LCI and S_{cond} (r^2=0.41, p<0.001) but not between LCI and S_{acin} (r^2=0.11, p=0.07). This relationship between LCI and S_{cond} was maintained at each time point on both visits. There were no significant correlations between FeNO and any of the measured physiological variables.

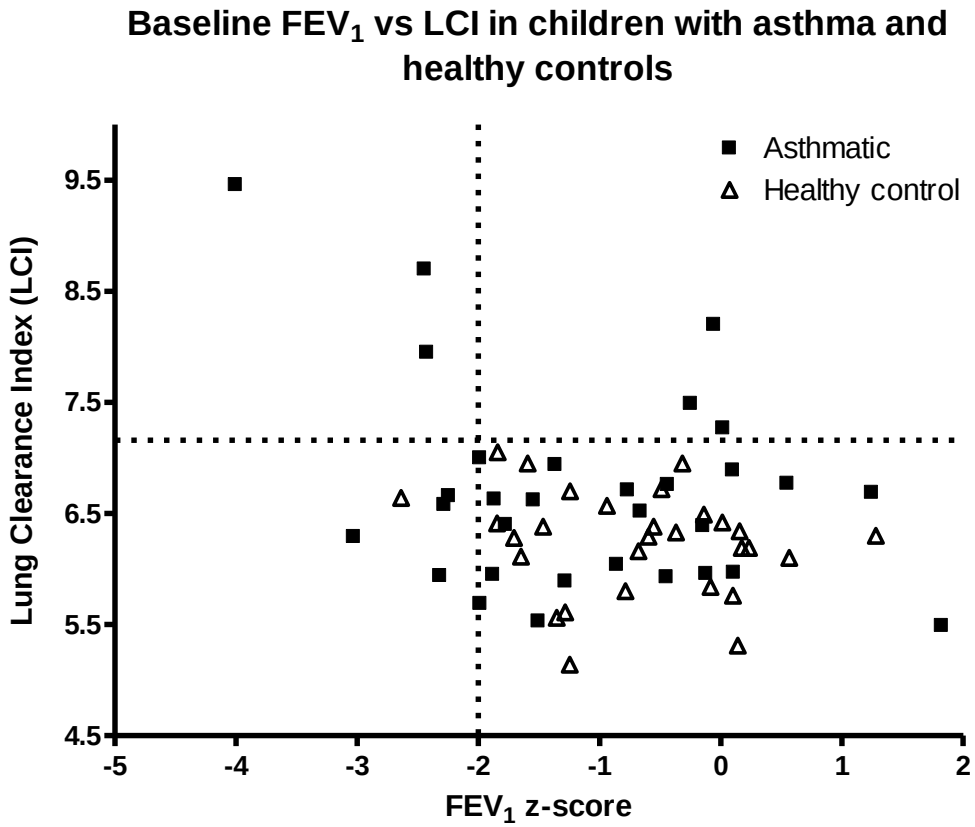


Figure 34 - Baseline characteristics FEV_1 vs. LCI in children with asthma and healthy controls. Dotted lines indicate limits of normality. LCI limit calculated from mean +1.96SD of healthy control population.

To assess repeatability of measurements in asthmatic patients, within subject coefficient of variation (within subject standard deviation divided by population mean, CV) was calculated for FEV_1 and LCI before and after placebo (4.17% and 5.39% respectively).

Salbutamol intervention

Following salbutamol administration there was a significant increase (6%) in mean (SD) FEV_1 z-score (-1.26(1.25) to -0.93(0.23), p=0.03) indicating additional reversibility despite patients having optimal control (no recent exacerbations and no increase in symptoms). No change was seen post-placebo (-1.04(1.14) to -0.97 (1.17), p=0.5). There was no significant change in mean LCI, S_{acin} or S_{cond} following either salbutamol or placebo (p>0.05). (* Significant *difference in post-salbutamol value compared with healthy controls (p=0.01)*

Table 10No significant ***differences***

Table 11)

Mean (SD) FEV_1 z-score after salbutamol did not differ significantly from healthy controls (-0.93 (0.23) vs. -0.69 (0.88) p=0.38). Mean (SD) post-salbutamol LCI remained significantly higher than controls (6.64 (0.69) vs. 6.24 (0.47), p=0.01) indicating residual overall ventilation heterogeneity despite improvement in FEV_1. Compared with controls, there was no difference in post-salbutamol S_{cond} (p=0.54) or S_{acin} (p=0.33). (* Significant *difference in post-salbutamol value compared with healthy controls (p=0.01)*

Table 10).

	Healthy controls	Asthmatic		
		pre	post	p (post vs. pre)
Mean (SD) FEV_1 z-score	-0.69 (0.88)	-1.26 (1.25)	-0.93 (0.23)	0.03
Mean (SD) LCI	6.24 (0.47)	6.82 (1.04)	6.64 (0.69)*	0.12
Mean (SD) S_{cond}	0.017 (0.02)	0.027 (0.02)	0.020 (0.04)	0.19
Mean (SD) S_{acin}	0.12 (0.06)	0.12 (0.06)	0.14 (0.10)	0.45

** Significant difference in post-salbutamol value compared with healthy controls (p=0.01)*

Table 10 - Measurements before, and 20 minutes after Salbutamol. Compared with healthy controls.

	Asthmatic		
	pre	post	p (post vs. pre)
Mean (SD) FEV_1 z-score	-1.036 (1.14)	-0.97 (1.17)	0.45
Mean (SD) LCI	6.69 (1.01)	6.71 (1.23)	0.83
Mean (SD) S_{cond}	0.033 (0.03)	0.033 (0.02)	0.90

Mean (SD) S_{acin}	0.14 (0.09)	0.16 (0.12)	0.46

No significant differences

Table 11 - Measurements before and 20 minutes after placebo

Discussion

In this study, children with well-controlled asthma had significantly higher LCI than controls, indicating abnormal gas mixing despite normal FEV_1. S_{cond} correlated with LCI at all time points, suggesting this abnormality may be located in the conducting airways (S_{cond}) as opposed to the acini (S_{acin}) although the trend towards a difference in S_{cond} between groups did not reach statistical significance (p=0.06).

In patients with ongoing inhaled corticosteroid therapy and high level of asthma control, salbutamol administration only minimally improved FEV_1, as is to be expected in a well-controlled asthmatic population such as this. Despite this, post salbutamol mean LCI did not change and remained significantly raised. This raises the suspicion that LCI may be able to detect airways disease that does not respond to salbutamol, and is not detected by FEV_1.

This is the first study to show evidence of ventilation heterogeneity in well-controlled asthmatic children with normal FEV_1. The residual abnormality did not appear to reflect persisting inflammation or bronchospasm as FeNO was normal, and heterogeneity persisted after salbutamol administration. It is possible therefore that LCI may at least partially reflect structural airway changes, consistent with previous observations made by bronchoscopic biopsy in children with asthma(Saglani et al. 2007, Sonnappa et al. 2011). My observational study was obviously unable to directly link ventilation heterogeneity with independent evidence of structural airway changes. Given the difficulty in performing these invasive measurements, we can only hypothesise as to the cause of this residual ventilation heterogeneity. Measurement of Airway Hyperresponsiveness (AHR), if measured, may also have provided further evidence of airway remodelling to support our hypothesis. If we had recruited pre-school children, we may have been able to determine the influence of age and length of time with wheeze on ventilation heterogeneity. This was not possible due to lack of validation of the Innocor system in younger children.

In adult asthmatics, ventilation heterogeneity has been demonstrated to correlate with AHR, independent of airway inflammation measured by exhaled nitric oxide(Downie et al. 2007). In a similar study in adults with mild asthma reported by Verbanck *et al,* S_{cond} and S_{acin} were measured before and after salbutamol. As in our study, there was evidence of ventilation heterogeneity in the conducting airways but not in the acini(Verbanck et al. 2003). The persistently raised S_{cond}, following salbutamol, reinforces the suggestion of non-bronchodilator responsive disease in the small conducting airways of well-controlled asthmatic patients. The elevation of S_{cond} was greater in both of these studies than we have seen. This may be due to poorer asthmatic control compared to our patients, or longer duration of asthma, leading to greater progression of airway structural changes. Gustafsson demonstrated elevated LCI and S_{cond} in a Swedish population of moderately severe asthmatic children, with a greater improvement in both of these variables than we have observed(Gustafsson 2007). Compared to the current study, however, these patients had a lower baseline FEV_1 (77% predicted) and a higher mean LCI (>8).

Inflammation is an important component of asthma. When exhaled nitric oxide or sputum eosinophil count are used to titrate inhaled steroid doses in adult asthmatics, better control can be achieved with fewer exacerbations and an overall lower steroid dose than treating using symptoms alone(Green et al. 2002, Smith et al. 2005). Similarly in children (6-18 years), using FeNO as the basis to increase or decrease inhaled steroid dose resulted in improved AHR and fewer exacerbations than those treated by symptoms alone(Pijnenburg et al. 2005). The abnormality in LCI we detected in the current study appears to be independent of airway inflammation, as FeNO was normal and did not correlate with LCI.

These findings are important because current treatment is normally decided by standard lung function (FEV_1) and FeNO. If these measures are unable to detect abnormalities related to progressive structural changes, it will be impossible to assess the efficacy of future treatments, targeted to managing airway remodelling. Therefore this study may enable other groups to begin development of novel treatment strategies aimed at preventing long-term lung function decline in children with asthma. Such treatments are only just being trialled in larger populations, and

involve targeted treatment of inflammatory proteins (IgE, IL-13). Data on longer term outcomes are awaited.

Critique of above conclusions

An alternative interpretation of the above results is that LCI is actually insensitive to mild asthmatic airways disease as most patients had normal results. Only 6 patients had an abnormal LCI, compared with 7 with an abnormal FEV_1. LCI did change in those with abnormal baseline results, not constituting an overall significant result. All the others had normal results and therefore no room for improvement.

This point is illustrated in Figure 35 below. This chart plots paired results (pre and post salbutamol) in asthmatic individuals, joined by a line. The progression is from pre to post in the direction of the arrow. If both FEV_1 and LCI improved, the arrow would move down and right, as is the case in a few individuals. It is also clear that a number of individuals have changes in FEV_1 indicating reversibility, but no change in LCI. While this results an overall increase in FEV_1, there is no overall change in LCI.

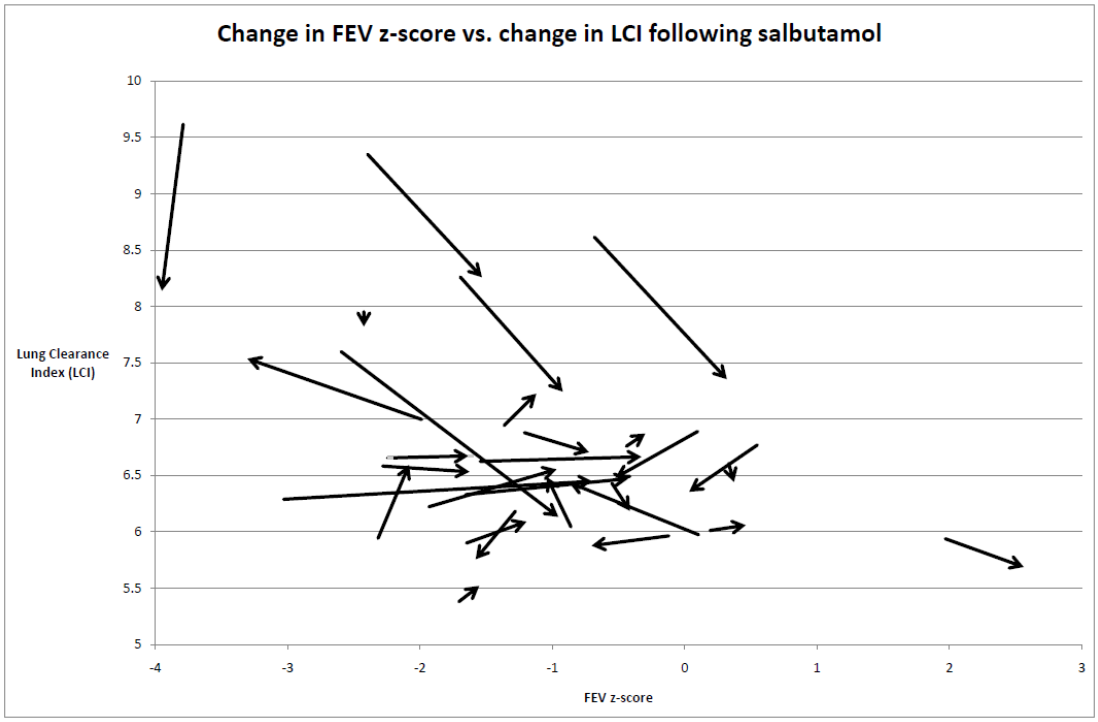


Figure 35- paired FEV1 vs. LCI in children with asthma following salbutamol administration. Pre to post measurements progress in the direction of the arrow.

To respond to this criticism, it is necessary to consider the fundamental principles of ventilation heterogeneity. LCI is thought to be a global measure of ventilation heterogeneity. As a disease of both the small and central airways(Hamid et al. 1997), there is interest in LCI as more appropriate measure of asthma, able to detect overall ventilation heterogeneity. As previously stated, FEV_1 is a measure of airway resistance at flow limitation in only the medium airways. Therefore LCI is likely to be better-suited, especially in those with well-controlled asymptomatic disease. This cannot be proved by this study.

While there are few patients with abnormal results, this may highlight the heterogeneity of the disease – so called asthma phenotypes. This study did not aim to select different phenotypes therefore conclusions are speculative. It could be that those with higher LCI, despite having the same level of control as others, represent a different phenotype. LCI did not change in the majority of patients, and remained raised compared with controls. Therefore the question arises: is it most likely that, if LCI is able to detect subtle residual disease even in this heterogeneous group and there was no overall change in LCI after salbutamol, this is because of lack of reversibility, rather than lack of sensitivity of the assay? To begin to answer this question, it is necessary to follow a well-controlled cohort, controlling for phenotypic features, to track progression of this abnormality over time. In addition – despite the complexity and invasiveness of such a study – pairing LCI results with bronchoscopic biopsy features would confirm the hypothesis that LCI is able to track these progressive structural airway changes.

Learning points and contribution to thesis

This was the first study conducted in children using the Innocor MBW system. While the technique is simple for children to perform, quality control issues needed to be learned. These issues have previously been described in chapter 2.

If repeating this study, it would be important to take into account the effect of one measurement on another. FeNO was consistently performed after spirometry despite ATS/ERS recommendations stating that forced expiration may affect FeNO('ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric

Oxide, 2005' 2005). This was an oversight in the study design, although on reviewing the literature there is conflicting evidence of the effect of Spirometry on FeNO(Gabriele et al. 2005, Kissoon et al. 2002). Notwithstanding this, FeNO was always performed after spirometry in this study and any bias is constant.

While still falling within the normal range, the mean(SD) FEV_1 z-score of the healthy control group is lower than expected (-0.69(0.88)). All were Caucasian and within the age-range of the reference population(Rosenthal et al. 1993). There are 3 possible explanations for this.

1. The reference population is different to the healthy control group

This is unlikely given that the reference population was a British Caucasian group.

2. Differences in method meant lower FEV_1 than expected

ERS/ATS guidelines recommend the use of a noseclip(Miller et al. 2005b). No noseclip was used in this study, which may have lowered the mean FEV_1.

3. Lack of experience

All healthy control children completing spirometry were naïve to the technique. This may have contributed to poor quality results. Lack of operator experience (all measurements were conducted by me) may have meant children were not all encouraged adequately to achieve flow limitation. Contrary to this hypothesis, there was no relationship between subject number ($R^2 = 0.03$) or age ($R^2 = 0.00003$) and FEV_1 z-score.

The above factors (particularly the lack of noseclip) may have affected FEV_1 measurements in this population. While this prevents comparisons between other healthy populations and is not ideal, both asthma and healthy groups were measured according to the same standards. Therefore, they can be reliably compared.

This first study conducted in children using Innocor was important in establishing the validity of subsequent studies. As a novel device for MBW in children, the suitability of the Innocor patient interface (mouthpiece and noseclip) and comparison of normative values with other devices is of particular interest. Innocor is simple to use, and did not require any servicing throughout the study. There were instances of

equipment malfunction (such as unexpected computer operating system re-boot, and accidental compression of the nafion sample tubing), but these were easily remedied. In this study, all children were able to perform MBW to a satisfactory standard, with 3 washouts being completed in 69% of occasions. Children were easily distractible using cartoons. Performing 6 washouts with a waiting period was tiring for some younger ones. With encouragement a good seal was maintained throughout the washin and washout periods.

A number of studies have been completed in normal populations, similar in size to this. A simple table of mean (SD) LCI using Innocor, compared with other SF_6 devices is shown below (Table 12).

Publication	Device, SF6 concentration	n	Mean [range] or (SD) age (yrs)	Mean (95% CI) LCI
This study (Macleod et al. 2009)	Innocor, 0.2%	29	11.1 [5-16]	6.24 (0.47)
Horsley et al (A. R. Horsley et al. 2008)	Innocor, 0.2%	48	33 [19-58]	6.7 (0.4)
Aurora (Aurora et al. 2005a)	Mass Spec, 4%	30	4.31 (0.84)	6.89 (0.44)
Aurora (Aurora et al. 2004)	Mass Spec, 4%	33	11.3 (3.1)	6.45 (0.49)
Gustafsson (Gustafsson et al. 2003a)	Mass Spec, 4%	28	11.4 [3-18]	6.33 (0.43)
Aljassim (F. Aljassim et al. 2009)	Mass Spec, 4%	18	18 [17-18]	6.50 (0.45)
Gustafsson (unpublished)	Mass Spec, 4%	44	41.1 (10.3)	6.6 (0.5)

Table 12 - comparison of recent publications. Healthy control adults and children. Adapted from Robinson et al(Robinson et al. 2009b)

Reassuringly, the results from this study were very similar to other published studies. While the basic setup of the mouthpiece and pneumotach was very similar, the concentration of SF6 used in mass spectrometer studies was 4%. This suggests that

gas concentration (with changes in viscosity) does not influence LCI, certainly in healthy subjects. The obvious conclusion from this is that results from Innocor and mass spectrometer studies can be compared.

In addition to reassuring normative results, this study provides evidence of increased sensitivity of LCI to residual airways disease in well-controlled asthmatic children. A clinical application of this would be in measuring and tracking changes in lung function over time in a larger population. This would only be possible in a large, multi-centre cohort. Innocor is likely to be more suited to this than a mass spectrometer for reasons already highlighted elsewhere.

Finally, this study allows us to make a power calculation for future interventional or observational studies in asthmatics. A change in LCI of 1 could be considered a meaningful change, based on the standard deviation of the population. Based on this study, a sample size of approximately 8 in each group would be able to detect a change in LCI of 1 with a power of 0.8.

Chapter 5 - Effects of airway clearance physiotherapy on ventilation heterogeneity in children with CF

Introduction

Chest physiotherapy is widely used for airway clearance in children and adults with Cystic Fibrosis. Improvements in survival of CF patients in the past 40 years are attributed, in part, to the introduction of effective airway clearance techniques. Despite a lack of robust empirical data to support this, recommendations currently state that airway clearance techniques should be introduced at the time of CF diagnosis (Konstan et al. 1994, Armstrong et al. 2005, Khan et al. 1995, Dakin et al. 2002a, Rosenfeld et al. 2001), but daily physiotherapy is time-consuming, adversely affecting compliance(Arias Llorente et al. 2008). A Cochrane Intervention Review, conducted to assess the evidence for the benefits of chest physiotherapy in CF, showed no long-term benefits. The only consistent outcome was a short-term improvement in mucous transport(van der Schans et al. 2000). In addition, no eligible studies showed a benefit in terms of improvements in lung function over the short or long term.

The lack of data recommending regular physiotherapy in CF may be because FEV_1 is relatively insensitive to acute change, especially in those with mild or stable lung disease. Forced expiration at flow limitation, thought to detect an overall increase in airway obstruction in medium-sized airways, may not be appropriate to detect early CF airway disease in small to medium sized airways. Ventilation heterogeneity (LCI) is likely to be more able to detect acute changes after an intervention such as chest physiotherapy. This study seeks to test this hypothesis.

CF lung disease is characterised by excess mucous production as a result of chronic infection and inflammation. Airway clearance is a fundamental treatment recommended universally for prevention of the long term consequences of destructive properties of retained mucous(Flume et al. 2009, Bradley et al. 2006). Most previous interventional studies have used FEV_1 as the main functional outcome following physiotherapy. Only 2 studies have so far measured lung clearance index before and after physiotherapy. One aimed to demonstrate that airway clearance does

not influence the short term variability of LCI in the clinical setting, rather than to prove a therapeutic benefit(Fuchs et al. 2010). There was no significant mean change in LCI following physiotherapy, suggesting it does not matter whether MBW is performed before or after physiotherapy. Another study used a measure of ventilation heterogeneity (single breath helium washout) to measure effects of physiotherapy in a small number of patients with cystic fibrosis(Darbee et al. 2004). Following positive expiratory pressure manoeuvres in 5 patients, the average gas mixing improved, indicating airway clearance had some positive effect on the function of the lungs.

Airway clearance techniques aim to move mucous proximally, to allow expectoration. Recommended techniques involve one, or a combination of, percussion, high frequency oscillation and postural drainage. Self-administered techniques that are popular with patients use thoracic pressure, rather than external forces, to mobilise mucous. Many patients use mechanical devices to increase expiratory resistance, provide oscillations or give non-invasive ventilatory assistance. Techniques are often combined and no one technique is recommended universally.

Theoretically, physiotherapy should be moving mucous proximally, resulting in changes in lung volume, however previous studies have not shown changes in FRC following airway clearance. Airway clearance should also have an effect on lung clearance index, where movement of mucous leads to changes in ventilation heterogeneity.

The primary aim of this study was:

- To assess the ability of MBW, compared with FEV_1, to detect a change in lung function following airway clearance physiotherapy in children with CF (active cycle of breathing technique, ACBT).

The hypothesis stated that following ACBT, there would be an improvement in ventilation heterogeneity compared with those who performed paired measurements without intervention, due to clearance of retained airway mucous. This small study contributes to an understanding of how ventilation heterogeneity changes following an acute intervention. In contrast, large numbers would be required to provide

definitive evidence supporting or refuting the current standard recommendation of regular chest physiotherapy in CF.

The secondary aims of this study are of importance to the thesis as a whole:

- To compare short term change in LCI in disease states with and without an intervention

To assess Innocor performance in a therapeutic study with multiple repeated measurements in one visit. Those with CF can have elevated LCI values and prolonged washout duration, which may amplify errors in the system. Previous studies have measured LCI in CF, but few have recorded short-term repeatability and acute response to interventions. This study therefore also aimed to demonstrate the suitability of Innocor for repeated prolonged washouts in those with measureable lung disease.

This study was designed and conducted in parallel with a similar protocol for adult patients with CF. The protocol for children was written with Elaine Dhoueib, Paediatric Physiotherapist at the Royal Hospital for Sick Children, Edinburgh. Results were submitted for a MSC in Physiotherapy qualification (Queen Margaret University, Edinburgh).
All data analysis and statistics for this study were performed by me.

Methods

This randomised interventional study recruited children (5-17 years) with CF attending two clinical centres at the Royal Hospital for Sick Children, Edinburgh. Subjects attended on one occasion and were randomised to perform MBW and spirometry before, and immediately after, physiotherapy intervention (ACBT) or a 30-minute waiting period.
All patients repeated MBW and spirometry immediately after the intervention, with an additional 2 MBW repeats, 1 and 2 hours after that. For a summary of the study protocol see
Figure 36.

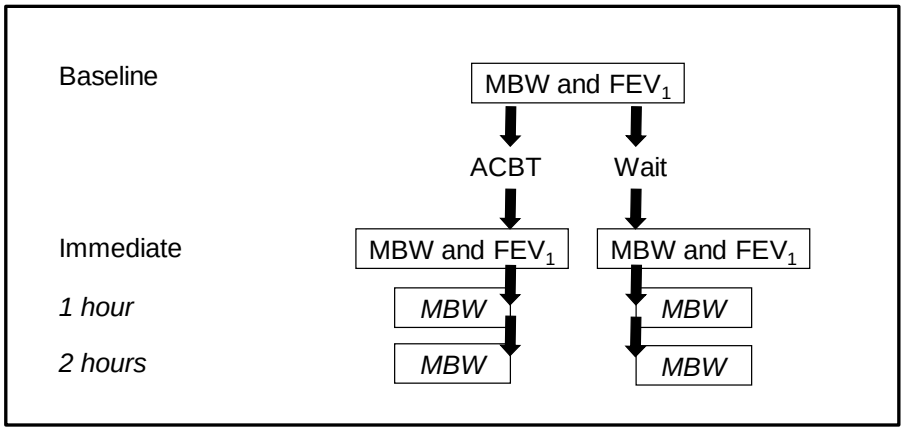


Figure 36 - Study flow chart

Active cycle of breathing technique (ACBT)

Active Cycle of Breathing was chosen because it is the technique children were most familiar with, and is recommended and taught by physiotherapists as standard care in the local hospital clinic. ACBT was supervised by a qualified physiotherapist or trained research nurse. Patients all received training prior to the study.

A brief description of ACBT is given here:

Children completed 20 minutes of standardised ACBT according to a locally agreed standard operating procedure, based on CF Trust guidelines. A set of 3 deep breaths with an inspiratory hold, followed by 1-2 forced expiration "huffs" at mid to low lung volume, was repeated 3 times. Patients were lying down throughout, and completed this process in supine, left and right lateral positions.

Those who waited sat and watched TV or a DVD for 30 minutes.

Multiple breath washout

MBW was performed and analysed as per standard operation procedure discussed in Chapter 2. Three repeat measurements were taken, with mean calculated, at all time points.

Spirometry

All patients performed standard spirometry, while sitting, immediately after MBW without a nose clip, (Easyone handheld spirometer, ndd, Switzerland). The highest value of 3 acceptable manoeuvres was defined according to ERS/ATS guidelines. Reference values were provided by recently published all age equations(Stanojevic et al. 2008).

Statistical analysis

Data were analysed using Prism software (GraphPad Software Inc., CA, USA). A t-test, or non-parametric equivalent where appropriate, was used to analyse differences between groups. Mean vs. difference before and after (Bland-Altman plot), with 95% limits of agreement was used to compare change in measurements following physiotherapy with those who waited. Paired measurements with a similar interval in healthy volunteers were not available; therefore, the limits of agreement between the first and third washouts, spaced approximately 30 minutes apart during normal testing in adults only, were chosen as an equivalent. These measurements were taken from a suitable healthy control population recruited for the clinical study in chapter 4.

No power calculation was possible at the time as there were no data on the short term variability of LCI in CF.

Ethical approval

Ethical approval for this study was granted by the Lothian Research and Ethics Committee (REC 06/S1103/66). Patients and parents, where appropriate, were provided with detailed information, and signed an informed consent form prior to commencing the study.

Results

18 patients with cystic fibrosis (median age 11.8, range 7-17) were recruited to the study, and randomly assigned (random number) to receive physiotherapy (n=10) or wait (control, n=8). 29 healthy control subjects (mean age 11.5, range 5-16) were used as a comparison group, recruited for the previous study (chapter 4) to complete one set of washouts on a single occasion. Three children were unable to complete all four time points due to time constraints, however paired measurements before and after the intervention were obtained in all patients.

All patients completed measurements during periods of clinical stability. This was defined as no recent exacerbation or increase in respiratory symptoms requiring extra antibiotic courses in the 2 weeks prior to testing.

Baseline measurements

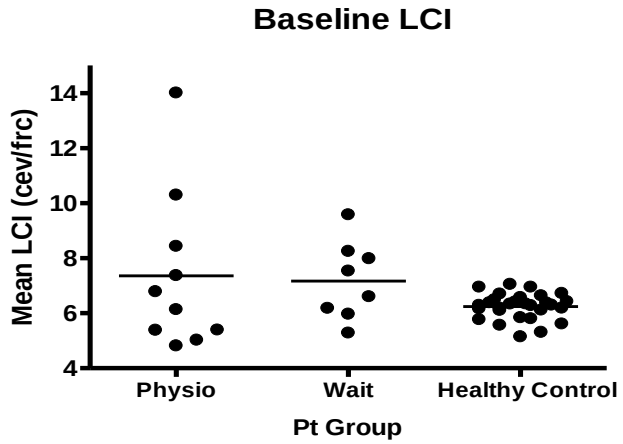
Baseline measurements and demographics for all patients are shown in table 13. Results are presented as the randomised CF groups (physiotherapy and wait) compared with healthy controls. There was no difference in mean (SD) FEV_1 z-score in either the physiotherapy or wait group compared with healthy volunteers (-0.56(2.75) and -1.05(2.20) respectively vs. -0.60(0.88), $p>0.05$). Mean(SD) LCI, however, was higher in both CF groups (physiotherapy and control) compared with healthy volunteers (7.36(2.90) and 7.17(1.42) vs. 6.24 (0.47), $p=0.047$ and $p=0.004$, respectively. See Figure 37.

Mean (SD) functional residual capacity (z-score) did not differ between either CF group and healthy volunteers ($p=0.58$ and $p=0.14$ respectively). Scond was significantly higher in both CF groups compared with healthy controls (0.061 (0.028) and 0.078 (0.030) vs. 0.016 (0.013), $p<0.0001$). This relationship was only seen in Sacin measurements in the group receiving physiotherapy (0.19 (0.065) vs. 0.12 (0.064), $p=0.03$), not in the wait group (0.15 (0.13) vs. 0.12 (0.064), $p= 0.40$).

	Cystic Fibrosis		Healthy Control
	Physio	Wait	
N	10	8	29
Sex (m/f)	7/3	5/3	18/11
Median [range] age (yrs)	14 [7 - 17]	11 [7 - 17]	11 [5 - 16]
Mean (SD) height (cm)	144.9 (18.61)	142.4 (11.4)	148.2 (19.1)
Mean (SD) FEV_1 z-score	-1.05 (2.86)	-1.54 (1.92)	-0.81 (0.97)
Mean (SD) FRC z-score	3.37 (4.04)	-0.35 (1.46)	1.49 (2.94)
Mean (SD) LCI (cev/frc)	7.36 (2.90)*	7.17 (1.42) †	6.24 (0.47)
Mean (SD) Sacin $V_t$adj	0.19 (0.07) †	0.15 (0.13)	0.12 (0.064)
Mean (SD) Scond $V_t$adj	0.061 (0.03) ‡	0.078 (0.030) ‡	0.016 (0.013)

*Significant difference to healthy controls * $p<0.05$, † $p<0.01$, ‡ $p<0.001$*

Table 13 - Comparison of baseline measurements between intervention and no intervention groups, and healthy control



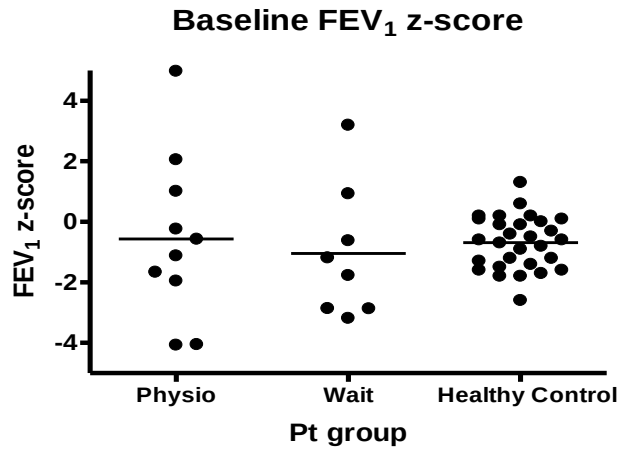


Figure 37 - Baseline measurements compared with healthy control population

Response to physiotherapy

After completion of ACBT, there were no significant differences in any measurements, compared with those who waited. This applied at all 3 post-physiotherapy time points. Neither were there any significant mean changes from zero. To summarise these data, mean(SD) absolute changes (with % change where relevant) are shown by time point in

No ***significant differences (p>0.5)***

Table 14. It can be seen that mean changes are very small; however the variability of change is relatively high. Especially with FRC, coefficient of variability (CV%) on the measurements immediately following physiotherapy was higher than the wait group (9.3 vs. 4.8). This suggests a trend towards increased change in lung volume following physiotherapy compared with waiting; however, the change is not consistent in one direction, and did not continue in the subsequent measurements. Change in LCI at all time points for both groups is illustrated in Figure 38. This demonstrates greater fluctuation of LCI in the physio group but no overall group differences.

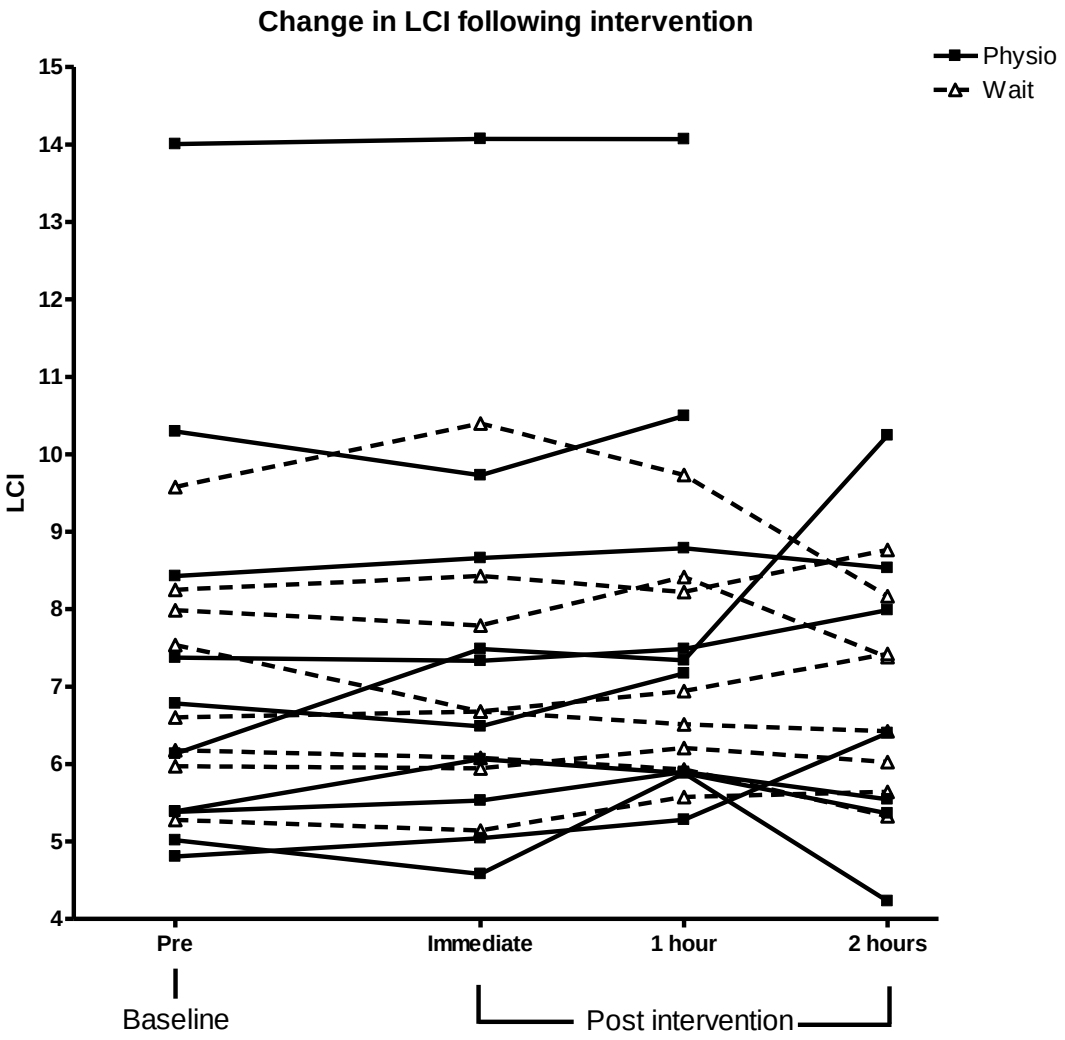


Figure 38 - Change in LCI following physiotherapy compared with wait group.

	Mean(SD) change from baseline - Immediate		Mean(SD) change from baseline – 1 hour		Mean(SD) change from baseline – 2 hours	
	Physio	Wait	Physio	Wait	Physio	Wait
FEV_1 z-score	0.16(0.62)	-0.28(0.49)	--	--	--	--
FRC	0.02(0.57)	-0.02(0.12)	-0.20(0.44)	-0.01(0.16)	-0.01(0.10)	-0.01(0.16)
FRC % change	3.35(16.77)	0.14(0.15)	-6.02(11.04)	0.50(12.50)	0.15(6.47)	-0.81(10.77)
LCI	0.14(0.56)	-0.03(0.47)	0.34(0.41)	0.02(0.48)	0.23(0.73)	-0.28(0.83)
LCI % change	2.63(9.07)	-0.83(5.61)	5.47(7.26)	0.48(6.60)	4.44(14.76)	-3.04(11.03)
Sacin	-0.014(0.06)	-0.021(0.06)	-0.023(0.05)	-0.030(0.08)	-0.025(0.05)	-0.041(0.14)
Scond	0.007(0.02)	0.006(0.02)	0.012(0.03)	0.006(0.02)	0.021(0.03)	0.007(0.02)

No significant differences (p>0.5)

Table 14 - Mean change in measurements following physio and in waiting controls.

There was no consistent correlation between change in LCI and change in FEV_1 z-score, Scond or Sacin. There was, however, a significant correlation between change in LCI and change in FRC. This is summarised in Table 15. It is expected that change in FRC would result in a concurrent change in LCI (FRC being the denominator when calculating LCI). However, despite both positive and negative changes in FRC, there is not always an associated "mathematical" change in LCI, e.g. fall in FRC does not necessarily correspond to a rise in LCI (CEV/FRC). This indicates a more complex picture. This will also be discussed.

	Change from baseline					
	Immediate		1 hour		2 hours	
	R^2 *	p	R^2 *	p	R^2 *	p
Physio	0.69	0.003	0.49	0.02	0.05	0.65
Wait	0.79	0.5	0.76	0.005	0.36	0.12

**Pearson's correlation coefficient*

Table 15- Correlation between change in LCI and change FRC

Discussion

In this study, neither LCI, FRC, Sacin, Scond and FEV_1 changed consistently following ACBT compared with baseline and control. While there was evidence of greater absolute change in those receiving physiotherapy compared with those who waited, the difference was not significant. Furthermore, change was not in one direction. Change in LCI did not correlate with change in FEV_1 and was not limited to those with a higher LCI or lower FEV_1.

Fuchs et al have recently measured short-term reproducibility of MBW measurements in healthy control children and adolescents(Fuchs et al. 2009). This showed an acceptable co-efficient of variability (CV%) of LCI after 1 hour. FRC reproducibility is not quoted in this paper. The same research group has published a study measuring LCI and FEV_1 before and 1 hour after physiotherapy or waiting in young people with CF. This was with the aim of determining to what extent performing physiotherapy before LCI and spirometry affects the results. This is obviously very similar to our study, although their aim is more concerned with test quality control in a clinical setting rather than airway physiology. Again, minimal

changes in LCI were seen following physiotherapy, which confirms the results we have collected. Table 16 compares data from these papers against those collected before and 1 hour after physiotherapy. No data are available from these articles about intra-individual, between test reproducibility of FRC.

Both previous studies have shown similar results. CV% is low (<5%) after 1 hour, even after 30 minutes of physiotherapy. CV% is higher post physiotherapy compared with those who waited, and it is also clear from this paper that the 95% limits of agreement are wider in those who did not receive physiotherapy, compared with those who did. We have confirmed the findings of Fuchs et al(Fuchs et al. 2009).

		This chapter		Fuchs et al post physiotherapy in cystic fibrosis		Fuchs *et al* healthy volunteers
LCI reproducibility (CV%)	Post physiotherapy	n=10	4.71 (3.7)	n=16	4.6 (3.1)	4.2 (3.7)
	Post wait	n=8	3.56 (3.0)	n=11	2.6 (2.1)	

Table 16 - Comparison with published MBW data. Coefficient of variability (CV%) of LCI measurements in individuals with CF before and 1 hour after physiotherapy or wait, and 1 hour apart in healthy individuals.

The marginal average changes in ventilation heterogeneity seen here are similar to those seen in a similar study by Neil Woodhouse and Jim Wild et al, Sheffield(Woodhouse et al. 2009). This group have developed inhaled Hyperpolarised Helium-3 MRI as a non-ionising method of imaging ventilated lung volume. This study measured lung volumes followed by spirometry before and 30 minutes after physiotherapy in 9 individuals, comparing results with paired measurements in 5 controls. There were no significant changes in volume and spirometric measurements, however higher variability was seen in those performing physiotherapy compared with the control group.

To attempt to explain these findings, I will discuss the effect of airway clearance on mucous in the lungs, and how this may affect ventilation heterogeneity.

Physiotherapy is considered to be an effective way of clearing mucous from the airways in CF. This study attempted to test a simple, regularly used airway clearance technique (ACBT) to see its effect on ventilation heterogeneity. There seems to be a

hypothetical connection between mucous obstruction and ventilation heterogeneity, and therefore measurable change. It was thought that this change could be measured more sensitively with LCI rather than FEV_1, because LCI is thought to measure global ventilation heterogeneity involving small as well as medium and large airways, rather than the flow limitation in the medium airways with FEV_1. LCI is also more able to detect subtle lung disease, and therefore may be more sensitive to acute change. This was seen after bronchodilator use in CF and asthma in a previously published study by Gustafsson(Gustafsson 2007).

CF is a complex airway disease, with a dynamic balance of factors influencing airway patency, as seen in an inhaled Hyperpolarised Helium-3 MRI study. Mentore et al demonstrated increased ventilation defects per image (VDI), i.e. the number of areas with no gas entering, after physiotherapy in patients with CF first treated with inhaled salbutamol(Mentore et al. 2005). While the baseline VDI measurements correlated with lung function (spirometry) there was only a weak correlation ($r = -0.13$) between change in VDI and change in FEV_1. This sheds some light on the processes underlying the results seen in our study. It appears that more regions of lung are blocked off after physiotherapy, however the authors admit that the scoring system exaggerates mild defects and dampens severe defects. It may be that very subtle differences result in a large increase in VDI. In addition, any de-stabilising effect of salbutamol on airway smooth muscle tone was not discussed. While a reduction in VDI was seen after the pre-physio salbutamol dose, the effect of smooth muscle relaxation could result in exaggerated changes after physiotherapy because smooth muscle is no longer involved in maintaining a balance of airway patency in parallel lung regions.

In this study, physiotherapy may have increased blocked off areas of lung by shifting mucous proximally as is proposed by Mentore et al. Simple shifting of mucous proximally would result in a reduction in FRC, but overall changes in our study were marginal, implying no effect or rather parallel processes of conflicting changes. It is somewhat disappointing not to be able to report results with clear conclusions. There are, however, important discussion points raised relevant to this thesis.

Study limitations

There are limitations to this study that may have contributed to the inconclusive results.

1. Study power.

With 8 subjects in 1 group and 10 in the other, only large and consistent changes in LCI will be detected. It is clear that this study is underpowered to answer the primary aim of the study. The results, however, are important in informing similar studies. Without clear statistical conclusions, any comments made here are essentially speculative. It is important to learn from the following study limitations in order to design future interventional studies in a complex airway disease such as CF.

2. Contribution of spirometry to variability in "wait" group.

Those patients who waited, rather than performing physiotherapy, did complete 3 forced vital capacity manoeuvres following the first sequence of MBW. While this was brief, and allowed comparison with previous studies, there is concern that these manoeuvres may have disrupted airway obstruction and at least partially cleared the airways. A solution to this would have been to perform FEV_1 at some point after the MBW manoeuvres (e.g. 2 hours), or not at all in those who waited.

3. ACBT not enough to clear on this occasion

ACBT was chosen as a familiar technique to most patients. This is the preferred method in the clinical centres involved in the study. It is assumed that one 30-minute session of ACBT will result in significant clearance. All subjects were clinically stable at the time of testing, and may not have had "clearable" obstructions (i.e. mucous). A large proportion of both physiotherapy and wait subjects (32% and 39%) had baseline LCI results below the upper reference limit. This suggests minimal baseline heterogeneity and little potential for improvement. Therefore, ACBT on one occasion, in these patients, may not have been enough to alter ventilation heterogeneity significantly.

An alternative method could have chosen those being treated for an exacerbation. Measuring acute changes in those with more active airway disease, and presumably increased mucous, may have produced results that were more impressive but less applicable to understanding the effect of daily physiotherapy to lung function.

4. Multiple parallel factors influencing change in ventilation heterogeneity

The primary aim of ACBT, in the absence of efficient mucociliary clearance, is to move mucous proximally allowing expectoration or swallowing. If mucous is completely cleared from a lung region, it would be assumed that ventilation efficiency would improve as overall heterogeneity is decreased. It may not be so simple. The following diagrams (Figure 39) seek to illustrate possible mechanisms for the minimal overall changes we saw following ACBT in this study.

The basis for these diagrams is that LCI measures ventilation heterogeneity, i.e. differences between washouts of parallel lung units. A simplified 2-compartment model, as described by Paiva and Engel(Engel 1985), proposes a model of well- and poorly-ventilated compartments. These compartments could exist at any airway level, but are probably most important at small-airway levels where CF airway disease is thought to begin. As stated in Chapter 2, the well-ventilated compartment washes out first, as inspired fresh air is preferentially distributed. Later, the poorly ventilated compartment washes out, requiring a larger overall number of lung turnovers to washout the SF_6 to the end-point. In this model, I have added a further compartment. It is completely closed during tidal breathing and therefore does not contribute to LCI during the 3 washouts of any one test. This corresponds to a known trapped gas volume in CF, shown by the differences between washout and plethysmography FRC(Hulskamp et al. 2006, Morris et al. 2001, Stocks and Quanjer 1995). I propose this “virtual volume” may be altered to greater or lesser degrees because of the postural changes and forced expiratory manoeuvres (“huffs” and coughs) during ACBT. Significant changes in FRC were not seen in this study, and no measure of trapped gas was made to confirm or refute this hypothesis. A reduction in FRC, as the denominator in the LCI equation (CEV/FRC), may cause a mathematical rise in LCI but the lack of consistent strong correlations between change in FRC and change in LCI indicate a more complex picture. A reduction in

lung volume does not automatically result in an increased LCI, because there is less lung volume to be washed out so that the CEV also decreases. Changes in volume following physiotherapy are probably in those lung regions most affected by disease. Therefore, the diseased components will contribute less to the washout. While LCI is a ratio, the numerator and denominator are not independent of each other.

To further discuss the dynamic changes within the lung following physiotherapy, Figure 39 shows a simplified 3-compartment model prior to, and following, chest physiotherapy. Green represents the well-ventilated compartment (no obstruction), yellow is poorly ventilated (some obstruction), and red is completely obstructed (i.e. not ventilated at all during tidal breathing).

Following ACBT, one of 3 scenarios is proposed.

1. LCI may decrease if obstruction is cleared from the partially obstructed region (previously yellow), decreasing heterogeneity. The closed region (red) is unchanged.
2. Alternatively, LCI may increase if mucous is partially cleared from the previously closed region (red), increasing overall heterogeneity.
3. The 3rd scenario is a mixed picture, with both 1 and 2 occurring in parallel. This seems most likely as these processes are multiplied throughout the lung and ACBT is unlikely to have a uniform effect on already heterogeneous airways.

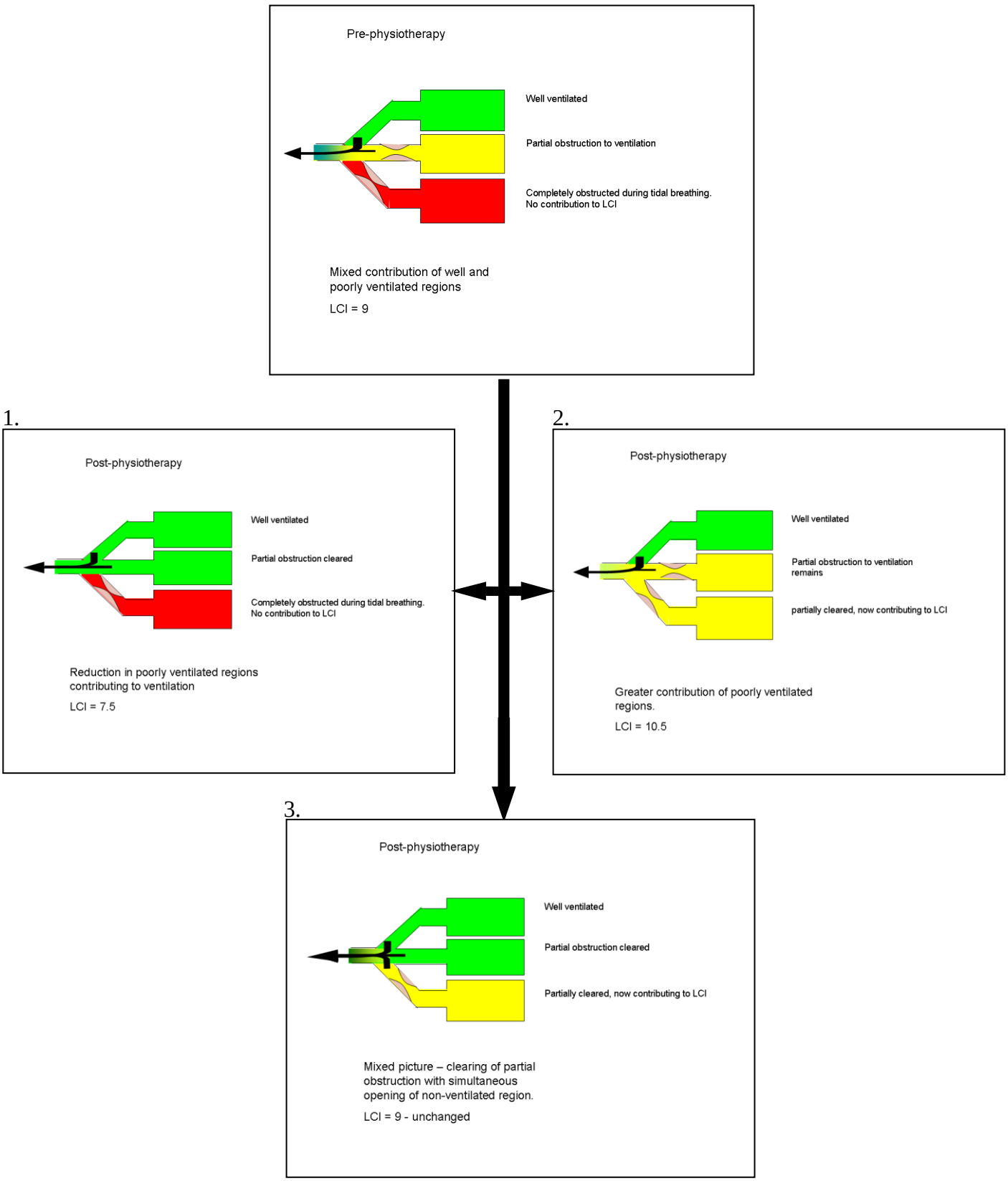


Figure 39 - Three-compartment model illustration proposing complex interactions of parallel airway regions on LCI following airway clearance

Learning points and contribution to thesis

This study raises important learning points relevant to the main theme of the thesis:
In planning the study, we used a standardised protocol, but did not factor in the effect of one measurement on another. It would have been wiser, in retrospect, to avoid spirometry (or at least 3 full forced expiratory manoeuvres) until after the post intervention MBW was completed. The study was underpowered, and future short-term interventional studies should take this into account. In addition, recruiting young people with stable CF increases the likelihood that one short term intervention will have no effect on stable airway disease.

LCI is already recognised as a promising technique for detection and tracking of CF lung disease and is better able to detect disease than FEV_1. This study suggests, however, that it may not be able to assess the effectiveness of acute interventions in CF without recruiting large numbers of patients. This may be due to the insensitivity of the assay, or the complexity of factors influencing global ventilation heterogeneity in the CF lung of even clinically stable individuals. The 3-compartment model I propose seeks to explain interactions of complex factors following ACBT in a simplified way. This is in no way a conclusive model, as this study was not designed to answer this question.

Finally, the Innocor device, used over 2 hours in each patient, performed well. There were no incidents of technical faults or random measurement errors seen. The measurements took place in another department of the hospital, with only stair access. The moving each day and setting up did not appear to destabilise the analyser or flow meter. This helps to validate Innocor as a robust, reliable device capable of regular and prolonged use without advanced technical support. There is therefore potential for use in a clinical setting once software and hardware validation has been completed in all ages.

This study does not have clear conclusions on the acute effect of physiotherapy on lung function in CF, mainly due to underpowering. Physiotherapy may have complex short-term effects on ventilation heterogeneity, which are not reflected in a

conclusive overall change. Conversely, one session of physiotherapy may not be enough to produce measureable change in children with stable CF lung disease. This is an important finding in terms of planning future clinical studies. In assessing long term change in a cohort of patients, the effect of the timing of daily physiotherapy is unknown, therefore should be kept constant at each time point. This recommendation is contrary to what has previously been published(Fuchs et al. 2010).

Chapter 6 - Effects of posture on LCI and FEV_1 in health and disease

Introduction

MBW is suitable for all ages to perform. Recent guidelines have focussed attention on younger age groups, primarily because of a paucity of longitudinal data tracking the progression of cystic fibrosis. Generally, infants perform MBW while supine, but in older children (>2 yrs) and adults, MBW is performed sitting. This may cause difficulties in longitudinal studies tracking changes in children from infancy to childhood, as it would not be clear whether any progression in lung disease was due to true lung changes or simple methodological alterations. Therefore, for lung disease to be tracked in this way, the effect of a change in posture should be examined.

Previous studies have documented changes in lung function in infants, children and adults whilst supine. Lung volume (FRC, Vt and FVC) is reduced in individuals when changing from sitting to lying supine(Gustafsson 2003, Moreno and Lyons 1961). In a study of healthy adult male volunteers, changing from sitting to supine or prone posture resulted in a fall in FEV_1 and FRC(Vilke et al. 2000). In a study of children with asthma and healthy controls there was a fall in FRC on lying, but no clear effect of the presence of airway disease on the magnitude of the fall. In the same paper, peak expiratory flow rate (PEFR) also fell in those with asthma, but not healthy controls(Greenough et al. 1991). No published studies have performed the same experiment in children with CF.

A few studies have looked at the effect of change in posture on ventilation heterogeneity parameters(Gronkvist et al. 2002, Cortese et al. 1976, Grönkvist et al. 2002, F Aljassim et al. 2009, Gustafsson 2003). Mostly these studies have been using phase III slope analysis, rather than LCI. Only one has involved children with cystic fibrosis(F Aljassim et al. 2009). In this study (currently presented only as an abstract) there was reduction in FRC and increase in LCI in older children, but importantly not in infants. In another study by Professor Gustafsson, FRC decreased after changing to supine posture from sitting in children with asthma and healthy controls. A greater than 20% reduction in FRC was seen, but there was no significant change in LCI in

either group. As CF has a different disease process to asthma, the results of that study do not necessarily translate to other conditions.

An important implication of a decrease in lung volume and function with supine posture is the connection between this and sleep disordered breathing. A number of studies have reported hypoxaemia in infants, children and adults with CF, even in the presence of stable lung disease(Villa et al. 2001, Bradley et al. 1999, Francis et al. 1980, Tepper et al. 1983). It is suggested this may be due to reduced respiratory drive while asleep, leading to lower tidal volume and impaired ventilation. Simulating the sleep posture while awake to assess the effect on lung function may shed light on an explanation for sleep disordered breathing in CF. The changes after altering posture may be the same as those occurring at night, however it is important to recognise that additional sleep-related effects due to changes in upper airway patency and respiratory drive will not be measured.

This study had the following primary outcome:

- Effect of change in posture from sitting to supine on lung volume (FRC), ventilation heterogeneity and spirometry (FEV_1) in healthy controls and children with CF

Secondary outcomes were:

- Correlation of posture-related changes in FRC and LCI to change in spirometry
- Effect of Disease (CF) on posture-related changes in lung volume and ventilation heterogeneity compared with healthy controls.

This is an important study for the following reasons:

1. When validating Innocor for use in younger children, the effect of a change in posture must be taken into account. In the scenario of repeated measurements over time, some children might perform tests lying down on one occasion, then sitting up. If there is a change in LCI only due to change in posture, this may affect the interpretation of results. The study presented by Professor Gustafsson's group(F Aljassim et al. 2009) showed no change in FRC in infants (when changed from supine to sitting) however there was a reduction in older children. No overall change in LCI was recorded in either infants or

older children. This may mean that there is no concern about following young children over time even if there is a change in posture. There may however be important methodological differences between Professor Gustafsson's study and the standard Innocor setup that negated the effect of the change in posture. This will be discussed later.

2. This thesis as a whole considers the wider physiological basis of ventilation heterogeneity. A number of studies have shown a change in FRC with change in posture, but the effect of a change in FRC on LCI has not been fully explored. This study, similarly to other studies in this thesis, aims to apply a standardised change in the respiratory system, and measure the effect of this on gas washout efficiency. It is hoped that this chapter will provide important information on how lung disease affects LCI, and how LCI and FRC interact in health and disease.

This study was conceived and designed by Dr Steve Cunningham and myself. It started as a medical student project but continued beyond the student project period. Chantal Ellis, then a 3rd year medical student, was involved in the study design discussions and assembled the paperwork for ethical committee and R&D application. The study protocol was written by Chantal, with supervision and advice from Dr Steve Cunningham and me.

Method

Patient selection

Children with cystic fibrosis and healthy volunteers were recruited to complete all measurements on a single day. Children with CF were recruited from those attending the RHSC and Stirling Royal Infirmary paediatric CF clinic. A letter was sent inviting them to take part with the relevant parent and child information sheets. A follow up phone call was made after at least one week to make an appointment date with willing participants. Healthy volunteers were largely recruited from the previous healthy volunteer group (chapter 4) and a proportion from children of hospital staff. Again, an invitation was made by letter with relevant information sheets. The follow up phone call or e-mail made a date with willing participants.

Inclusion and exclusion criteria

Healthy volunteers were selected as in the previous study (chapter 4). They were to be healthy, without chronic respiratory history and in the age-group shown. Children with CF were clinically stable, defined as no exacerbation requiring antibiotics in the 4 weeks preceding the testing date. They had mild to moderate lung disease (FEV_1 >40%), characteristic of this age-group. Those with severe lung disease were excluded as it was thought the tests may be too difficult to perform and there was little experience with this particular population. Those with a significant co-morbidity that may have confounded the results (previous prematurity, neuromuscular or congenital cardiac defect) were also excluded.

Measurements were performed by myself, Chantal Ellis (4^{th} year medical student), Kay Riding (research nurse), Debbie Miller (research nurse) and Dr Helen Sheridan (clinical research fellow) between October 2008 and May 2011.

Inclusion criteria

Healthy volunteers

(a) Children aged 5-16 years.

(b) No history of respiratory disease.

(c) No significant medical history.

(d) Non-smoker.

Children with Cystic Fibrosis

(a) Children aged 5-9 years.

(b) Confirmed diagnosis of cystic fibrosis (positive sweat test or genetic testing).

(c) Clinically stable – no recent exacerbations (requiring antibiotics) in preceding 4 weeks.

(d) FEV1 > 40% predicted.

Exclusion criteria

Healthy volunteers

(a) Any past history of: recurrent wheezing episodes, pneumonia, cystic fibrosis, pertussis or tuberculosis.

(b) Previous diagnosis of asthma or taking anti-asthma medication.

(c) Previous hospitalisation for respiratory infection.

(d) Born before 34 weeks gestation.

(e) Neuromuscular weakness or bone disease likely to affect respiration.

(f) Congenital cardiac defects requiring treatment.

CF paediatric patients

(a) FEV1 < 40%.

(b) Recent chest exacerbation requiring antibiotics (in past 4 weeks).

(c) Born before 34 weeks gestation.

(d) Neuromuscular weakness or bone disease likely to affect respiration.

(e) Congenital cardiac defects requiring treatment.

Study design

This study has a simple design. MBW and FEV_1 were performed whilst sitting up, then lying down. It was felt that to allow time for airway changes to take place, there needed to be a 30 minute period of lying before repeat measurements were taken. The study protocol is summarised in Figure 40. The sequence was identical for CF and healthy control groups.

Prior to commencing the measurements, a brief patient history was taken to ensure inclusion and exclusion criteria were adhered to, with details of CF treatment and recent changes documented. Height (and weight) was measured to calculate FEV_1 reference values.

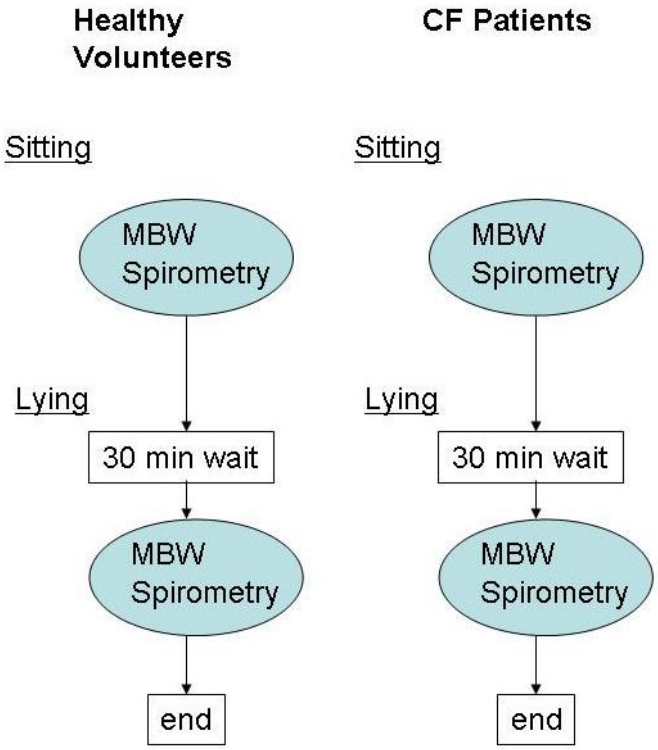


Figure 40 - study protocol summary flowchart

Statistical analysis

Basic washout data were analysed according to methods described earlier in this thesis, with results tabulated using a standard spreadsheet (Excel 2007, Microsoft, USA). Mean LCI, FRC, Vt and CEV were taken from 3 washouts. Individual washouts were excluded if FRC values differed by more than 10% of the next nearest result. FEV_1 values were converted to z-score using a spreadsheet plugin. Data for this are published in Stanojevic et al (Stanojevic et al. 2008), with the plugin available from www.growinglungs.org. Mean baseline (sitting) results were compared between children with CF and healthy controls using the Student's t-test. Paired sitting and lying results were also compared using a paired Student's t-test. Statistical calculations were performed using Prism version 4 (Graphpad software, USA).

Ethical approval

Ethical approval was obtained from the Lothian Research Ethics Committee (NRES 08/S1101/29)

Results

Twenty children with cystic fibrosis (median age[range] 7.5[5.1-14.8] years) were recruited, and analysed along with data from twenty-one healthy volunteers (median age[range] 11.8[5-17.8] years). Data from two children with CF were excluded as measurements on lying were too highly variable to be acceptable. Mean baseline sitting measurements in these 2 children were not extreme compared with the rest of the group (FEV_1 z-score -1.49 and -1.92 respectively, LCI 5.22 and 7.22 respectively).

Baseline measurements

Mean basic information and baseline sitting measurements are shown below for the subjects included in the final analysis. Mean(SD) FEV_1 z-score was lower and LCI was higher in children with CF compared with healthy controls (-1.39(1.5) vs. -0.21(0.9), $p<0.01$ and 8.28(2.2) vs. 6.23(0.4), $p<0.0001$). Importantly, mean FEV_1 was still within the "normal range" (>-2). Comparing baseline FEV_1 z-score and LCI (

Figure 41), 6 (33%) children with CF have raised LCI (>7.4) but normal FEV_1z-score (>-2). This indicates, as has previously been shown (chapter 4), that LCI appears to detect more abnormality in children with CF than FEV_1.

Mean %predicted FRC was similar between groups. Both Tidal volume and cumulative expired volumes were the same between groups, but because of height differences, it is incorrect to draw any further conclusions.

	Healthy volunteers (HV)	Cystic fibrosis (CF)	p (HV vs. CF)
Number	21	18	
Sex (m/f)	13/8	7/11	
Median Age (yrs)	11.8	7.6	
[range]	[5.0 - 17.8]	[5.1 – 14.8]	
Mean (SD) Height (cm)	147.2 (22.4)	129.1 (17.4)	<0.01
Mean (SD) FEV_1 (L)	2.50 (1.1)	1.36 (0.5)	<0.005
Mean (SD) FEV_1 z-score	-0.21 (0.9)	-1.39 (1.5)	<0.01
Mean (SD) FRC (L)	1.87 (0.9)	1.15 (0.4)	<0.01
Mean (SD) % pred FRC	101.0 (21.1)	98.79 (19.5)	ns
Mean (SD) LCI (cev/frc)	6.23 (0.4)	8.28 (2.2)	<0.0001
Mean (SD) CEV (L)	11.69 (5.4)	10.04 (5.3)	ns
Mean (SD) Vt (L)	0.48 (0.2)	0.40 (0.1)	ns

Table 17 - Baseline (sitting) measurements from healthy volunteers and children with CF. Mean (SD) values shown with between group comparison in the right hand column (*ns, not significant*).

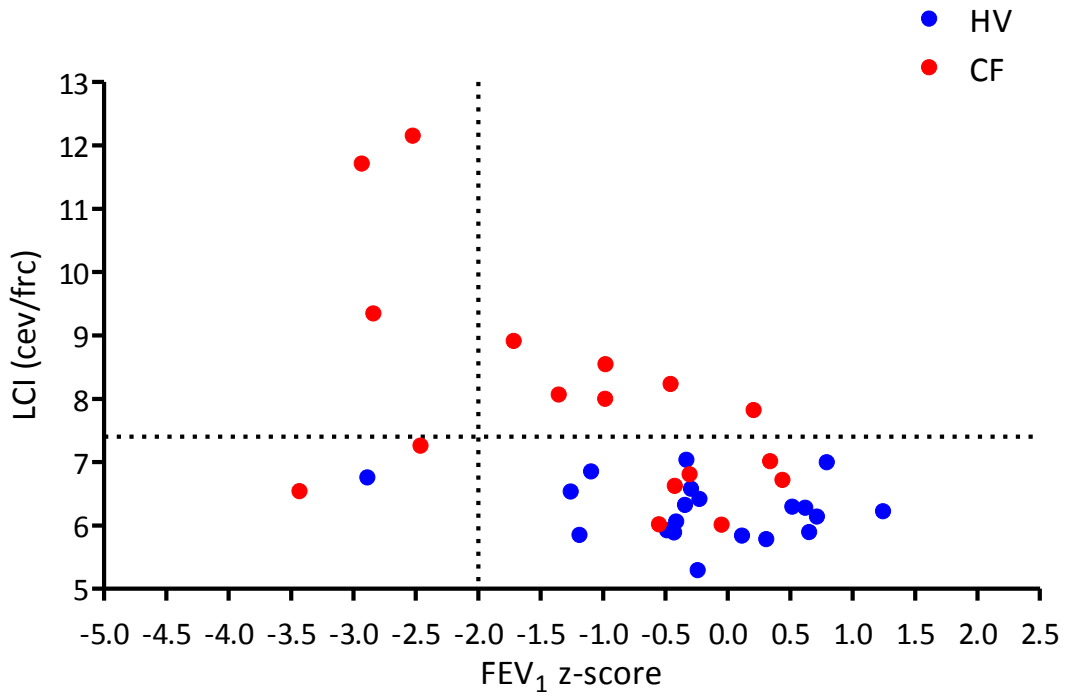


Figure 41 - Comparison of baseline (sitting) FEV1 z-score and LCI in children with CF (red) and healthy controls blue). Dotted lines indicate limits of normality: -2SD below reference

population mean for FEV1 and greater than 7.4 for LCI (based on previous local, as well as international data using other devices).

	Mean [95% CI] difference after change in posture		HV vs. CF	
	Healthy volunteers (HV)	Cystic Fibrosis (CF)	Are groups different?	Are variances different?
FEV_1 (L)	-0.21 [-0.3 – -0.1]*	-0.12 [-0.2 – -0.04]§	no	no
FEV_1 z-score	-0.67 [-1 – -0.4]*	-0.37 [-0.9 – -0.03]‡	no	no
FRC (L)	-0.60 [-0.8 – -0.4]*	-0.30 [-0.4 – -0.2]*	p=0.002	p=0.002
%pred FRC	-31.4 [-37.5 – -25.3]*	-24.2 [-28.3 – -20.9]*	p=0.03	p=0.049
LCI (cev/frc)	0.29 [0.1 – 0.5]†	0.64 [0.2 – 1.1]‡	no	p=0.003
CEV (L)	-3.29 [-4.3 – -2.3]*	-1.81 [-2.7 – -1.0]†	p=0.02	no
Vt (L)	0.01 [-0.05 – 0.06]^{ns}	-0.05 [-0.08 – -0.02]†	p=0.09	p=0.007

Table 18 - Change in measurements after change in posture in healthy volunteers and children with cystic fibrosis. Symbols show level of significance within groups: * p<0.0001, † p<0.005, § p<0.01, ‡ p<0.05, ^{ns} *not significant.* Significant differences between groups are shown in the right-hand columns.

Change in measurements with posture

There was a significant mean change in FEV1, FRC, LCI and CEV in healthy volunteers and children with CF. Only CF patients showed a significant change in tidal volume when supine, with a mean fall of 11% (50ml). There was a similar change in FEV1 and LCI (p>0.05), however while between group variance in FEV_1 was similar, there was a significantly greater variance in change LCI, indicating greater changes (positive and negative) in LCI in those with CF compared with healthy volunteers. There was a greater fall in absolute and %predicted FRC in healthy controls than those with CF. There is also a dissociation between change in FRC and change in LCI (CEV/FRC) as those with CF demonstrated a greater mean rise in LCI (although not significant). Intuitively, if reduction in FRC is the sole contributor to change in LCI the above would not be seen.

The magnitude of change in CEV and Vt cannot be compared between groups as both are growth related values. The observation that Vt changes significantly in children with CF, but not in healthy controls is still an important finding, however.

Figure 42 below shows the association between change in FEV_1 z-score and change in LCI. In healthy volunteers there is a reduction in FEV_1 and increase in LCI, but

not to the extent of becoming abnormal. The graph suggests that in those with a raised LCI, there is a greater change (in both directions) in LCI following change in posture. Because of the numbers involved it is not possible to perform sub-analysis, but the greatest change in LCI appears to be in those children with a moderately raised sitting value, rather than in those with the poorest lung function. Performing Pearson r correlation, there is no association between change in FEV_1 and change in FRC, LCI, CEV or Vt, following change in posture.

This is illustrated in Figure 43. Average LCI (sitting and lying) in each subject is plotted against magnitude of change on the y axis. This is an approximation of the Bland-Altman plot, with bias and 95% limits of agreement of change in LCI (mean change +/- 1.96*pooled standard deviation) with the solid and dotted lines on the Y-axis. The dotted line on the x-axis indicates the upper limit of normal LCI. The greatest change in LCI is mainly limited to 6 (33%) of subjects with CF and a moderately raised LCI. This change is not consistent as there are 2 others with moderately raised LCI and no change. A minimum change in LCI is seen in subjects with CF with a normal LCI and healthy controls. This indicates that the presence of lung disease contributes to changes in LCI following change in posture.

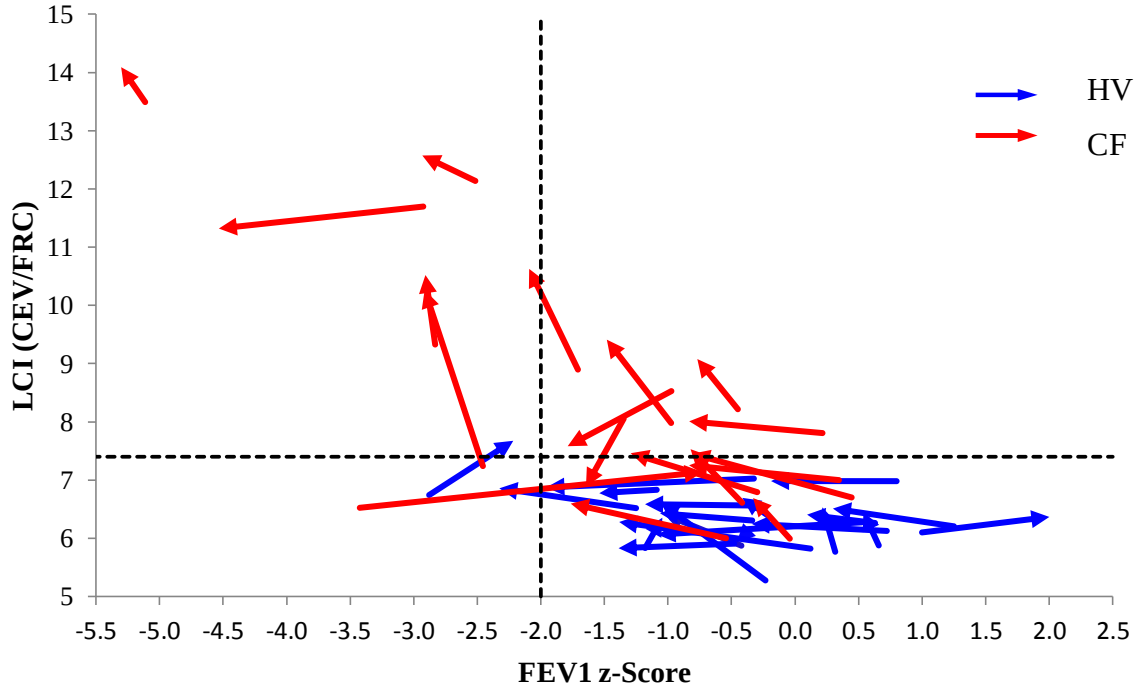


Figure 42 – Effect of change in posture on children with CF (red arrows) and healthy volunteers (blue arrows). Direction of change is indicated, as is association between change in FEV1 z-score and change in LCI. Direction towards the top left, indicates worsening of both LCI and FEV_1.

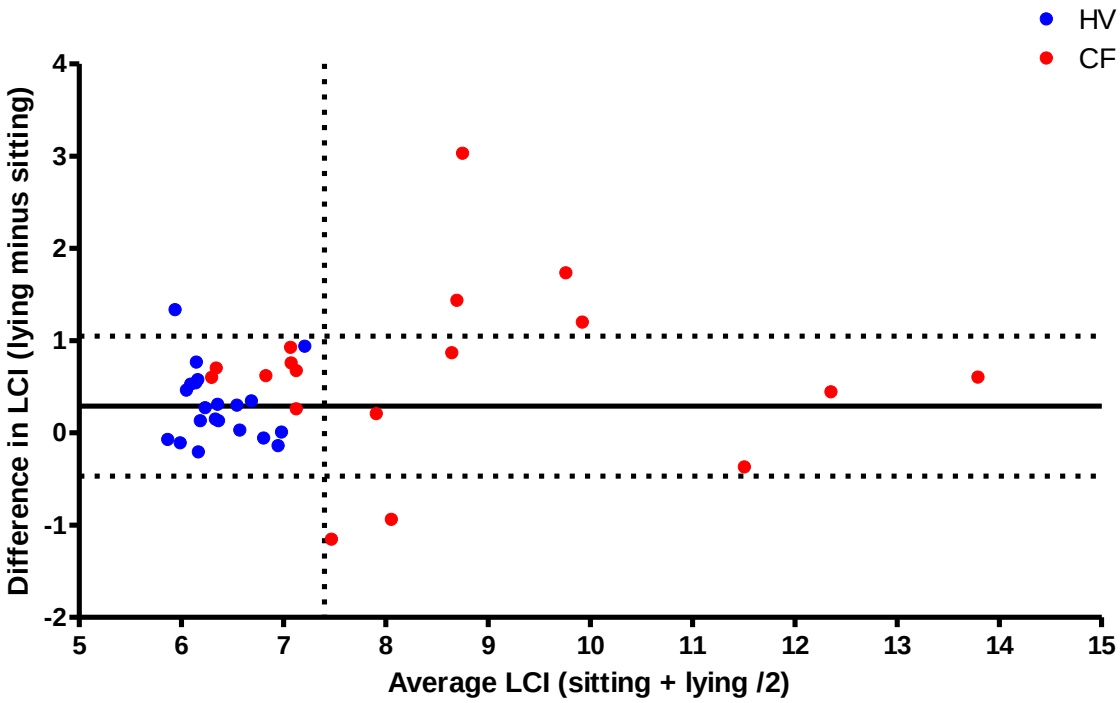


Figure 43 - Modified Bland-Altman plot showing magnitude of change in LCI based on average value. Y-axis lines show bias and 95% limits of agreement, with x-axis line showing upper limit of normal LCI. Greater changes are seen in those with a moderately abnormal LCI

To investigate associations with change in LCI, correlations calculations (Pearson r) indicate that change in LCI after lying did not correlate with change in FEV_1 z-score, FRC, CEV or Vt in healthy volunteers. In children with CF, change in LCI only weakly correlated with change in CEV ($R^2 = 0.27$, p=0.03) and did not correlate with change in FEV_1 z-score, FRC or Vt.

Conclusions

This study shows that the effects of change in posture, measured by LCI, are different between healthy controls and those with lung disease. In both groups of children, FRC and FEV_1 fell and LCI rose after 30 minutes of supine posture compared with sitting. Importantly this change was significantly more variable in the children with CF than healthy controls; with the greatest individual changes limited to those with measurable lung disease (raised sitting LCI) compared to those with normal baseline measurements. Change was not in one direction, in that LCI both fell and rose beyond the 95% limits of agreement of healthy volunteers.

This is the first study to show such results, and has implications for the use of LCI in young children where measurements are generally performed supine. In the scenario of following longitudinal change in lung function in infants, those who progress over time from lying to sitting to perform the test may have a disproportionate change in lung function from one test to another, depending on the extent of lung disease present. It may be hard to interpret to what extent this is a worsening in lung function, limiting the clinical application of the test.

Previous studies have shown that change in posture reduces resting lung volume (FRC). The increased effect of supine posture on LCI in those with a raised LCI has not previously been demonstrated. It is not clear why Aljassim et al demonstrated no change in LCI in children with CF(F Aljassim et al. 2009), whereas we have shown the opposite. In another paper by the same group, LCI did not rise in asthmatic children or healthy controls following a more prolonged supine period (1 hour)(Gustafsson 2003). Professor Gustafsson uses a standard operation procedure that is slightly different to that described in chapter 2. He distracts all subjects with a cartoon or film, but in older children he displays tidal volume targeting. This information is from discussion with him about general MBW principles, rather than

from the published article, where no mention is made of tidal volume targeting. It may be that with targeted tidal volume breathing, any disruption of supine posture to ventilation heterogeneity is negated by compensating with deeper breathing, although this does not explain why in our study healthy volunteers saw a rise in LCI, and no change in tidal volume. Respiratory rate was not measured in this study, and may be another compensatory measure for disruption to ventilation.

In our study, LCI did not change as a function of reduced FRC. Also, there was a reduction in tidal volume in those with CF, but not in healthy controls. The only association with rise in LCI, was increase in CEV, and only in children with CF. Interestingly, FRC and CEV both fell more in the healthy control group than CF. This suggests that there are intra-airway differences in disease groups compared with healthy controls that cause a fall in tidal volume, presumably due to airway collapse. The presence of airway inflammation and alterations in airway architecture (bronchiectasis) may contribute to a reduction in airway muscle tone which leads to increased airway collapse following a reduction in resting lung volume upon adopting supine posture.

Study limitations

This study is limited in that it did not set out to investigate respiratory effort or minute ventilation. It is not clear why there are differences between CF and healthy groups, and a further study involving detailed respiratory rate and minute ventilation measurements would help to understand the relationship between supine posture and resultant changes in breathing pattern. Larger numbers would be able to investigate whether degree of ventilation heterogeneity leads to increased changes following change in posture.

A criticism of the observation that the greatest changes in LCI were seen in those with moderately raised LCI, is that intra-test variability is higher in this group anyway. Therefore even without a change in posture, there would be a bigger intra-test positive or negative change in LCI. Importantly, in those with the highest LCI values, where test variability would be expected to be highest, there was minimal change following supine posture. Also, correlating sitting and lying intra-test

variability (CV%) with between posture variability showed no association ($p>0.1$). There was also no association between sitting LCI and between-posture variability ($p>0.1$).

Studies of sleep hypoxaemia and disordered breathing in children and adolescents with CF postulate that increased airway resistance with supine posture, increased V/Q ratio, and decreased respiratory drive contribute to impaired oxygenation(Tepper et al. 1983, Villa et al. 2001). This appears to occur mostly during deep (REM) sleep. A reduction of tidal volume in deep sleep due to decreased muscle activity reduces resting lung volume, thereby increasing airway closure and decreasing V/Q ratio, leading to desaturation. In this study we have simulated sleeping posture but while awake. We have still seen an overall increase in LCI in healthy control subjects as well as those with CF. The greatest changes were seen in CF patients with measurable lung disease. An increase in ventilation heterogeneity after changing to supine posture may go some way to explaining why hypoxaemia occurs in children with CF. This study indicates that it may be gravity-dependent, as well as sleep related factors, which lead to desaturation during sleep. With a future carefully designed study, LCI may prove to be an important measurement to help decide which patients are at risk of sleep-induced hypoxaemia, as sleep studies are complex and time-consuming to perform.

Learning points and contribution to thesis

In conclusion, care should be taken when interpreting MBW results in young children over time, as posture may contribute disproportionately to a rise in LCI in those with lung disease, compared with those with normal values. This is important in research and future clinical use. While there is only one published longitudinal study in CF involving younger age-groups(Kraemer et al. 2005), it is expected that with wider use, more groups are ready to publish data in larger populations over longer periods of time. These data should be interpreted with posture taken into account.

This study is also important for the wider themes of this thesis. Previous chapters have already demonstrated that Innocor shows promise as a tool for detecting and tracking change in lung disease over time in children with lung disease. This study

matches previous lung function and posture studies, in that a decrease in FRC was seen. Increased ventilation heterogeneity (interregional) is also previously documented in healthy men(Gronkvist et al. 2002). This study used LCI as a measure of overall heterogeneity, and there was a mean increase in healthy control and disease groups. There was a bigger increase in the CF group although this did not reach statistical significance. Variability (sitting vs. supine) was greatest in those with moderate lung disease. LCI measured with Innocor appears able to detect posture-related changes within the airways.

This study was the first to use trained nursing staff and another clinical research fellow to perform the washouts. The additional staff were trained locally after observing many washouts and becoming familiar with the equipment. Because my post as clinical research fellow came to an end during the study, most of the CF patients were tested by other staff. There was no visible difference between their data and mine, in terms of washout quality. No more washouts were discarded following the change in operator. While occasional technical support was required from me, it was mainly due to software issues, rather than analyser issues. To restate the main reason for Innocor validation, Innocor is a more compact, robust, affordable alternative to a mass spectrometer. It is also simpler to operate, as demonstrated by this study. A mass spectrometer operator needs to attend a formal training session to be able to use this equipment. While adequate formal training is essential in the context of a larger multi-centre study, it is clear from this study that good quality results can be obtained without the need for on-site technical knowledge.

Chapter 7 - Longitudinal change in LCI in children with progressive lung disease.

Introduction

This chapter describes a large clinical study conducted on behalf of the UK CFGTC. While the work of this group does not contribute to the central theme of this thesis, it is for reasons of completeness and general interest I have included much of the descriptive information about the "Run-in Study". The findings of this study have much to contribute to the central thesis aims. It would not have been possible to obtain these data without the consortium initiative. The following describes some background to the CF gene therapy "run-in" study.

In 2001, the CFGTC was established to investigate and develop agents for use as pulmonary gene therapy for Cystic Fibrosis. In the first 5 years of the consortium, essential basic scientific work established the principles of gene therapy for cf – a plasmid containing a genetic code for CFTR, and a non-viral vector to carry the plasmid into the airway cells. Despite the large number in the past 17 years, clinical trials of gene therapy have so far been limited to a small numbers of patients measuring a cellular or electro-chemical response(Griesenbach et al. 2009) . Positive results from these studies have stimulated progression from basic to clinical science. Instead of only assessing cellular responses alone, a clinical benefit must also be elicited.

In CF the most commonly quoted clinical outcome is survival. Fortunately for younger patients this end-point is many years in the future as overall life expectancy is increasing. Either the trial of treatment would need to be very prolonged, or the follow up period set far in the future. This incurs a lot of expense and very slow progression of scientific knowledge. In recent years, the response to the difficulties of measuring slow disease progression has been to investigate surrogate markers of long term outcomes. The ideal assay is simple and repeatable, representing a true assessment of disease progression, and how a new intervention has influenced this severity

The CFGTC conducted the tracking study in 2006. This study recruited adults and adolescents with CF during a respiratory exacerbation. Measurements were

performed at the beginning, end and 2 weeks after a course of intravenous antibiotics. A main aim of this study, in addition to collection of valuable data on assay variability, was development of the best available surrogate assays relevant to CF in adolescents and adults, individuals most likely to receive Gene Therapy in future trials. Given that these assays were complicated to set up, perform and analyse, the tracking study provided an opportunity to practice in a real clinical research situation. Assays in this project were selected to measure all aspects of CF lung disease – lung function, inflammation, structure, infection, symptoms and quality of life/ daily functioning.

In 2008, the Run-in study commenced. Using data and experience from the tracking study, assays were selected if tolerable to patients and most likely to detect a change following treatment with gene therapy. They needed to be reproducible and repeatable in real CF patients. One of these assays is Lung Clearance Index. I was employed by the CFGTC in 2006 primarily to recruit and retain paediatric patients to this long-term study in Edinburgh.

This chapter briefly describes the Run-in study method, but will only concentrate on MBW and spirometry results performed in children and teenagers recruited by me, as other assays were not managed by me, and have not yet been completely analysed. Many assays will be listed, but not explained as results are not presented here. The run-in study will in due course progress to a clinical trial of gene therapy, and recruitment from those who completed the run-in study has begun at the time of writing this chapter.

In the context of this thesis, the whole run-in study is of limited importance. However such a study assesses the practicalities of using Innocor in a long-term study in larger numbers of subjects, i.e. how robust the equipment is, and how acceptable it is to patients. In addition, data from the run-in study is able to assess how well LCI detects change in lung function compared with FEV_1. These are some of the first data to measure longitudinal change in ventilation heterogeneity (LCI) in young people with CF. In another important study which helps to inform the run-in study, Kraemer et al previously published annual clinical and lung function data in 142 children and adults with CF (aged 6 to 20 years)(Kraemer et al. 2005). They

demonstrated that LCI, along with FRC_{pleth} and MEF_{50} had the steepest slope of progression over time. FEV_1 progression was least steep.

Scientific basis for inclusion of LCI in the run-in study

Previous clinical studies measuring LCI in CF have mainly been limited to single measurements or very short term change, with some groups beginning to collect longitudinal data(Robinson et al. 2010, Robinson et al. 2009a, Gustafsson et al. 2008, Kraemer et al. 2005, Aurora et al. 2004, Fuchs et al. 2010, Riedel et al. 2009). In developing LCI as a potential assay in future gene therapy trials, sensitivity to change over the planned gene therapy trial needs to be estimated. LCI must therefore be able to measure the slow, gradual decline often seen in CF, the rate of which is highly variable between patients. A complicating factor is how acute events such as exacerbations contribute to this. For this reason the run-in study was designed to perform serial measurements over time, in clinically stable individuals. The hypothesis was that if a predictable change in LCI is seen over time (12-18 months), repeatable between visits, and correlating with change in other assays (e.g. structure, function, symptoms), then it may be suitable for detecting a halt in decline, or even improvement following repeated doses of inhaled gene therapy.

As stated previously, LCI is thought to be a global measure of ventilation heterogeneity, contributed to by airway changes in the small to medium airways. If CF lung disease progresses from the small to medium airways, LCI is intuitively more likely than FEV_1 to be a more suitable assay.

Prior to starting the run-in, the results of which are not complete at the time of writing this chapter, the proposed broad selection criteria for the future gene therapy trial were:

1. Those in which CF lung disease is severe enough to measure with the available assays.

CF begins in infancy with early evidence of airway inflammation(Armstrong et al. 2005, Armstrong et al. 1997, Armstrong et al. 1995). The earliest changes appear to be only evident in invasive broncho-alveolar lavage studies, or on CT scan, with associated radiation risks with repeated measurements (de Jong et al. 2006, Armstrong et al. 2005, Dakin et al. 2002a, Dakin et al. 2002b, Gustafsson et al.

2008). Most other tests are normal, and remain so until late childhood. Because LCI appears to detect mild or early airway disease, it was added to the list of assays for the run-in study and gene therapy trial.

2. Those in which lung disease is not so severe that there is too great a barrier to inhaled gene transfection.

Mucous biolayers and inflammation cause thickening of the wall between air and cells. In advanced disease it may be that this barrier is too thick, rendering transfection of the inhaled gene product unsuccessful(Griesenbach et al. 2006). The obvious caveat to this is that a "no signal" result from a gene therapy trial may be due to lack of availability of the product due to intra luminal barriers to transfection, or simply no response because the product is defective.

The consortium summarise those patients with measureable disease, which is not too severe for successful transfection of inhaled therapy as the "can deliver, can measure".

It is likely that those in the adolescent age-group will be those in the "can deliver, can measure" group. Intuitively it makes sense to treat those earlier in the disease to get the most benefit. However, concerns about the ethical propriety of recruiting young children to the first multi-dose gene therapy trial resulted in a minimum age of 12 years for the first multidose trial. For the run-in study, children as young as 10 were recruited with the realisation that the run-in will take 18 months to 2 years to complete.

Chapter aims

This chapter uses FEV_1 and LCI data from all paediatric patients in the Run-in Study at the Edinburgh RHSC site as these are measurements I performed and analysed.

The primary aim is to:

- Assess the sensitivity of LCI measured using Innocor compared with FEV_1 on multiple occasions over 18 months in cystic fibrosis lung disease.

Secondary aims are:

- Long-term reproducibility of LCI measurements performed using Innocor on healthy controls.

- Suitability of Innocor for larger clinical studies. In particular, how robust is the equipment, and how acceptable is the test to children with lung disease?

Methods

The protocol for the run-in study was assembled by a large team of experienced clinical and laboratory researchers over many months. My role was refining the protocol for the LCI in children, along with Dr Alex Horsley, the clinical research fellow responsible for recruitment of adult patients. While I had some input to the assay order, timing of testing, protocol writing and data analysis, this is not my study and apart from the MBW assay, the protocol is not mine. The following is a description of the methods for the run-in study, but in addition there are important data from healthy volunteers that were not part of the run-in study. These children were recruited initially as part of other studies included in this thesis, and the study protocol was my responsibility.

Children with CF

According to inclusion and exclusion criteria, paediatric patients in centres local to Edinburgh were invited to take part in the run-in study, with the added information about the forthcoming gene therapy trial. Local centres were identified where travelling time to the study site (Royal Hospital for Sick Children, Edinburgh) was deemed acceptable. Four clinical sites were contacted: Edinburgh (Royal Hospital for Sick Children), Glasgow (Royal Hospital for Sick Children, Yorkhill), Stirling (Stirling Royal Infirmary) and Dundee (Ninewells Hospital). Together with responsible consultants and specialist nurses, families were identified who fitted the inclusion criteria. Information was sent out, with the option of a telephone discussion regarding the details of the study. A slightly different approach was chosen at each site, depending on the responsible consultants' preferences. Some preferred to select families they thought would be suitably interested and motivated. Other centres preferred to be as equitable as possible, giving all eligible families the opportunity to opt in.

Interested families returned the invitation and a date was set for the first visit, after checking inclusion and exclusion criteria. Patients were considered recruited at the time of commencing the first study visit.

Visits were estimated to take around 3 hours. 2 visits could take place per day, with time in-between to clean the room and ensure low risk of cross-contamination of colonised bacteria.

Parallel recruitment of adult patients from Scotland took place at the Western General Hospital, and because some assays required laboratory staff support, only 2 days per week were allocated as paediatric study days, with a total of 4 possible study visits per week. A 16 week lead-in time was built in to the study timetable for completion of all first visits. Therefore, the maximum possible number of paediatric patients in the Royal Hospital for Sick Children was 64; however it was clear that many appointments would need to be changed at the last minute because of exacerbations and other variations to management affecting the strict stability criteria. Therefore flexibility was built into the timetable to allow the freedom to change appointments if patients were not able to attend.

The run-in study was conducted over 18 months, with patients attending 4 times over that period for a uniform series of measurements. The time period in between the 1st and 4th visit was 12-15 months. The study was conducted over 3 sites – the Royal Brompton Hospital, London (paediatric and adult), and the Western General Hospital (>16 years) and Royal Hospital for Sick Children (RHSC), both in Edinburgh.

Patients were recruited with the following inclusion and exclusion criteria (taken from the study protocol):

Inclusion

1. Cystic fibrosis confirmed by a combination of clinical symptoms/ signs and sweat test, genetic analysis or nasal potential difference measurements.
2. Forced expiratory volume in 1 second (FEV_1) > 40% of predicted values.
3. Clinical stability at entry defined by:
    a. Not on any extra antibiotics (excluding routine, long-term treatments) for the last 2 weeks.
    b. No increase in symptoms such as change in sputum production/ colour, increased wheeze or breathlessness over the last 2 weeks.
    c. No change in regular respiratory treatments over the last 4 weeks.
4. Aged 10 years and above.

5. Written informed consent from patient (aged 16 years and above) or parent if younger child; assent from child where appropriate.
6. Permission to inform GP of participation in study.

Exclusion

1. Current participation in any other interventional clinical trial.
2. Infection with *Burkholderia cepacia* complex organisms or MRSA unless local infection control guidelines can be adhered to.
3. Awaiting/ referred for or post-lung transplant.
4. Chronic day-time oxygen requirement.
5. Previous spontaneous pneumothorax unless patient has had a subsequent pleurodesis.
6. Recurrent severe haemoptysis.
7. Current smoker (will be assessed by measuring urinary cotinine level or exhaled CO prior to study entry).
8. Significant comorbidity including:
    a. Moderate/ severe CF liver disease (varices or significant, sustained elevation of transaminases: (ALT/ AST>100 IU/l).
    b. Significant renal impairment (serum creatinine > 150 μmol/l).
    c. Significant coagulopathy.
9. Receiving 2nd line immunosuppressant drugs such as methotrexate, cyclosporine or intravenous immunoglobulin preparations.
10. Pregnancy.

Therefore, these criteria were designed to select those with confirmed CF, and were likely to select those with measureable lung disease, without being too severe to be able to benefit from the treatment in a subsequent gene therapy trial.

Assays performed at each visit are shown below. A detailed description of each test is not provided, as results are not available for this chapter. It is shown here only as an outline description.

Test	Assays	Visit 1	Visits 2 - 4	Pre- Gene Therapy Trial
Sputum expectoration	Microscopy and culture Inflammatory assays	✓	✓	✓
Spirometry	FEV_1 FEF_{25-75} FVC	✓	✓	✓
Blood tests	Standard clinical biochemistry Full Blood Count CRP Research Inflammatory cytokines	✓	✓	✓
Exhaled Breath Condensate	pH, nitrite and ammonia	✓	✓	✓
Urine	Research inflammatory assays	✓	✓	✓
Quality of Life Questionnaire	Standardised Questionnaire	✓	✓	✓
Multiple Breath Washout	LCI FRC	✓	✓	✓
Shuttle Walk Test	Modified 6-minute walk test	✓	✓	✓
Hypertonic Saline Sputum induction	If no spontaneous sample obtained	✓	✓	✓
High Resolution CT scan	Various scored items	✓	×	✓

Additional interim measurements

Pedometer	Steps taken each day in the week prior to testing
Weekly Spirometry (Piko-6 handheld spirometer)	FEV_1 and FVC (FEV6) performed on the same day, weekly between measurements

Table 19 - Full schedule of gene therapy run-in study

The study was designed to perform the assays at a time of clinical stability. To ensure this was the case, a standard phone questionnaire was conducted with each patient or parent approximately 1 week prior to each visit. The stability criteria shown below refer to the week prior to the visit, except the last question relating to routine medication:

- No new courses of iv or oral antibiotics
- No fever greater than 38^{o}C (temperature was asked to be taken with "tempa-dot" thermometer whenever fever suspected)
- No unscheduled absence from school or work due to CF illness
- No increased cough
- No major change in sputum volume or consistency or change in chest symptoms
- No increased shortness of breath
- No new haemoptysis
- No other clinical issues that may affect the measurements
- No change in routine respiratory medication in previous 3 weeks

If there were any stability issues, the appointment was re-scheduled for 3 weeks later, and stability re-checked. The sequence of questions was not a validated technique, but was formulated to make sure patients adhered to inclusion and exclusion criteria, and to allow the best comparison of results between visits.

Only spirometry and MBW data are presented in this chapter, therefore the methods of other measurements are not described. Spirometry and MBW data were collected at every visit, according to methods described in chapter 2.

MBW was performed using Innocor to collect flow and gas concentration data for calculation of FRC and LCI. The setup and analysis was identical to that described in Chapter 2.

FEV_1 was performed using the EASYONE device (ndd, Switzerland) as described in previous chapters. Measurements were performed standing, without a noseclip. Subjects were asked to inhale to full vital capacity, and then forcefully exhale without a pause to residual volume. As previously described, 3 good quality forced

expirations were performed at flow limitation, according to ERS criteria; with the highest FEV_1 value dictating which manoeuvre to select.

Healthy control population

To assess long-term reproducibility of measurements in healthy controls, data from a cohort recruited to 2 previous clinical studies were analysed. In 18 cases, the same healthy children were recruited to the asthma study (chapter 4) and posture study (chapter 6). As measurements in these studies were completed approximately 24 months apart, this is a convenient sample to study.

This represents a retrospective analysis and would not be valid if there were changes to the test standards or equipment during this time. Importantly, the same MBW test protocol was used for the asthma, posture and run-in studies – that outlined in chapter 2. Similarly, FEV_1 was performed to the same standards in each study.

Statistical analysis

Measurements were expected to fluctuate over time due to natural variability and temporal changes in gas mixing (to be thought of as random variation). The purpose of this analysis is to assess the ability of LCI to detect longitudinal change in CF lung disease compared with Spirometry (FEV_1), independent of these fluctuations. For this purposes of this chapter, this change is thought of as true progression in disease over time, although this implies that random variability is reversible, and underlying disease progression is not. With this major limitation in interpretation in mind, the following statistical analysis was performed.

Students t-test comparison of 1^{st} and 4^{th} measurements is the most basic way to assess these data. The null hypothesis is that the overall change in LCI and FEV_1 over 18 months (4 measurements) is no greater than two standard deviations from the baseline measurement.

Basic t-test analysis assumes that the 1^{st} and 4^{th} measurements are not influenced by random fluctuations, by not taking into account the 2^{nd} and 3^{rd} measurements. Analysis of variation (ANOVA) allows analysis of more than 2 measurements to see if an observed difference is likely to be greater than that expected from random variability, and is a ratio of within and between test variability(Altman and Bland 1996).

To assess the reproducibility of measurements in health controls, within-subject variability of LCI measurements (Coefficient of variability, CV%) is measured between 2 visits, similarly spaced. Variability of FEV_1 z-score measurements is also assessed, although CV% is not appropriate in this case due to the distribution of values around zero.

FEV1 and height z-scores are taken from published normal populations(Freeman et al. 1995, Stanojevic et al. 2008), using Microsoft Excel plug-ins to calculate values (www.growinglungs.org.uk). %predicted FRC is taken from an age-appropriate reference population(Stocks and Quanjer 1995).

This study as a whole was powered with the gene therapy trial in mind. No statistical power calculation was done. A maximum of 100 patients were able to be recruited to the future trial, and to take into account attrition and future exclusions from the trial, twice as many were recruited to the run-in study. In the paediatric arm of the study in Edinburgh, the maximum number able to be recruited was 36, given the manpower and time taken to complete each visit.

Ethical approval

The complete run-in study was approved by the Kings College Hospital Research Ethics Committee (07/Q0703/78). Data from patients in this study were taken for inclusion in this chapter.

Results

34 paediatric patients (median age 12.3 [range 10-17] years) were recruited to the run-in study and completed the first visit. Basic demographic information is shown in Table 20. One child was not eligible following visit 1 because of Cepacia growth in induced sputum. One subject did not complete v3 and v4, and one did not complete visit 4, because they were too old and had moved to the adult CF service. Two further subjects did not complete v4 because unable to attend due to exacerbations and breaching stability criteria. Therefore, 29 patients (88%) have complete Spirometry and MBW data for 4 visits. Data are included for all 34 patients in the following tables and graphs.

18 healthy control subjects (median age 19.2 [range 5 – 16]) are included as a convenient control to assess long-term reproducibility of MBW measurements in children.

	Run-in Study	Healthy controls
Number	34	18
Male/female	22/12	12/6
Median age (years) [range]	12.3 [10 – 17]	10.2 [5 - 16]
Median height (cm) [range]	152.0 [137 – 184]	146.0 [118 – 180]
Median height z-score [range]	-0.17 [-2.3 – 3.1]	0.86 [-1.5 – 2.7]
Mean (SD) FEV_1 z-score [range]	-1.62 (1.3) [-5.4 – 0.5]	-0.82 (1.1) [-3.1 – 1.3]
Mean (SD) FRC (L) [range]	1.99 (0.6) [1.3 – 3.7]	2.01 (1.1) [0.8 – 4.5]
Mean FRC % predicted [range]	98.54 (17.7) [69.7 – 128.4]	111.7 (21.4) [74.0 – 145.7]
Mean LCI (cev/frc) [range]	9.02 (1.8) [6.7 – 14.2]	6.39 (0.55) [5.3 – 7.4]

Table 20 - basic demographic information. CF and healthy control groups

In this CF population Mean (SD) LCI (9.02(1.8)) was significantly higher, and FEV_1 (-1.62 (1.3)) was significantly lower, than healthy controls (6.39 (0.6), p<0.0001 and -0.82 (1.1), p=0.03).

27 patients (80%) had abnormal LCI (>7.4) at baseline. 13 patients (38%) had abnormal FEV_1 (<-2). These cross-sectional data demonstrate that increasing age appears to be associated with abnormal LCI and FEV_1 (Figure 44 Figure 45).

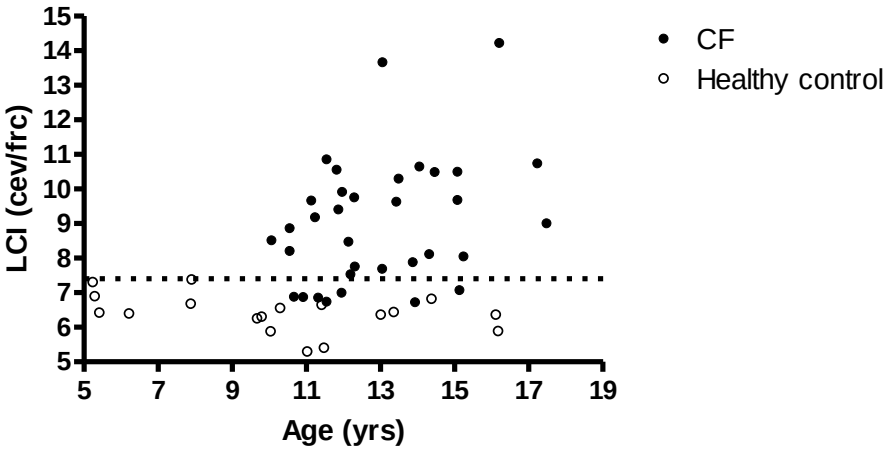


Figure 44 - Baseline (visit 1) LCI measurements by age

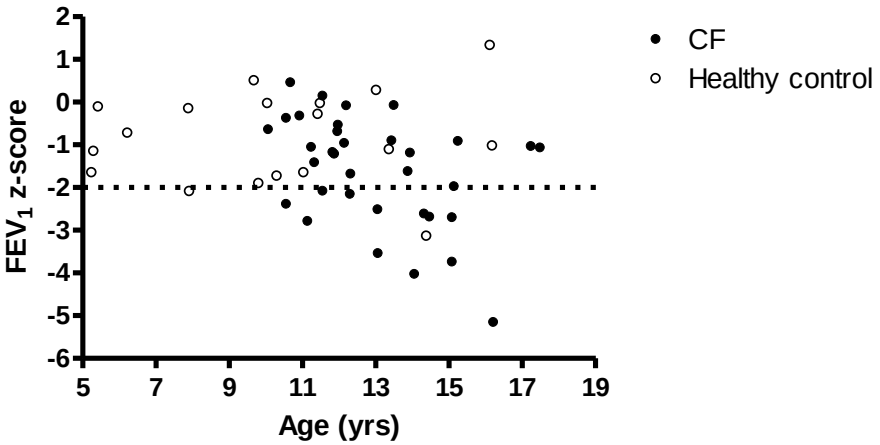


Figure 45 - Baseline (visit 1) FEV_1 z-score measurements by age

Comparing baseline (visit 1) FEV_1 and LCI measurements, most either have both a normal FEV_1 and LCI, or abnormal FEV_1 and LCI. 14 subjects (41%) have an abnormal LCI but normal FEV_1, whereas only one with a normal LCI had a

marginally abnormal FEV_1. Figure 51 shows baseline FEV_1 vs LCI. There is a significant correlation between these measurements (R^2= 0.41, p<0.0001).

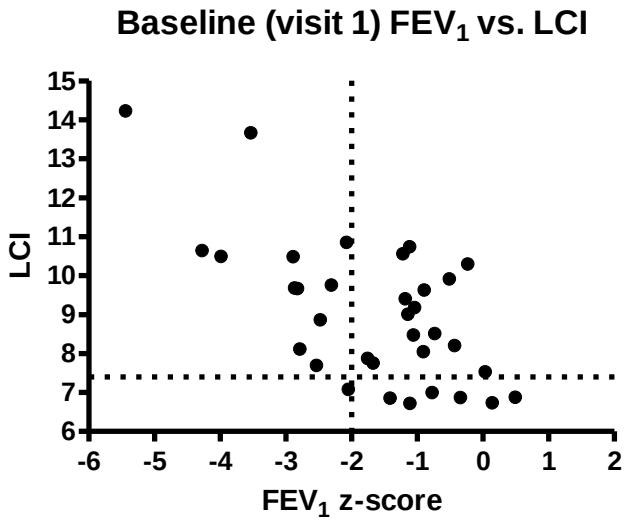


Figure 46 - baseline FEV1 standard deviation score vs. LCI in children with CF

Comparison of CF with healthy controls – long-term change and reproducibility

	Run-in subjects			Healthy controls		
Number	29			18		
	Visit 1	Visit 4	t-test	Visit 1	Visit 2	t-test
Median Age (yrs)	12.3	13.4		10.1	12.3	
[range]	[10 – 16]	[11 – 17]		[5 – 16]	[6 – 18]	
Median time between measurements (months)	13.6			22.2		
[range]	[10.6 - 16.3]			[16.3 – 31.8]		
Mean (SD) LCI	8.96 (1.9)	9.14 (2.1)	p=0.38	6.39 (0.6)	6.31 (0.5)	p=0.57
[range]	[6.7 – 14.2]	[6.1 – 15.5]		[5.3 – 7.4]	[5.3 – 7.0]	
Mean (SD) FRC	1.97 (0.6)	2.10 (0.7)	p=0.049	2.01 (1.1)	2.20 (0.9)	p=0.048
[range]	[1.3 – 3.7]	[1.3 – 4.5]		[0.8 – 4.5]	[0.9 – 4.1]	
Mean (SD) %pred. FRC	97.90 (17.8)	92.86 (15.8)	p=0.07	111.75 (21.4)	129.5 (30.6)	p=0.01
[range]	[69.7 – 126.5]	[71.2 – 136.8]		[74.0 – 145.7]	[81.4 – 181.3]	
Mean (SD) FEV_1 z-score	-1.56 (1.3)	-1.85 (1.4)	p=0.09	-0.83 (1.1)	-0.24 (1.0)	p=0.05
[range]	[-5.2 – 0.5]	[-5.5 – 0.1]		[-3.1 – 1.3]	[-2.9 – 1.3]	

Table 21 - Comparison of CF subjects with healthy controls. First and last measurements in both groups are compared. Significant differences (students t-test) are shown in bold (p<0.05).

Table 21 shows how measurements change over time. There is a significant change in FRC, which is to be expected in growing lungs. There is a rise in %predicted FRC in healthy controls, with a trend towards a fall in CF. There is a trend towards a rise in FEV_1 z-score in healthy controls (p=0.05), with a slight fall in CF patients (p=0.09), although neither reach statistical significance. Importantly, there is no significant change in LCI in either group.

Analysing change in LCI and FEV_1 in healthy controls shows that LCI performs well. Variability (CV%) within subjects over 2-3 years is <5%. This is illustrated in Figure 47 and 48. FEV_1 z-score does appear to increase over the time period in healthy controls; however this trend did not quite reach statistical significance.

Figure 47 and 48 illustrate the direction and variability of change in healthy controls over time.

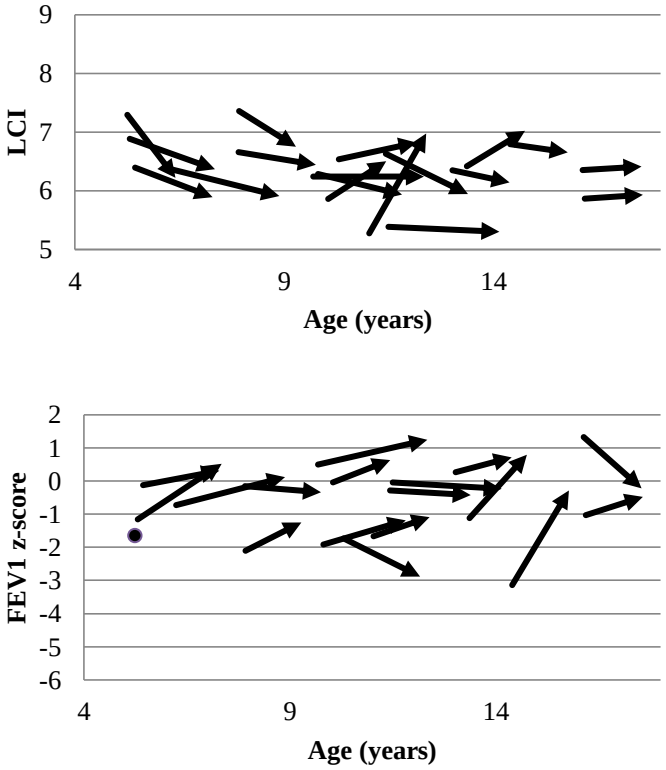


Figure 47 and Figure 48 - LCI and FEV1 z-score at 2 time points plotted against age in years. This chart shows change in individual LCI over time in healthy controls. The arrow shows the direction of change. In one subject only one FEV1 result was available due to technician error (indicated by a dot)

Longitudinal change in LCI and FEV_1 in children with CF

Performing One-way Analysis of Variation (ANOVA) shows a significant increase in LCI at timepoint 3. There is no significant change in FEV_1 at any timepoint (Table 22). Performing ANOVA for trend however, shows no significant change in LCI over the 4 timepoints (p=0.2) whereas FEV_1 has a significant, if small, trend over the time period (slope -0.06, p=0.03). In this population, therefore, there is an overall decrease in FEV_1 over the time period, whereas LCI was not able to detect a change.

	V1 (Baseline)	V2	V3	V4	1-way ANOVA for trend p
Number	34	33	32	29	
Mean (SD) FEV_1 z-score	-1.62 (1.29)	-1.55 (1.51)	-1.75 (1.74)	-1.85 (1.36)	0.03
Mean (SD) LCI	9.02 (1.83)	9.30 (2.40)	9.62 (2.04)*	9.14 (2.11)	ns

Table 22 - Mean FEV_1 z-score and LCI values over 4 visits in children with CF. There was a trend towards a decline in FEV_1 over the time period. * significant increase in LCI only at this timepoint (1-way ANOVA, p=0.02).

Figure 49 illustrates the complexity of overall trend in LCI and FEV_1. The top plots show the overall change from v1 to v4. The bottom plots show the degree of variability of measurements over the 4 timepoints, illustrating the importance of multiple repeated measures to detect a significant change, independent of period "random" fluctuation.

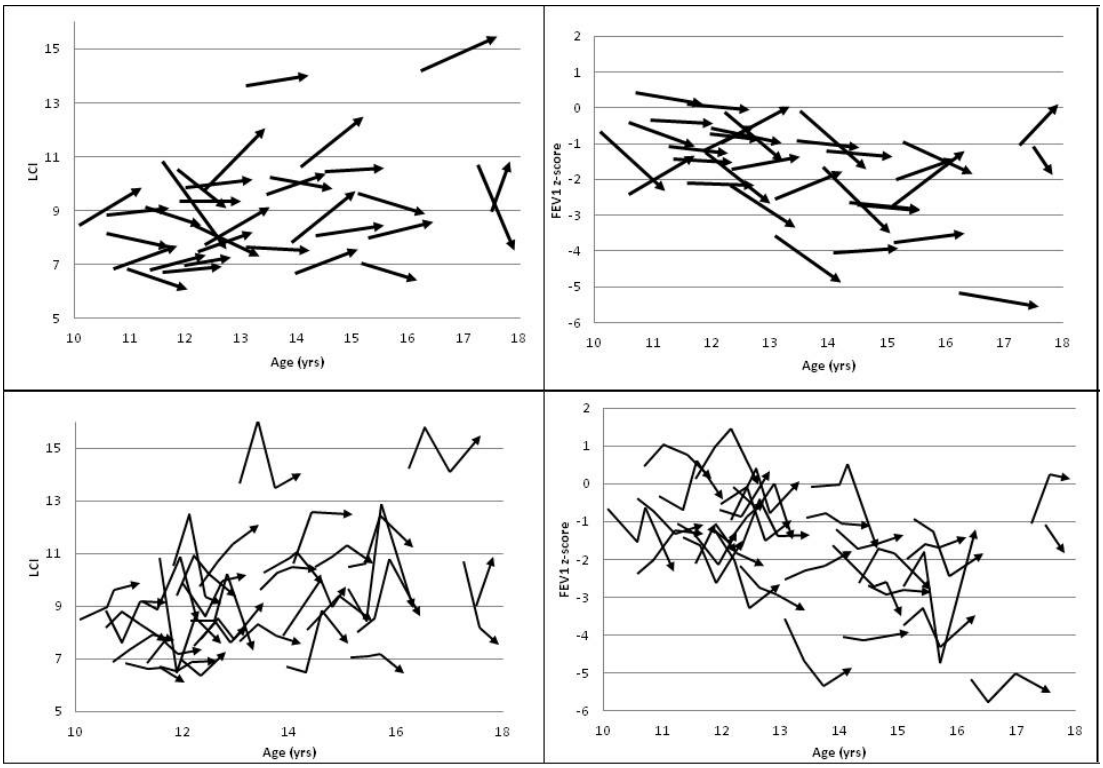


Figure 49 - Change in FEV_1 z-score and LCI. Top plots draw line between 1st and 4th measurements. Bottom plots connect all 4 measurements to show inter-visit variability

The plots above aim to illustrate the variability of FEV_1 and LCI measurements between visits. Despite patients being tested at a period of clinical stability, defined by the locally developed criteria, there is still a great degree of fluctuation of values (mean [SD] CV% of within-subject LCI over 4 visits = 8.95[5]). This highlights the inherent difficulties of performing lung function tests in CF. Drawing a straight line between visit 1 and visit 4, ignoring the measurements in-between may underestimate the significance of a change.

An alternative analysis strategy is to look at all FEV_1 z-score values plotted against LCI in individuals (Figure 50). A linear regression line is drawn using the 4 values from each individual, so that the overall trend can be seen. No direction is indicated in this plot, but an association is seen where lines follow upper left to lower right direction (increased in LCI with fall in FEV_1 z-score), or vice versa (decrease in LCI with rise in FEV_1 z-score). Lines which follow a perpendicular trajectory to this indicate a dis-association in overall trend. In this scenario, there is overall a weak association between change in $FEV_1$and change in LCI (R^2=0.18, p=0.02).

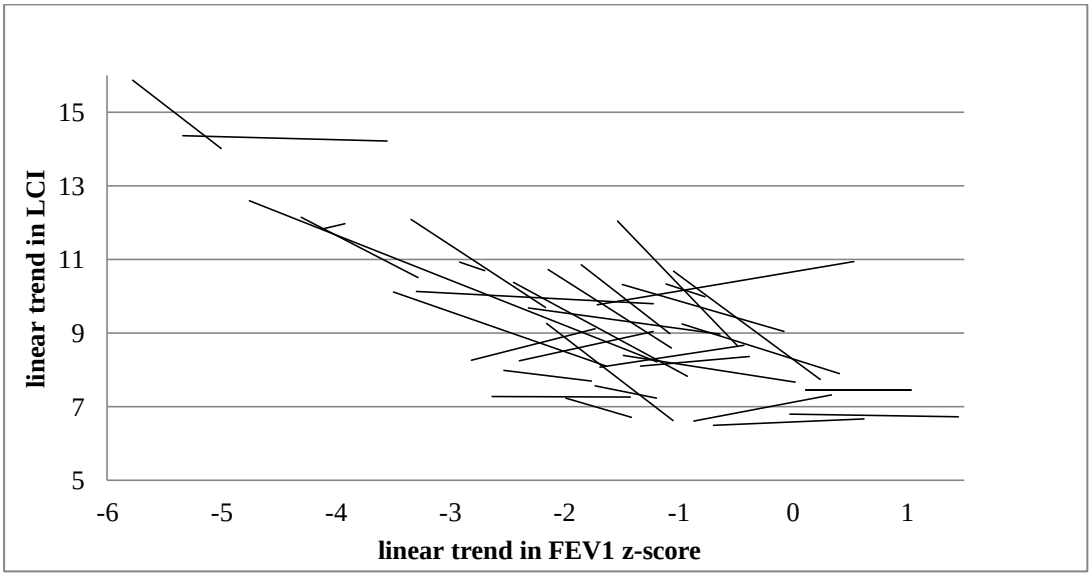


Figure 50 - FEV1 plotted against LCI at each timepoint (individual points not shown). A linear regression line drawn for each subject shows an association between change in FEV1 and LCI. Direction of change not indicated in this graph.

Finally, a comparison of average monthly change in FEV_1 vs. LCI shows that 25 (75%) subjects demonstrated an average rise or no change in LCI over 4 visits. FEV_1 did not change in the same way, in that only 19 (58%) subjects demonstrated an

expected average fall, or no change, in FEV_1 z-score. Most subjects show an associated change in both measurements, as illustrated below

Figure **51**), however some show a rise in LCI, with an associated rise in FEV_1. None showed a fall (improvement) in LCI with a fall (worsening) in FEV_1.

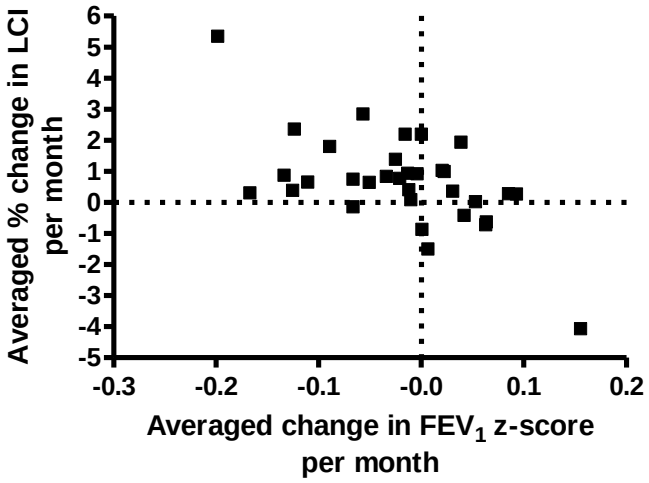
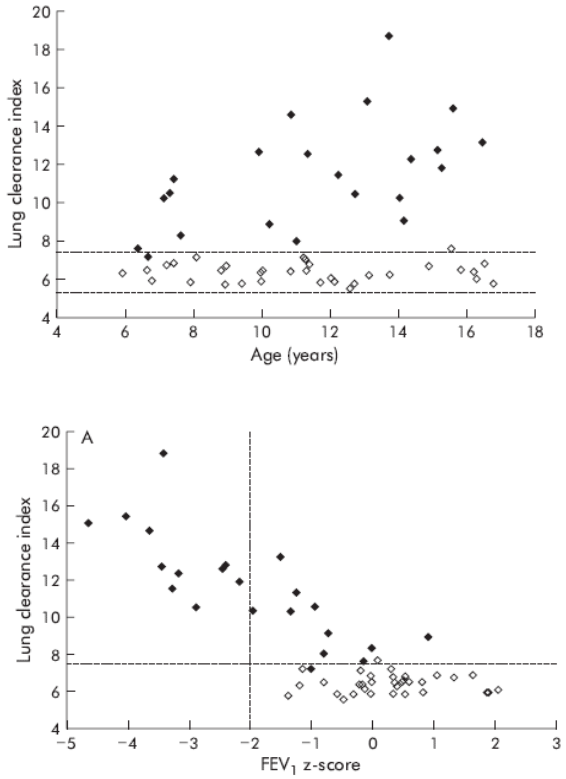


Figure 51 - Average change in FEV1 vs. average change LCI per month. Indicating the association between average change in measurements.

Conclusions

Lung clearance index in children with CF is higher than healthy controls, and LCI appears to be more able to detect lung disease than FEV_1, in that abnormalities were detected in more subjects.

Baseline measurements showed a cross-sectional trend towards increased LCI and decreased FEV_1 z-score in older children. This trend has previously been reported by Aurora et al (Aurora et al. 2004), (Figures 52 - 53). Their study from 2004 was conducted in 22 children with CF and age-matched healthy controls aged 6-16 years. Those with CF had an increased LCI and, importantly, only 1 child with CF had a LCI value within 2 standard deviations of the healthy control mean. This study, with others by the same authors, generated a great deal of interest in the scientific community for LCI as a marker of CF lung disease. This marked distinction between healthy control and disease populations is not commonly seen in physiological measurements, but the run-in study data do not show the same clear difference. There are a number of possible explanations for this that have relevance for this study, as well as future studies in CF.



Figures 52 and 53 - data from Aurora et al 2004, showing cross-sectional association between increasing age and worsening lung function. No effect of age in healthy controls. Clear distinction between disease (CF, black symbols) and healthy controls.

The population chosen for the run-in study was defined as those with mild to moderate CF lung disease (FEV_1 ≥40% predicted). In addition, measurements were only performed while clinically stable, according to clinical criteria. Finally, those with Cepacia colonisation – associated with increased decline in lung function – were excluded. Subjects in the Aurora et al study were recruited opportunistically from outpatient clinics and in-patients, and therefore were not controlled for exacerbations or changes in treatment. Patients in the run-in study were tested at least 4 years after those in the Aurora et al study. In a different population with different treatment this may explain some of the disparity in the cross-sectional data.

There was a significant trend of decreasing FEV_1 over the 4 timepoints, whereas LCI did not change. Average direction of change in FEV_1 matched that of LCI in all but 6 (18%) patients. In these 6 cases, there was an improvement in FEV_1, but with an increase (worsening) in LCI. In no cases did LCI improve where FEV_1 worsened.

Therefore, this indicates that LCI may be measuring an aspect of airways disease also measured by FEV_1, but that it may also be measuring a different aspect of airways disease (possibly medium to small airways disease). These data are unable to examine this, therefore this can only ever be speculation.

In healthy controls, LCI did not change after 2-3 years. Reassuringly, results are very reproducible (CV <5%), adding to the validity of the changes seen in those with CF. There appears to be a trend for increased FEV_1 z-score over time, which did not reach statistical significance in this cohort. It cannot be proved from these data what this related to. There may be inaccuracies in the z-score algorithms or simply better results due to improved subject technique on the second occasion, even though FEV_1 is effort independent at flow limitation.

The results presented here were conducted as part of a large clinical programme leading to a clinical trial of pulmonary gene therapy for CF. The other data have not yet been analysed and are not presented here. This study will yield important information for selection of patients for the future trial but also contributes, as a stand-alone study, to the understanding of CF as a progressive airway disease.

The data presented support the use of LCI as an outcome measure for a trial of gene therapy. LCI seems to be measuring, in part, a similar aspect of airway disease as flow-limitation spirometry. Measurements change slowly in this population, as may be expected. LCI is able to detect change in measurements over time. More frequent measurements over a longer period of time would help in understanding the reasons for fluctuations in in measurements in clinically stable individuals. Other data collected as part of the run-in study – e.g. weekly home FEV_1 measurements, inflammation measurements, exercise capacity or infective colonisation – will also contribute to this. These data have not yet been analysed.

LCI is proposed as a surrogate marker of CF disease progression. It is assumed that CF progresses (i.e. gets worse) over time but also there are periods of poorer lung function (e.g. an exacerbation) that responds to treatment (e.g. antibiotics). It can be difficult to separate these two elements if measurements are taken without controlling for exacerbations or changes to treatment. These were controlled for in the stability criteria check prior to testing.

In using these data to plan for a gene therapy trial there are multiple aspects complicating the analysis. CF is a highly heterogeneous condition, in that there are a variety of severities, with variable progression within severity levels based on a number of factors (e.g. pseudomonas colonisation or number of exacerbations). All patients over the 18 month time period had episodes of poorer health. There were changes to medication, exacerbations, new interventions and presumably variable adherence to prescribed therapy. Because there is no guarantee that the initial measurement represents the true disease state at that point, independent of periodic fluctuations, a line cannot be drawn between visit 1 and visit 4 without including observed fluctuation over that time period. Additional measurements taken during the run-in visit – symptom scoring and inter-visit FEV_1 measurements – will help to distinguish fluctuations from "true change", but were not available for this thesis.

Learning points and contribution to thesis

Concerning the aims of this thesis, the run-in study provided a great deal of information on the utility of Innocor in a larger clinical study, as there are important practical considerations when validating a lung function device.

During the run-in study, Innocor was used for approximately 360 washouts. It did not require extra servicing during this study, and there were no technical problems that meant the test couldn't be performed, or where the validity of the results was in doubt. Innocor is also portable in that the self-contained flow meter and analyser can easily be transported to another site. The limitation to this is transporting the pressurised gas tank, as this is bulky and subject to a number of health and safety regulations. There is obviously the possibility of having gas tanks available in multiple locations.

To perform the test to the highest standard, ensuring adequate washin and washout, one complete manoeuvre can take up to 20 minutes. Performing 3 is obviously time-consuming. MBW was one part of a long series of tests in the run-in study, taking over 3 hours to complete. This may have implications for the use of MBW in some studies, depending on the number of other assays being performed. The MBW test was acceptable to all subjects who took part in the study. It was obvious that the washin period causes a dry mouth, and this is worse in those with advanced disease

because of prolonged washin time. Other than that, the test is simple, if boring, to perform. Distraction in the form of an appropriate video was all that was required. If this test was to be performed on a regular basis, there may be some compliance issues, which were masked in this study by the novelty of the test and the increased motivation of being part of a Gene Therapy project.

In summary, this chapter describes a large clinical study, however only analysing a small portion of data. Innocor performed well during this long study period with a relatively large number of patients and no technical concerns. The results are largely consistent with previously published work. LCI is higher in CF compared with healthy controls, and appears more able than FEV_1 to detect mild to moderate lung disease. LCI measured during periods of clinical stability increased over the study period despite high variability between time points. Concerning the wider thesis question of Innocor validation, this chapter adds strength to the argument that MBW using Innocor is able to accurately estimate and track ventilation heterogeneity in lung disease and distinguish children with lung disease from healthy controls.

Chapter 8 - methods II: Innocor hardware modifications for use in young children and infants

Introduction

The first part of this thesis has described the validation of Innocor for older children and adults. Building on previous work carried out by Dr Alex Horsley, adult clinical research fellow, I was able to set up the equipment for use with older children (≥5 years). The methods and clinical studies contributing to the wider theme of Innocor validation have already been described.

A further question that arose from these initial studies was whether the perceived limitations to the Innocor system that prevented its use in younger children and infants could be overcome to allow studies to be conducted in this age-group. The following section of the thesis concerns this important validation question.

As explained in previous chapters, Innocor is a promising technology for MBW measurements. It has the following advantages over similar equipment (e.g. A mass spectrometer):

- Ease of use
- Portability
- Relative affordability
- Relatively high gas analyser signal to noise ratio

There are concerns over the limitations to Innocor that may invalidate its use in young children and infants, the age group in which many feel there is greatest potential application. The main limitations are:

- Slow analyser response time
- High gas sample flow rate

These concerns were raised following discussion with experienced researchers, who used MBW equipment on a regular basis. Information from detection and tracking of disease progression from infancy to adulthood is of interest to research groups and

clinicians. If the above limitations to the Innocor device cannot be resolved, invalidating its use in children less than 5 years, there will be only limited future research and clinical relevance. For MBW measurements conducted on Innocor to become accepted in the wider academic world, these specific concerns needed to be resolved.

This chapter describes the Innocor technology in detail, and how the above limitations contribute to error in MBW measurements conducted in infants and young children. There is a description of work completed previously by Dr Horsley, as part of his PhD thesis, to provide background to the current experiments.

Section 1 - Background and previous research

Initial Innocor modifications

The standard "off the shelf" Innocor setup is not designed for MBW measurements. Its licenced purpose is to measure flow and exhaled gas concentration during exercise testing. While there are similarities between multiple breath washout and non-invasive cardiac output measurement – washout of gas is followed in both cases – the equipment requirements and data analysis process are different.

Initial investigation in previous research revealed the following factors that prevented use in MBW testing.

- The flow meter has a large equipment dead space (Figure 54) to allow automatic switching between gas and air supplies. This is appropriate for exercise testing as large tidal volumes are expected. During resting tidal volume breathing this large dead space may introduce error unless accounted for
- The large dead space risks accumulation of CO2 with prolonged testing. This risk increases in younger patients with smaller tidal volume
- The gas sampling capillary placement is optimised for automation of re-inspired gas tests including switching from test gas to air with a valve system. This set-up is not appropriate for MBW gas sampling

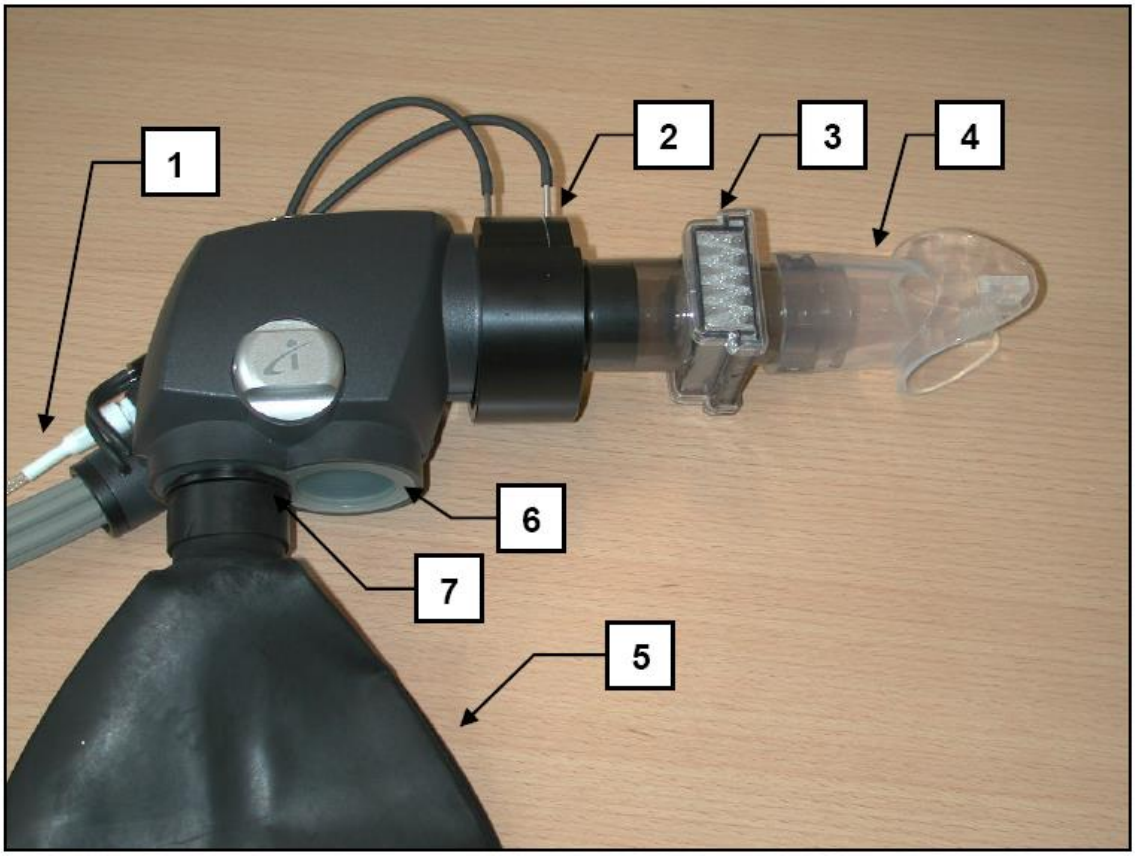


Figure 54 - Standard Innocor rebreathing valve unit (RVU) with connections.
1 gas sample line, 2 flowmeter, 3 respiratory bacterial/viral filter, 4 mouthpiece, 5 rebreathing bag, 6 breath-by-breath port, 7 rebreathing port. Total equipment dead space approx. 130ml. Taken from Innocor user manual(Innovision 2005-05) (COR-MAN-0000-001-IN / UK, May 2005).

To resolve the initial problems with the standard setup and to increase similarities with other centres performing MBW measurements, a replacement low deadspace screen pneumotach was chosen instead of the standard Innocor Pneumotach to measure flow. Consistent with the MBW setup seen at other centres a sample capillary was sited inside the pneumotach using a metal needle(Gustafsson 2005, A. R. Horsley et al. 2008, Morris et al. 2001, Aurora et al. 2005b).

To establish the principle of Innocor in MBW measurements and to begin validation of the device, Horsley and Gustafsson also conducted a series of studies on the Innocor device in adults [unpublished data]. The device was initially tested against the mass spectrometer to confirm its ability to accurately estimate gas concentration and breath volume during tidal breath tests. A series of washouts was performed,

simultaneously collecting Innocor and mass spectrometer gas concentration signals. When the signals are overlaid there appeared to be a close match, although calculations of expired and inspired gas volume showed large differences. The expired SF6 volume was 4% less when measured through the Innocor. Inspired concentration was also greater when measured by Innocor.

Data comparing results with The mass spectrometer were also collected from adult washouts at a faster respiratory rate (~30/min). It was shown that at lower breathing rates normally seen in adults the two devices are comparable. However, it was observed that while the signals overlap well, additional error was introduced at slightly raised respiratory rates. The error may also be greater in disease states because of longer washout duration. This was not studied.

To confirm stability of the concentration measured by the internal Photoacoustic Gas Analyser (PGA), the signal was tested at low SF_6 concentration. High signal to noise ratio is also required throughout the concentration range measured during the washout and this was confirmed(A. R. Horsley et al. 2008). Most other devices use 4% SF_6, out with the Innocor upper range. For simplicity 0.2% to 0.005% was chosen as this was the range used in the standard Innocor exercise manoeuvres and the signal is linear. In addition, there is reduced gas consumption. Data from these experiments were published by Horsley et al(A. R. Horsley et al. 2008).

Building on Dr Horsley's experiments, I began to assess Innocor

Why does slow rise time lead to error in expired gas volume estimation?

Despite high gas signal quality and simple setup for MBW testing on Innocor, the method of gas analysis is slow compared with the mass spectrometer. The speed of a gas analyser is quantified as the rise time or response time after a step-change in gas concentration. A graphical representation of the effect of signal response after a sudden step change in concentration is delivered to two different simulated devices is shown in

Figure 55. A step change is the actual change in concentration if a sudden flow of gas at maximum concentration is delivered to the analyser. All devices lag behind this

step change to a greater or lesser degree. In this illustration it can be seen that the simulated machine with the fast response reaches the peak concentration earlier than the other, closely representing the true step change. The slow analyser has increased error in measured concentration.

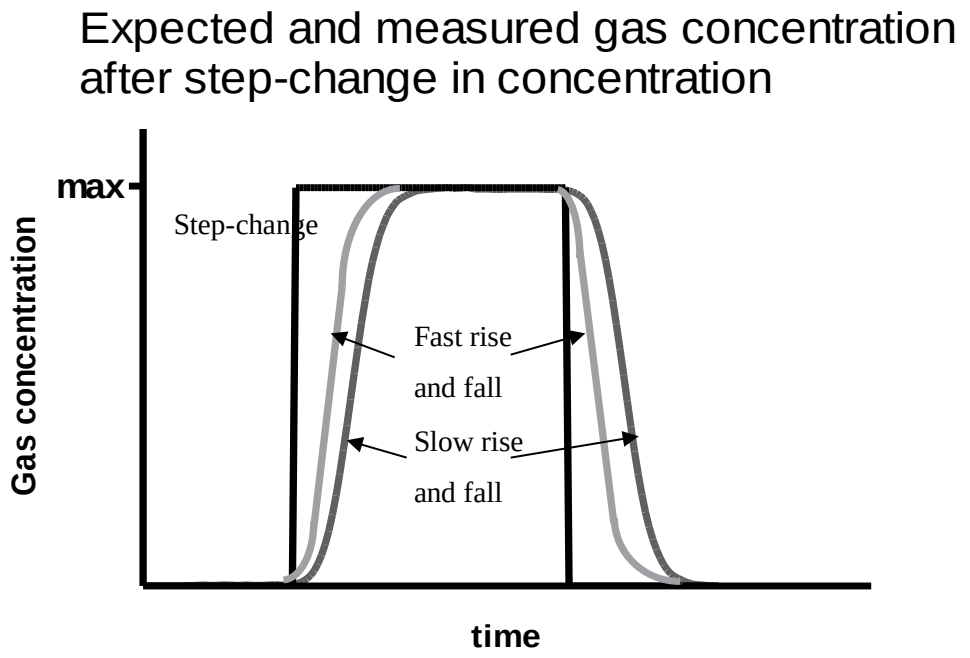


Figure 55 - simulated step-change in gas concentration with the output response from 2 devices with different signal rise times

Innocor has an approximate signal rise time (10-90%) of 160ms. The standard rise time of the mass spectrometer equipment is 80ms. This slow Innocor SF_6 signal response causes error in volume measurements (e.g. FRC) through additive effects on estimated gas volume.

Net expired gas during a breath by breath test is calculated in the following way:

$$net\ expired\ gas\ volume = expired\ gas - re\ inspired\ gas$$

Re-inspired gas is that which is left in the equipment at the end of each breath.

A slow rising gas concentration leads to underestimation of expired volume. This error is not cancelled out by overestimation of the falling gas concentration, as may be expected. Slow falling gas concentration adds to the error as flow is reversed

during inspiration. Underestimated expired volume is thus further reduced by overestimation of reinspired gas volume. The final net estimated FRC is therefore greatly underestimated due to additive errors in both inspiration and expiration(Arieli and Van Liew 1981).

This error in gas signal response was initially investigated in this department (A. R. Horsley et al. 2008). It was clear then that the response time (time for signal to rise from 10-90% of full concentration) is slower than A mass spectrometer (160ms vs. 80ms) and that this contributed to error in volume estimation during washout tests.
The initial research question posed by Alex Horsley to resolve this is given here:

Given the effect of slow rise time on gas volume estimation, can the SF_6 signal be manipulated to improve signal response time, and therefore the accuracy of MBW tests?

This first question was addressed in the initial validation steps, but is revisited in my thesis when considering Innocor for use in younger children (<5 years) and infants. Children and infants pose a considerable additional problem when considering analysis of exhaled breath, as expired volume is low and expiratory time is short. The options below were proposed by Dr Horsley. I have included them as an introduction to further Innocor modifications, and also because I worked with Dr Horsley on the initial Innocor validation for use by the UKCFGTC.

Early improvement proposals

Option 1 - mathematical correction of the SF6 signal to improve the rise time.

The principles behind this originate from earlier studies conducted primarily in CO2 analysers(Sasse 1985, Schena et al. 1984, Block and McDonald 1992, Linnarsson and Lindborg 1974, Arieli and Van Liew 1981). In a key paper by Arieli and Van Liew, mathematical formulae were applied to gas signals to improve signal response time(Arieli and Van Liew 1981). Because concentration curves tend to follow predictable mathematical sigmoid forms, a formula that steepens this rise has the

effect of increasing the estimated expired gas volume. Arieli et al conducted a modelling study that generated timed step changes in gas concentration while a mass spectrometer measured the gas concentration. Also, syringe breaths were delivered to simulate human breaths. A conclusion of this study was that to optimally correct for slow analyser response time, a mathematical 2^{nd} or 3^{rd} order correction should be applied to the gas concentration signal as this improved the estimated gas volume experimentally. Errors of as much as 4% in gas volume were seen with an unaltered signal; however this was reduced to less than 0.5% with optimal mathematical correction. Error in volume estimation was reduced with larger breaths (1.5L vs. 0.5L). Arieli and Van Liew also found that, while matching the change in flow with a later point in the rise to maximum gas concentration (>50%) improved the error in unaltered gas signals, matching to an earlier point in concentration rise (20%) improved the error in mathematically optimised signals. The recommendation is that a mathematical alteration including an adjustment in flow gas delay compensation reduces signal error.

Assumptions to be made when mathematically altering concentration signals are:

- The concentration curve is sigmoid and can be manipulated without compromising signal quality. A high signal to noise ratio needs to be maintained for data analysis.
- The measurements after a sudden step change in gas concentration are equivalent to the more gradual changes seen in washouts.
- The Innocor internal signal processing does not already have filtering or signal altering. Further mathematical modification would greatly increase noise in the signal.

Horsley applied first and second order manipulation to washout signals and compared this against a mass spectrometer signal. The first order correction did not make a great difference to expired gas volume and the second order produced excessive noise making analysis of washouts impossible [unpublished data, Alex Horsley].

A concern with mathematical manipulation such as this is that it is not physiological. The true rise in gas concentration in human washouts with each breath is not a step-change because of phases of airway emptying. Therefore mathematical manipulation may simply be introducing a cosmetic improvement without actually improving accuracy in real washout situations. Arieli and Van Liew also state that the measurement error seen in these published studies is greatly dependent on flow rate past the sample capillary. At higher flow rates in "real" breaths, any error may change due to faster rise or fall in concentration.

Internal Innocor signal processing

When considering if the Innocor gas signal should have mathematical alteration, a missing consideration of Dr Horsley's investigations was that the Innocor analyser already applies a first-order mathematical "speed-up" filter to the gas concentration signal. Further alterations, in addition to the internal Innocor signal processing, introduced additional error and excessive noise This issue was discovered some time later by me, and confirmed by Knud Pederson at Innovision..

Conclusion

In conclusion, it is felt that to improve the Innocor signal for infant washouts, mathematical altering of the signal is not appropriate. While the principles of speeding the signal may seem to improve the response of the equipment, this may be purely cosmetic. Mathematical filter speeding sacrifices signal quality, potentially leading to poorer representation of the concentration curve over the whole breath. The improvements demonstrated by Arieli et al were not in infant washouts. Any recommendations based on simulated washouts would need to be conducted using infant lung parameters.

Finally, signal speeding is not possible with the current Innocor setup. Removal of the signal filter would have to be performed by the device manufacturers.

Option 2 - speeding of the Innocor signal to align it to the mass spectrometer signal.

Given the difficulties associated with mathematical signal correction, an alternative solution to the problem of slow signal rise was investigated and subsequently adopted into the Innocor MBW setup. As the Innocor signal lags behind The mass spectrometer due to slow rise time, and the signal is aligned to the flow at calibration, it was appropriate to left-shift the Innocor signal relative to the flow. For the rising gas signal, this has the effect of matching the change in flow with a later point in the SF_6 rise, as suggested by Arieli and Van Liew(Arieli and Van Liew 1981). Conventional signal alignment matches change in flow with 50% rise or fall in gas concentration as this recognises that gas concentration rises or falls as a function of "spreading" within the sample tubing. The alternative "equal area" technique aligns the zero point in flow with the 50% of the area under the signal curve. Both attempt to measure the true rise in gas signal, whilst compensating for error during transit of gas. The equal area technique is thought to be more accurate as gas concentration does not necessarily rise linearly during tidal breathing. However, since gas signal alignment is concerned only with spreading of the gas signal, it is reasonable to conclude this spreading is linear.

Signal speeding improves underestimation of expired gas volume by aligning the zero flow point later in the rising or falling gas signal – once the concentration is 80% (rather than 50%) of max. This increases the measured volume of expired gas on the rising SF_6 portion of each breath.

Using comparison washouts from the Innocor and The mass spectrometer (see above), the calculated Innocor FRC using this speeding method was very close to the mass spectrometer FRC [unpublished, A Horsley]. This was repeated using a model lung, and measuring known "FRC" volumes with the speeded signal alignment(A. R. Horsley et al. 2008). This method was therefore validated for use in humans performing washouts but includes the following limitations:

1. The effect of slow rise time may be influenced by respiratory rate and breath volume

A number of published papers have shown that expired gas volume estimation in breath tests is actually greatly influenced by respiratory rate and breath volume(Arieli and Van Liew 1981, Linnarsson and Lindborg 1974, Tang et al. 2005, Brunner and Westenskow 1988). Brunner et al, in particular, showed that when step-changes in concentration are delivered, respiratory rates or 40bpm caused a 7% error in estimated volume and rates of 60bpm resulted in errors of 16%(Brunner and Westenskow 1988). Therefore, it may be that adjusting signal alignment is not accurate when considering infant washouts.

2. Reinspired gas volume cannot be measured

Speeding the gas signal improves accuracy only in the rising gas portion. Re-inspired gas is the small volume residing in the equipment dead space that is breathed back in at the start of inspiration. In a low dead-space system the re-inspired gas volume is low but must be taken into account in infants otherwise FRC will be overestimated. With the modified MBW setup, the SF_6 sample capillary was sited close to the end of the pneumotach. This has the effect of minimising (negating) the volume of re-inspired gas. The volume of pneumotach space re-inspired with each breath is approximately 5ml and with adult and older child tidal volumes of greater than 200ml, this volume is negligible and is ignored in volume calculations as proposed by Dr Horsley. It is not clear, however, whether there is increased error in this system as the tidal volume reduces in younger children. At lower tidal volumes seen in infants (20- 75ml) this 5ml volume cannot be considered negligible.

Summary of early Innocor validation

Previous experiments have shown that Innocor, despite being designed for a different physiological test, was able to accurately estimate lung volumes in adults and older children. Simple modifications to the gas signal alignment (signal speeding) compensated for slow gas analyser response. This method was devised based on previously published comparing Innocor against the current MBW standard device (mass spectrometer). As a result, data published in healthy control adult and older

child populations showed very similar results to other populations measured using different equipment(A. R. Horsley et al. 2008, Aurora et al. 2004, Gustafsson 2007, Gustafsson et al. 2003a).

Despite the improvements to the Innocor and reassuring experimental and clinical data from adults and older children, there is much less confidence in this modified Innocor setup for use in infants and younger children (<5yrs):

1. At faster respiratory rates (>30/min), the slow response of the analyser may greatly under-estimate the cumulative expired volume, with a possible increased error in patients with lung disease as more breaths are required to washout the SF_6 in less efficient airways.
2. The post-capillary dead space re-inspired with each breath may not be negligible and should be taken into account during cumulative expired volume calculation. This means signal speeding cannot be applied in this age group.

When considering solutions to these problems, there is high risk of including multiple errors if all aspects of the equipment are not fully investigated. It can be seen from these summarised problems, for example, that overestimation of re-inspired gas and underestimation because of slow analyser response may in fact cancel each other out in the results. This could lead investigators to consider equipment highly accurate if not investigated rigorously. This equilibrium of errors may not be balanced throughout all ages and may be affected by the presence of disease. Operating a system with two potentially large errors is therefore unacceptable if they cannot be quantified in-vivo. Further validation must therefore recognise sources of error and correct accordingly.

Section 2 – basis for Innocor hardware modification for infant washouts

Innocor limitations in children

The above Innocor problems were carefully considered for this thesis from basic principles, aiming to extend the use of Innocor to younger children. A lower limit of 5 years prevents detection and tracking of early lung disease in infancy without using a different device in younger age groups.

Intuitively, it is expected that the most accurate validation involves comparing Innocor directly with a mass spectrometer device in young children and infants. In preparation for this in-vivo comparison validation in young children and infants, a series of initial lab-based in-vitro experiments was conducted to resolve the main concerns about the Innocor equipment.

This section describes investigations into further Innocor modifications, and the justification behind them prior to in-vivo mass spectrometer comparison. Advice for this was given by Innovision, the manufacturers of Innocor. Some direction was offered regarding hardware modifications, but Innovision was unable to alter the software or fundamentally change the assembly. This is presumably due to regulations surrounding CE European safety marking and wider company marketing policy.

All the following work was led, and conducted by me in the Department of Child Life and Health, Edinburgh.

Basic breath by breath gas analysis principles

This chapter has already stated the limitations of the Innocor system, particularly the lack of confidence about its lower age limitation. These concerns are primarily about the relatively slow gas analyser response time and the position of the gas sample capillary that eliminates the need for analysing re-inspired gas in the current “adult” setup.

There are limited data on which to draw evidence for an upper signal rise time limit that prevents use of any particular gas analyser for MBW in young children. An official ERS statement document, published in 2007, contains comprehensive

information on many different lung function techniques(Beydon et al. 2007). This was in response to the increased use of these techniques with very few equipment and analysis standards. One chapter, written by Professor Gustafsson details the use of MBW equipment, quoting 100ms as the acceptable upper rise time limit. It is from this statement that Innocor is judged to be "too slow" for MBW in small children and infants.

Critique of 100ms as the upper acceptable limit for gas analyser rise time

Professor Gustafsson proposed 100ms as the upper acceptable limit for gas analysers used to perform multiple breath washout of inert gas(Beydon et al. 2007). This figure is a statement in the online supplement of an influential standards guideline of the American Journal of Respiratory and Critical Care Medicine. This paper concerned MBW in infants and young children, but is the only recommendation for MBW so far. No reference is given to support this statement.

An important paper by Brunner et al will be discussed in the following paragraphs(Brunner and Westenskow 1988). This article sought to establish the accuracy of both end-tidal gas concentration and integrated gas volume at different rise times. Far from recommending 100ms as the upper limit, this paper found that any device with a rise time of greater than 20ms would not be able to accurately estimate expired gas volume after a step change in concentration. As expired concentrations are not step change, reaching the maximum over around 70ms (see below), there are actually no data with which to recommend an upper rise time limit. A major aim of Innocor modification is to demonstrate accuracy at different signal rise times so as to refute this arbitrary upper rise time limit.

Influence of signal rise time on gas analyser accuracy

Understanding Innocor simply as a flow meter with a gas analyser, rather than a complex cardiac output device, simplifies the problem of how to overcome the slow rise time and error in gas signal. For MBW, the flow meter must accurately measure flow within the range expected in infants. This flow signal is integrated with the SF6 concentration to calculate expired SF6 volume. Flow accuracy can be tested during calibration and is standardised by using appropriate equipment. Pneumotach

standards are readily available in published ATS/ERS guidelines(Frey et al. 2000a) to aid flow measurement accuracy. As a result, the gas analyser can be considered separately, according to first principles. This is explained in the following paragraphs.

Slow signal rise time

Gas signal rise time is influenced by factors that disrupt the gas front. At the extreme, a step change in gas concentration should change from 0% to 100% instantly but anything that causes mixing or turbulence spreads the gas concentration so the measured signal appears to rise more slowly. Figure 56 below illustrates how a sudden change in gas concentration delivered to a sampling gas analyser may be "read" as a gradual rise due to spreading of gas.

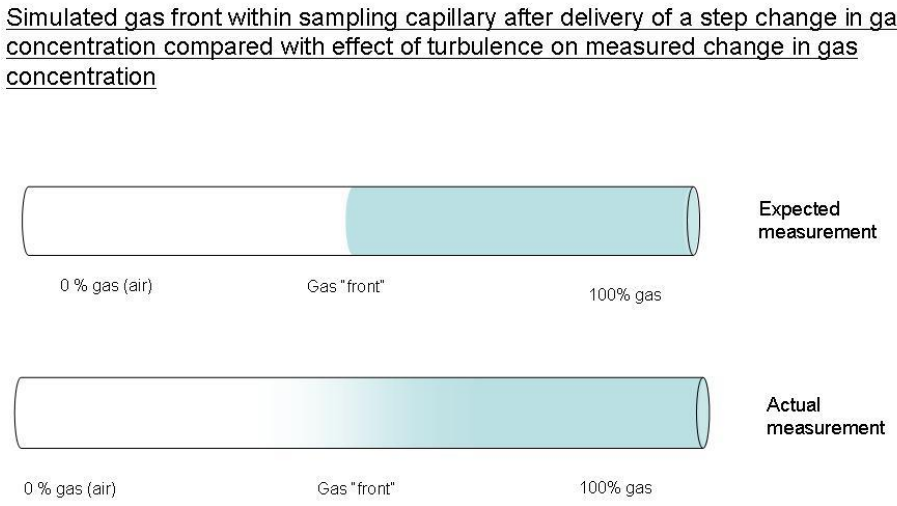


Figure 56- illustration of how a sudden change in gas concentration is measured as a slower rise in measured signal due to sample tubing

Slow rise time is contributed to by turbulence within tubing and connections. The likelihood of turbulence within a tube can be calculated using Reynolds number as reviewed by Rott(Rott 1990). The Reynolds number is a ratio of inertial to viscous forces in dynamic fluids (including air). This is limited to flow within a uniform tube. It is clear that any sudden widening or narrowing will cause turbulence and spreading of the gas front. Using a convenient online calculator (www.pipeflowcalculations.com/airflow/index/htm), the nafion itself has a low

Reynolds number, with the conclusion that if turbulence within connections can be minimised, any spreading of gas due to turbulence can be minimal.

Turbulence is not the only factor affecting spreading or mixing of gas during analysis. Any analysis step, such as the chamber where the gas concentration is measured, may also alter the true gas concentration during a rise or fall in signal(Tang et al. 2005). All sampling analysers have a degree of mixing due to such factors. The mass spectrometer device, for example, has narrow, high pressure low-flow tubing, meaning that turbulence is very low. All the signal rise time (40-60ms) is thought to be due to the sample analysis rather than spreading within the tubing(Davies and Denison 1979).

With the aim of reducing signal rise time, the relative contribution of turbulence within sample tubing and other causes of gas mixing was investigated in Innocor.

Gas flow system and tubing connections in Innocor

Innocor is constructed to measure gas concentrations during exercise and re-breathing manoeuvres. The flow of gas from the subject is as shown (Figure 57):

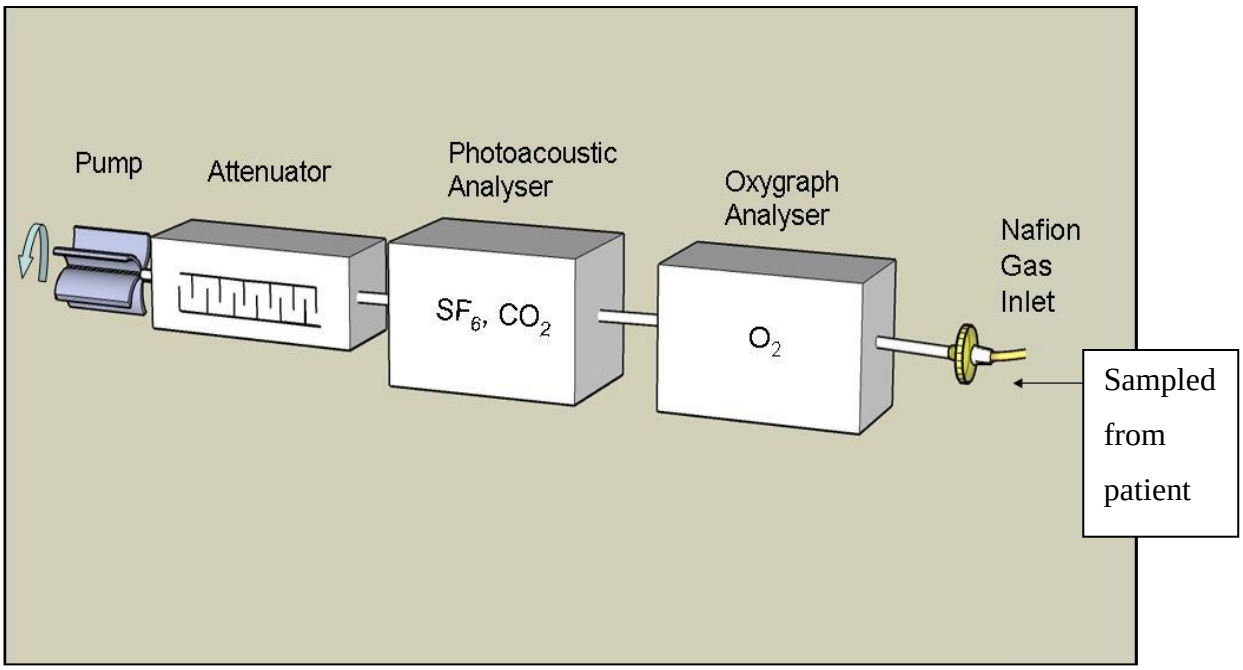


Figure 57 – Sequence of sampling within Innocor device. Gas is drawn in via the nafion tube to the Oxygraph O2 analyser, then to the photoacoustic analyser, PGA (SF6 and CO2). The pressure is generated by a pump, with an attenuator to prevent vibrations disrupting the gas signal.

As the Innocor method of analysis (photo-acoustic response) cannot be changed, the mechanical limitations in Innocor that may contribute to slow signal rise are:

- Innocor samples a relatively large volume of gas from the breath stream (2ml/second) and therefore has a higher sample flow rate. High sample flow increases the risk of turbulence and spreading of gas in non-linear tubing.
- The nafion tubing has a number of connections including a dust filter. These may introduce turbulence and spreading of the gas front.
- Innocor was designed to measure O2 concentration simultaneously with other gases, therefore the analyser places this separate O2 analyser before the photoacoustic device in series (Figure 57). The gas front therefore undergoes spreading before reaching the PGA.
- Spread of gas as it passes through the PGA sensor chamber.

Washout modelling and quantification of gas analyser error

All gas analysers have limitations. Publications quantifying the effect of these limitations on MBW tests are not currently available. While the priority is to validate the Innocor device, the following experiments are important for all breath-by-breath gas analysers.

To begin to quantify the effect analyser limitations have on accuracy of multiple breath washout tests in paediatric subjects, a basic model was developed. This consisted of a mathematical curve generator to reflect expected curves from devices with different rise times and to quantify how these contribute to measurement error.

Accurate gas concentration measurement is dependent on respiratory rate and breath volume. If the respiratory rate is high, the expiration time will be short. If the rise time of the equipment is slow, the concentration may not reach its peak before the concentration falls again at the new breath. At a respiratory rate of 60, the expiration time is approximately 0.3ms. The rise time therefore needs to be able to reach a plateau within this time(Sasse 1985).

Furthermore, if the breath volume is low, the error in expired gas volume may be large. Figure 58 illustrates the effect of slow rise time when a step change in gas

concentration is delivered. The expected signal is shown, but if there is a slow rise in concentration as shown by the sigmoid curve, the estimated expired volume (area under curve) may be lower than reality (blue area). The difference in volume between measured and expected is the area under the curve shown in pink. The proportion of this pink area to the total measured volume increases with faster respiratory rates (shorter expiratory time). Therefore, error in expired gas volume due to slow signal rise contributes greater error in younger paediatric subjects.

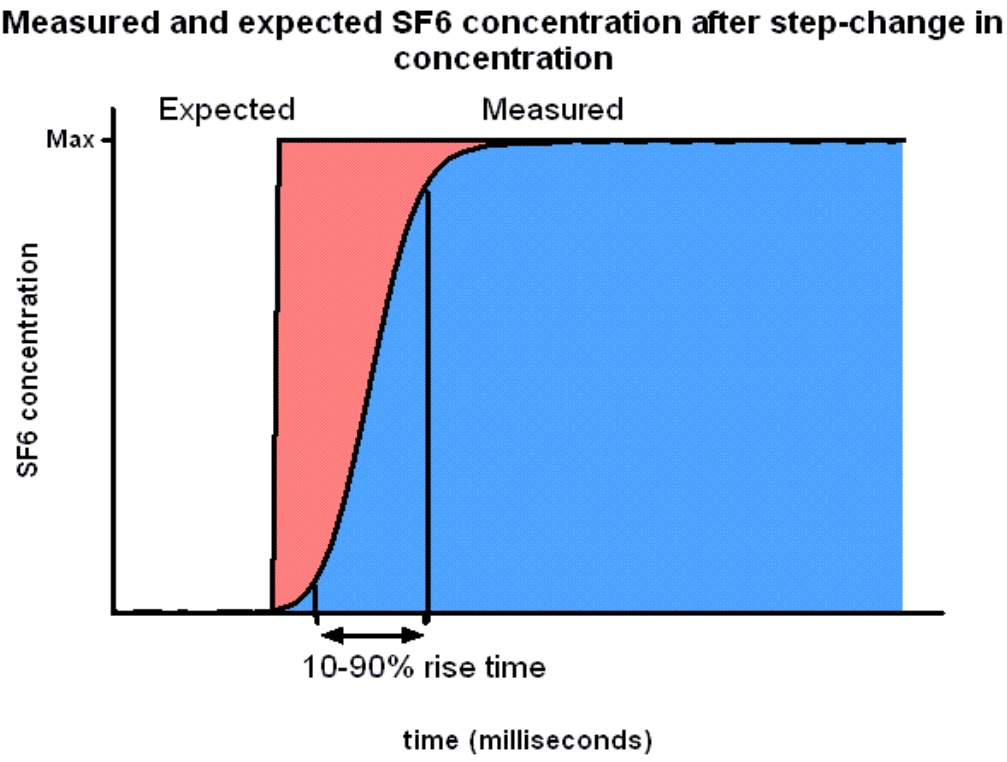


Figure 58 - illustration of how delayed rise time contributes to error in area under curve calculations during washout testing.

The difference between measured and expected volume can be quantified by %error:

$$\%\ \text{error} = \frac{(\text{expected} - \text{measured})}{\text{expected}} \times 100$$

For each breath and for FRC calculation, 5% error is considered the limit of acceptability (measured vs. expected breath volume or FRC volume)(Brunner and Westenskow 1988).

The effect of rise time on expired volume can be modelled in a number of ways. Brunner et al generated step changes in gas concentration, simulating the most extreme concentration change possible(Brunner and Westenskow 1988). While in-vivo washout tests do not generate such extreme concentration changes, it was felt that any device that is accurate with a step change in concentration will also be accurate after more gradual changes. A number of different CO_2 gas analysers were compared and only those with a very short rise time (40ms) were capable of measuring expired gas volumes to within 5% of expected volume.

Mathematical lung model

As tidal breath tests differ greatly from step-change tests, a more appropriate model was designed to assess the Innocor concentration signal. During human washouts, gas concentrations do not change in a step-wise manner. Spreading of the gas front during expiration causes the slow phase II rise in concentration. Even during inspiration, residual gas in the pneumotach mixes with inspired air, leading to a sloping fall in concentration. It is difficult to separate fall due to delayed response and real slope in concentration on side-stream analysers, therefore a sample curve was simulated. Mathematical concentration curves were also generated representing varying equipment rise times. The accuracy of this curve compared to the real concentration change was the outcome measure.

True concentration change

As a representation of a "real" washout curve, the response time from a sample infant washout signal was taken from a mainstream device with negligible rise time (molar mass signal, Ecomedics). The response of this equipment is approximately 3ms. The signal rise and fall were measured and used as the true change expected in this age group.

The sample infant washout data below shows typical washout curve (Figure 59). The signal rise time is negligible (<5ms) and therefore represents the true rise and fall in gas concentration during a washout. The graph shows that the steepest part of curve is falling concentration (no gradation due to airway anatomy). Normally this would

be quantified as 90-10% fall time. Here, response is measured as 90-30% fall due to gas concentration not falling to 10% in the molar mass concentration signal. According to Brunner et al(Brunner and Westenskow 1988) this is equivalent to a fall time (90-10%) of 70ms. A mathematical curve was generated using the response time.

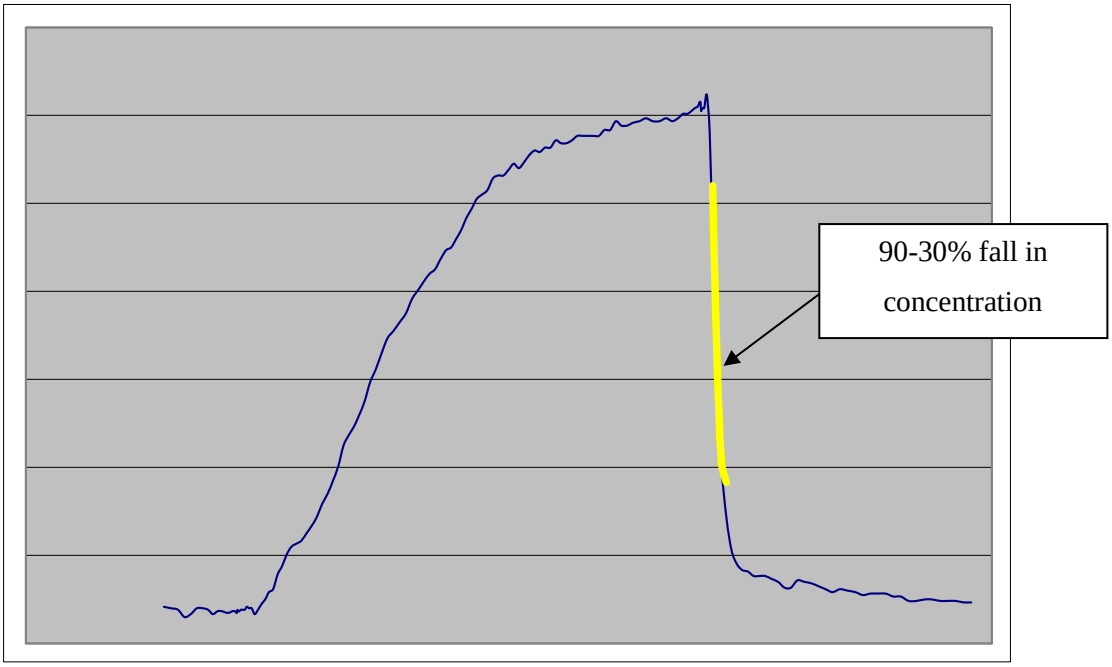


Figure 59 – Sample of infant breath (rise in concentration during expiration, with sudden fall during inspiration). Measurement of gas signal fall time in device with negligible response (Ecomedics, Berne, Switzerland)

Expired breath simulation

Mathematical sigmoid (Gompertz) curves, generated using Maple mathematical software (Maplesoft, Waterloo, Canada), were assembled by Dr Treven Wall, Department of Mathematics, Edinburgh University. Figure 60 shows hypothetical curves from a sequence of simulated devices with varying response times (units of volume over time, units.t^{-1}). The area under each curve is equivalent to the estimated expired volume of gas (units).

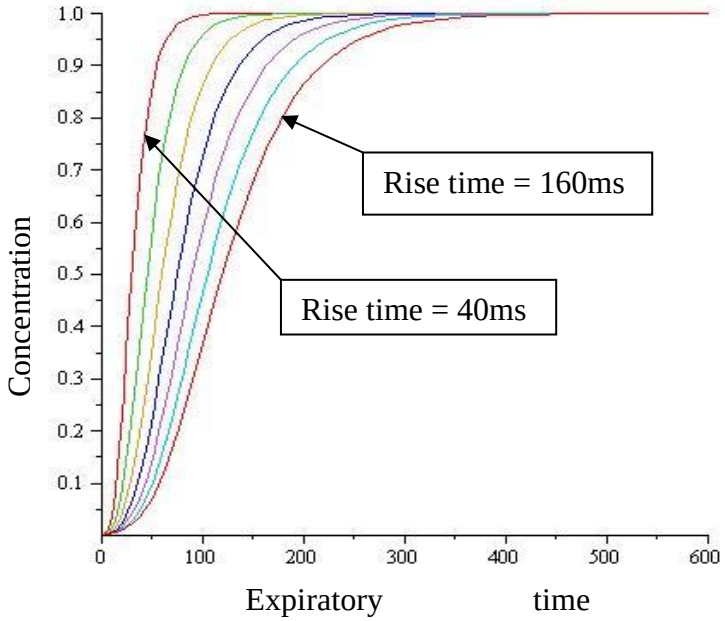


Figure 60 - simulated sigmoid concentration curves. Each curve represents a signal rise at a different device rise time. Concentration is in relative units, with area under the curve to calculate expired volume.

It was assumed that if the machine rise time is faster than the true gas concentration rise there will be zero error as the rise in signal is a true reflection of the gas concentration rise. Device rise times slower than the true subject concentration rise will introduce error in gas volume calculations. This assumption is valid only if rise time is purely due to the analyser, rather than spreading of the sample up the sample line (in which case the rise time would add to the true rise in signal). The modifications made to the hardware described seek to minimise spreading of the gas front and therefore rise time can be thought of as originating only from analysis and electrical delay within the analyser. This represents a simplification of a complex picture.

Error is quantified using the mathematical model and the real-life curve seen above. By calculating the area under the expected and measured curves, the % error can be calculated. The fall time in the real life patient above was 70ms (fall and rise in concentration taken to be equivalent). Therefore, any equipment rise time longer than 70ms will introduce error in volume calculation. Error is also dependent on breath duration. An expiratory time of 600ms was chosen, representing 60% of a 60/min

cycle (normal I:E ratio is 1:2, however 600ms simplifies calculation). This is the maximal expected rate in young infants, as respiratory rate is highly variable in this age group. At 60 bpm, a device with an equipment rise time of 70ms or faster will have zero error in calculating the volume of gas of each breath in this simulation.

Table 23 summarises the expected error in gas quantity estimation with increasing equipment rise time. A device with an 80ms rise/fall time would have a ~1.5% error, and a 100ms rise time will introduce a ~3.1% error. It can be seen that even small increases in equipment rise time result in large volume estimation errors. Furthermore, shortened expiratory time (increased rate) also contributes to increased error. This illustrates error in the measurement of a single expired breath, not error in FRC calculation, which is dependent on additive error over a number of breaths and is subject to other influences and assumptions (chapter 2).

Equipment rise time (ms)	40	60	80	100	120	160
Resp rate (min^{-1})	Error in gas quantity estimation (%)					
60	0	0	1.54	3.07	7.49	13.49
70	0	0	1.78	5.34	8.90	16.02
80	0	0	2.07	6.21	10.35	18.62

Table 23 – Estimated error from different rise times and respiratory rates, compared against a true concentration change from an infant washout breath, corresponding to a response time of 70ms.

From this modelling experiment it appears the Innocor with the standard rise time of 160ms demonstrates unacceptable error when measuring simulated infant washouts. Current standards recommend a maximum rise time of 100ms(Beydon et al. 2007). Even at this speed, there would appear to be large errors in gas quantity estimated at faster respiratory rates.

In light of these data, it was clear that prior to any comparison between Innocor and mass spectrometer improvements would have to be made to the Innocor gas analyser. To be within recommended limits the analyser speed should be less than 100ms, but the greater the reduction in rise time, the greater the accuracy of gas estimation.

Hardware modifications

Two factors contribute to gas signal rise time:

1. Spreading of the gas front in the sample tubing and analyser due to turbulent flow.
2. Delay due to internal electrical and analysis steps.

The method of analysis cannot be changed so the focus was in minimising spreading of the gas front by altering the following issues:

1. The sequence of analysers places the oxygen module before the SF6 module, contributing to spreading of gas. Once the oxygen module is removed, the rise time should improve.
2. The nafion tubing that samples gas has a number of connections that may introduce turbulence. If practical, improvements to these connections should improve rise time.

As previously explained, the Innocor is manufactured with specific analysis steps to measure cardiac output during exercise testing. MBW testing requires optimisation of gas concentration, specifically SF_6, rather than other gases – O_2, CO_2 or N_2O. The two gas analysers are assembled in series, with the oxygraph analyser first to receive the gas sample. Therefore the gas front is disrupted by passing through the oxygraph before reaching the SF6 analyser.

Delay in signal rise is also influenced by type of analysis, which cannot be altered. The 10-90% rise time according to Innovision is documented as <200ms. The most simple and feasible option to improve this was to bypass the oxygraph analyser step, so that gas was sampled straight into the PGA. This was proposed to Innovision and basic instructions were sent by them with internal system diagrams.

Experiments

Aims

- To measure the improvement in 10-90% rise time by bypassing the oxygen analyser and improving sample tubing connections.
- To achieve a target rise time of 100ms, according to published guidelines(Beydon et al. 2007).
- To assess the relative contribution of gas spreading and analyser delay to the total rise time.

Methods

Rise time measurement

SF_6 rise time was assessed using a modified technique published by Brunner et al(Brunner and Westenskow 1988). This involves creating a step change in gas concentration using a specially prepared syringe.

The syringe is illustrated below (Figure 61). The volume behind the plunger is filled with 0.2% SF_6. The plunger is pushed quickly past the side port, where the sample capillary is sited right at the entrance to the syringe. There is constant gas sampling and once the plunger has passed, 0.2% SF_6 is sampled immediately. This technique is simple and easily reproducible. Other methods, for example, have use automated devices that suddenly insert a sample capillary into a stream of flowing gas(Bates et al. 1983). This technique has the advantage of multiple repeats but is more complicated to set up.

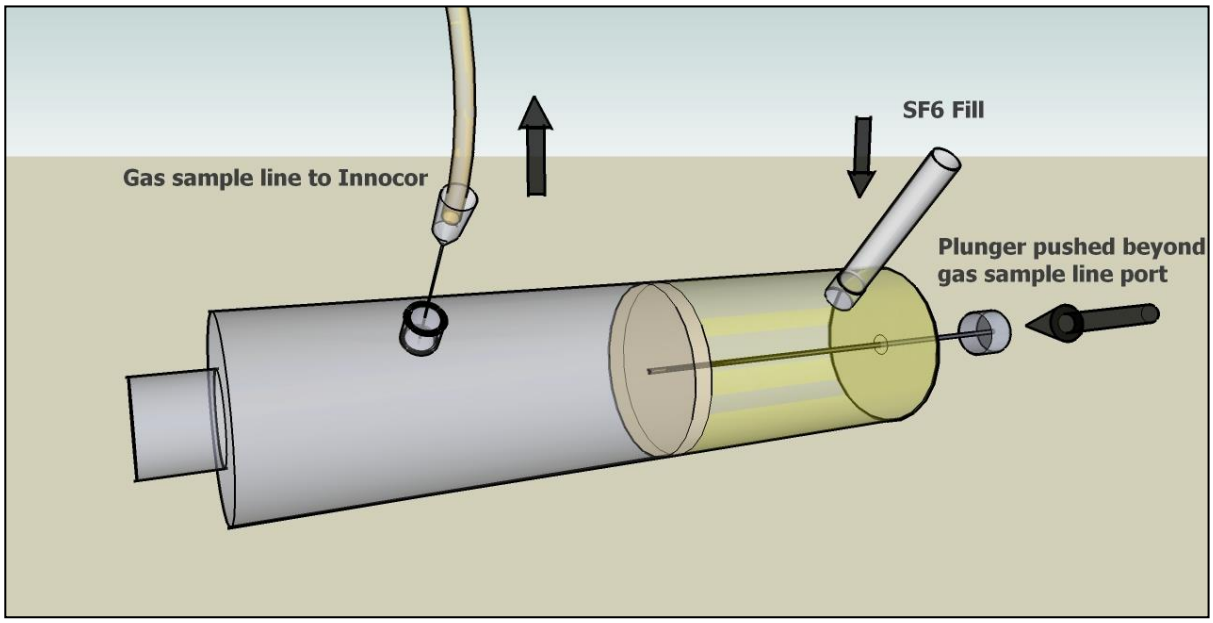


Figure 61 - Illustration of rise time syringe. A volume syringe with 2 ports is filled behind the plunger with SF_6. As the plunger passes over the sample port, there is a sudden rise in gas concentration (step change).

The resulting gas concentration signal can be exported from Innocor and displayed as individual signal values with time. If a graph is drawn of concentration vs. time, the rise can be seen. Subtracting time taken to get from 10-90% of the final concentration is the rise time.

Hardware modification

After consultation with Innovision and the company-produced Innocor Service Manual(Innovision 2007-01) the oxygraph analyser was bypassed by diverting the tubing which feeds from the nafion straight to the PGA. No input is provided to the Oxygraph. A full description is given in a hardware alteration manual (Appendix 3).

Nafion Alterations

The standard nafion length is ~180cm. This is the standard length but is not essential for optimal gas sampling. Reductions in length are therefore feasible. Leuer-lock connections are fixed as standard onto the tubing to allow simple connection of components. The standard nafion has a filter placed in-line, just before gas enters the Innocor, to prevent dust particles damaging the gas analysers. The sample capillary, inserted into the side of the pneumotach port, is fashioned from a shortened i.v. line needle (16g). The hub of this needle, relatively large in comparison to the nafion diameter, may contribute to turbulence of flowing gas.

It was hypothesised that the connections and the length of the nafion may affect the rise time by allowing turbulence and spreading of the gas sample front. Shortened nafion tubing was used with the nafion inserted inside the filter and sample needle hub by rubber bungs seemed to avoid changes in diameter likely to encourage turbulent flow. The sample capillary was also altered to make the needle extend inside the nafion, thereby encouraging a streamlines flow of gas whenever possible. The dust filter remains as this cannot be removed to protect the analysers.

Turbulence is reduced by siting the nafion as close to the filter mesh as possible. Modifications are shown in Figure 62.

The nafion must have the plastic protective mesh stripped from both ends.

Rubber bung is a short length of rubber tubing, just big enough to fit the stripped nafion.

The bung is pushed into the capillary hub or filter with minimal space left for turbulence of sampled gas.

The bung can be held in place with putty or similar to ensure a good seal.

A sample capillary can be made from a white intravenous cannula needle cut to size.

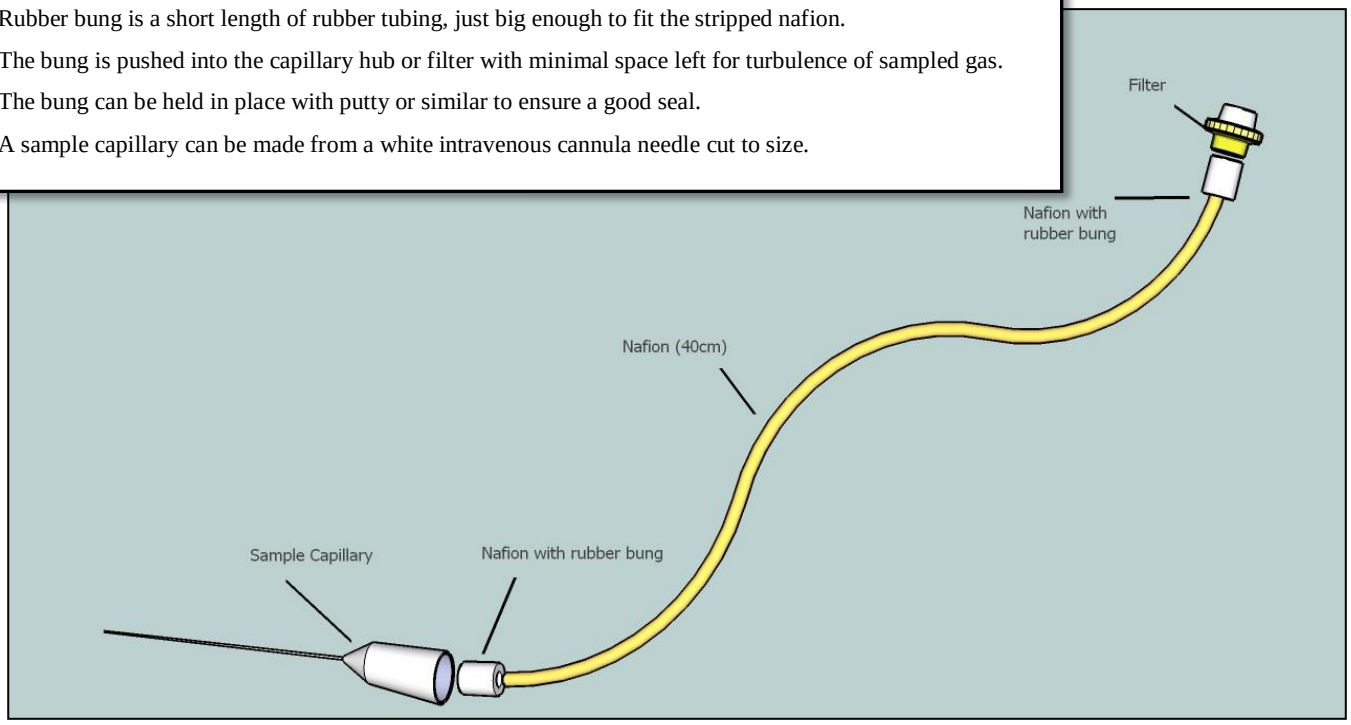


Figure 62 - nafion tubing modifications. Made to improve gas flow and minimise turbulence

Sequence of testing

The rise time was measured before and after oxygraph bypass. All other parameters (e.g. nafion tubing) were initially unaltered. Following this, further experiments were performed using the oxygraph bypassed Innocor with different lengths of nafion and improved connections to ensure there was minimal risk of turbulence due to wide bore connectors or port fittings.

Results

Pre-modification rise time was measured 16 times, with an average value recorded. Post-modification, rise time measurements were repeated approximately 10 times following each modification. Values are recorded to the nearest 10ms as the gas concentration resolution is 100Hz.

	Number of repeats	Mean 10-90% rise time (ms)	SD	Range (ms)
1.Pre-modification				
Standard Innocor setup	16	168.75	7.19	160-180
2. Post-modification				
Oxygraph bypass	10	139.00	5.68	130-140
+ shortened internal tubing	10	133	4.83	130-140
3. Nafion Shortening (oxygraph bypassed)				
Standard nafion length (176cm)	8	122.5	4.63	120-130
90cm	9	108.89	3.33	100-110
60cm	12	120.83	2.89	120-130
30cm	12	97.50	4.52	90-100

Table 24 - sequence of testing following sequential Innocor modifications. Values shown are the mean of all repeats, standard deviation and range. Less than 10 repeats were completed in some cases. However this does not represent discarded data.

Bypassing the Oxygraph alone improved the rise time by 17.6%. Improvements of 42% in total were seen when other improvements were added. These modifications do not seem to have caused any increased variability of rise time.

Once these improvements were made, it is now assumed that all the remaining rise time comes from the analysis step, rather than spreading within the tubing as connections have been optimised. It was shown by Davies et al that sample tubing per se does not contribute to slow signal rise(Davies and Denison 1979), therefore only the filter can be contributing to slow rise time within the tubing. Experimentally, removing the filter (not possible for washouts) caused a 16% reduction in rise time, indicating that >80% of the best practical rise time originates only from the analysis step.

Conclusions

The total increase in signal rise time achieved with these modifications was approximately 42%.

It is clear from these experiments that large improvements to signal rise time are possible with fairly moderate adjustments to the Innocor hardware. The biggest

change to the equipment was bypassing of the Oxygraph analyser. The largest changes in rise time, however, came from improvements to nafion tubing and connections. This demonstrates that, while the extra analysis step causes slowing in rise time, presumably due to gas front spreading, poor connections and long sample lines in themselves also contribute significantly to the rise time.

It is not clear which components contribute to the final 97.5ms response time. With close, tight tubing connections and a low pressure sample flow it is unlikely there is much spreading of the gas front. The gas analysis within the PGA may contribute additional spreading, but also electrical and mechanical delays add to the signal rise time. It is likely that this setup is the fastest response time possible. Innovision have conducted experiments with higher sample flow rates. This does improve rise time but has disadvantages during MBW testing in infants due to high lost gas volume. The concept of lost gas is discussed later.

As recommendations state that the minimum signal rise time should be <100ms, it is encouraging to achieve this improvement in the Innocor. I have stated my opinion about this figure, but to allow further validation to progress, achieving a rise time of less than 100ms is advantageous. It is important to note that the published recommendations are not based on in-vivo experiments in MBW washouts, and it has still to be shown exactly how slow signal response affects gas concentration measurement accuracy in an in-vivo setting.

Consequences of Innocor improvements

These improvements to analyser rise time introduce new complications to the equipment:

- Signal alignment for MBW analysis.

To align flow and concentration signals during analysis, an internal Innocor calibration programme is used. This programme relies on simultaneous oxygen analysis. Therefore if the oxygraph analyser is bypassed to improve signal rise time, an alternative method for aligning signals must be sought.

- Equilibration of humidity in shortened nafion tubing.

Nafion tubing is specifically designed to avoid adjustment for humidity differences between exhaled and ambient inspired air. A reduction in nafion length may affect its ability to equilibrate humidity.

- Sample flow rate remaining high in relation to infant breath volumes.

Increasing sample flow rate improves signal rise time. A reduction in sample flow rate is preferable to avoid affecting infant washout calculations. To maintain the fast analyser speed, the sample flow rate could be compensated for in washout analysis.

Experiments assessing the above problems are described below. I performed these prior to measuring the effect of Innocor improvements on MBW measurements, and whether in-vivo comparisons of Innocor versus a mass spectrometer are justified.

Signal alignment for MBW analysis

This section deals with signal alignment, i.e. how the raw flow and gas concentration signals are analysed. Both are recorded as separate signals and any delay in one or the other means that, at the time of analysis, they will be misaligned.

In Innocor there is a delay in gas signal measurement due to time taken for gas to reach the analyser and to be analysed. This causes a misalignment of flow and gas concentration. If left unaltered there is an error in gas concentration measurement which is proportional to the degree of misalignment. Time constants within the respiratory system affect the waveform of gas concentration produced during breath by breath assessments. In systems with shorter time-constants, a greater proportion of the tidal volume is expired early in the breath. The effect of misalignment of gas and flow signals is therefore more profound in respiratory systems with shorter time-constants (less compliant lungs). This source of error has potentially greater effects in younger patients and those with respiratory disease(Tang et al. 2005).

Correction of flow and concentration misalignment is most often achieved by applying a constant time-shift to one of the signals. Calculating this correction is more difficult.

As there are phases to each expiratory breath, gas concentration does not rise instantly as each breath begins. Therefore, the flow gas delay cannot be measured on a breath by breath basis. Also, flow gas delay varies depending on gas composition and temperature, so ambient changes may affect the value(Brunner et al. 1985, Eberhard et al. 1999). Flow gas delay in Innocor is determined as part of device calibration on a daily basis using a standardised manoeuvre.

Standard Innocor manoeuvre

According to the published standard operating procedure (Appendix 1), Innocor calculates flow-gas delay in a way similar to that described by Brunner et al(Brunner and Westenskow 1988). After a step change, measured concentration rises in a sigmoid fashion after the analyser delay. The 50% rise in concentration is considered the mid-point of the gas front. The time taken from the change in flow, minus the dead space portion between air and sample capillary to the 50 % rise in gas signal, is the flow gas delay. The 50% rise is calculated using the equal area technique, where the areas under the curve before and after are equal. This is shown in the shaded area in Figure 63. Delay time is shown under the time axis. The reference to “Rise Time T70” above the graph is not relevant for this section. The concept of rise time is discussed separately.

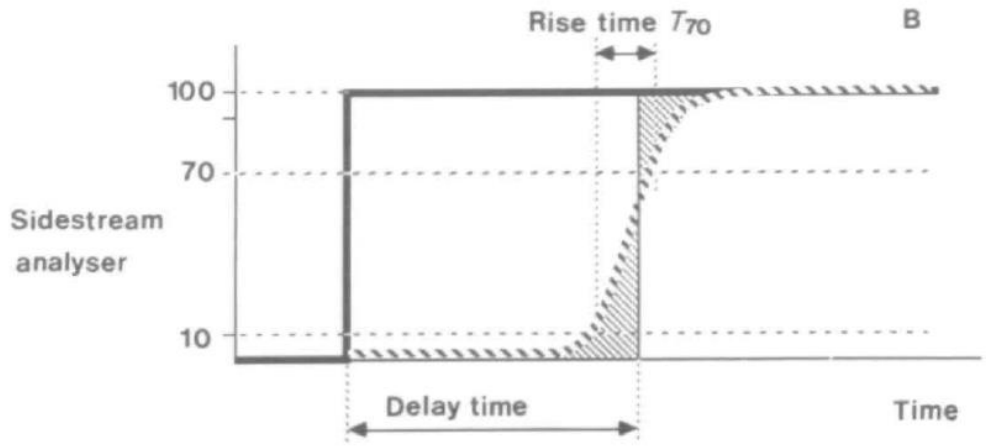


Figure 63 - calculating the time delay (flow gas delay) between a change in flow (solid line) and the 50% rise in gas signal (shaded area). Taken from Brunner et al(Brunner and Westenskow 1988).

To achieve a sudden change in flow coinciding with change in concentration, a standard manoeuvre is incorporated into the Innocor software. This involves aligning a sudden fall in CO_2 and change in flow at the time of a sharp intake of breath. The operator breathes out slowly to achieve a CO_2 plateau, then sharply breathes in. 10 repeats are performed to obtain an average. Clearing of the equipment dead space is taken into account in the calculation(Innovision 2005-05).

The assumptions with this method are:

- CO_2 and SF_6 responses are equivalent.
- Fall and rise times are equivalent.
- Delay is not dependent on flow.

For the purposes of MBW in adults and older children, small errors in flow gas delay do not contribute to large errors in FRC or end-tidal gas concentration. However, as infants and young children have shorter respiratory system time constants, a larger proportion of the breath is expired early in the expiratory phase. Therefore there is less confidence with this protocol in younger patients. Also, a large flow is generated when an operator performs this manoeuvre; much larger than is seen in infant tidal breathing. This may not affect the accuracy of the signal alignment but it is difficult to test. It is intuitive for routine calibration to mimic the flow change seen in the

patient to be tested. Bypassing the oxygraph module during rise time shortening alterations renders the Innocor unable to measure flow gas delay by the standard manoeuvre. This is a "fault" with the software but cannot be resolved. Furthermore this manoeuvre may not be suitable for infant calibration. Alternative methods for deriving flow gas delay must therefore be sought.

Alternative methods for deriving flow gas delay

Generating a sudden change in gas concentration by opening an electronically controlled valve very close to a gas sample capillary reveals the time taken for the gas signal to rise. This method is adopted by Per Gustafsson in the mass spectrometer MBW setup. This is possible because the gas signal is inputted to a laptop controlling the electronic valve. Therefore the signal rise can be timed. As the Innocor is not able to output a real-time signal, the timed valve approach cannot be used.

Equilibration of humidity in shortened nafion tubing

The standard Innocor setup uses a 180cm length of nafion with leuer-lock attachments to connect to the sample capillary and Innocor inlet. Contrary to a published study measuring mass spectrometer rise time with varying lengths of tubing(Lerou et al. 1990), shortening the nafion length appeared to improve rise time. The obvious concern is that the shortened nafion is unable to equilibrate humidified exhaled gas in the time it takes to pass through the tube. Advice from Innovision was that 180cm is a convenient length, rather than being the minimum requirement.

Humidity and gas volume

Gas volume and pressure are related according to Boyles Law. Pressure and volume are also influenced by temperature, density and gas composition. Water vapour (humidity) decreases density and therefore volume increases at a constant pressure and temperature. This is important during gas sampling as temperature and humidity fluctuate as sampled gas changes from relatively dry and cool room air to relatively humid and warm expired air.

Without adjusting for these variables, gas concentrations would be incorrectly estimated. Innocor adjusts for temperature fluctuations by heating the internal gas

analyser and sampled gas. Humidity is eliminated by the use of a Nafion sampling line.

Nafion

Information taken from Permapure website(Permapure 2009).
Nafion is a complex co-polymer, developed by the Du-pont company in the 1960's. It is modified from Teflon, and therefore has very high tolerance to heat. It was the first synthetic polymer with ionic properties. As such, it is able to act as an ion and proton exchange membrane. It is also widely used as a drying membrane due to selective and high permeability to water vapour in humidified gases. These unusual properties make it ideal for equilibration of humidified sampled gas. Nafion does not degrade over time and, while the surface may become coated with organic residues, the selective permeability to water is largely unaffected.

In theory, a Nafion membrane is able to completely dry a gas to less than 1% relative humidity. This is only possible with a dry gas passing over the outside of the tubing, which is not practical or necessary for Innocor gas sampling. For MBW testing, sampled gas needs to equilibrate with the air (approx. 30-40% relative humidity) in the time it is within the tubing, prior to entering the analyser.

Publications investigating nafion gas drying properties

Few studies have investigated the efficiency of nafion tubing in drying humidified gas. Leckrone et al measured the ability of nafion tubing with varying diameters and lengths to completely dry humidified helium of different temperatures(Leckrone and Hayes 1997). This was to determine the factors that most influence the efficiency of nafion gas drying. Importantly humidified gas was passed through the tube, with dry gas passed round the outside, creating a large humidity gradient.

The findings of this study were that nafion is able to completely dry (to <1% RH) saturated humidified gas efficiently. Efficiency is determined by temperature, diffusion coefficient of water vapour (cm^2/s, T_D) and the time gas is in the tubing

(length and flow rate, T_R). The length of nafion required to dry humidified gas is determined by the following equation.

$$L \geq F_c(10^{3.8}/120\pi D)$$

L = length of tubing

F_c = flow of gas within nafion (120ml/min)

D = diffusion coefficient of water at 1 atm pressure (0.260 in air at 23^{o}C)

Therefore, according to this paper, the length of nafion required to completely dry sampled gas in the Innocor system would be:

$120(10^{3.8}/120\pi 0.260) = 120(10^{3.8}/98.018) = 7724$mm i.e. 7.7metres.

This experiment has limited application as it was designed to make the gas sample completely dry (dew point <-40, RH<1%). This is not necessary for MBW gas samples as the nafion is only be expected to equilibrate with air (RH 20-40%) in a reasonable length of tubing without dry gas carrier passing over the outside. Graphs from this study indicate that 70% removal of water (approximate equilibration) is achieved in nafion lengths as low as 22cm, with flow rates of 95 ml/min (Innocor 120ml/min). With a length of nafion 40cm, the residence time of gas within the tubing, at a flow rate of 120ml/min is approximately 4 seconds. Taking into account diffusion coefficient of water (Td) in air at 1 atmosphere pressure, the T_R /T_D is 1.44. 70% removal of water is theoretically possible at this length, according to this paper. Using a 30cm length of nafion results in a Tr/Td of 1.3, which visually from the graph indicates only 65-70% removal of water.

Experimental data

Previous work in this department has demonstrated the stability of the SF_6 signal with different humidity levels(A. R. Horsley et al. 2008). This was done using heated, humidified gas and full length nafion tubing (180cm). Shorter lengths of nafion appear to be advantageous in terms of signal rise time. Therefore, to

determine whether shorter nafion lengths are able to equilibrate humidity to room levels, as required in MBW testing the following assumptions were made:

1. Humidity causes a decrease in density of gas and increase in volume. Therefore SF_6 concentration will decrease if humidity is not equilibrated.
2. Equilibration happens in both directions. Humid expired gas loses water to room air and dry inspired gas (from tank) takes water from room air.

Aims

1. Assess change in measured gas concentration between dry and humidified gas using different nafion lengths.
2. Find the minimum length of nafion suitable for use in the infant Innocor setup.

Method

Equipment:

- Innocor gas analyser.
- SF_6 supply – 0.2% (+/-5%).
- DeVillbis nebuliser – cold humidification.
- Fischer and Paykel Healthcare MR750 humidifier – warm humidification.
- Elephant tubing to conduct gas.
- Nafion tubing of various lengths. Fitted with leuer lock ends and metal or plastic sampling capillary. Dust filter in place proximal to Innocor inlet as standard (Figure 64).

Sequence

Gas concentration is measured with each length of nafion in turn from longest to shortest length. A stable concentration of dry gas was established first, which was then humidified.

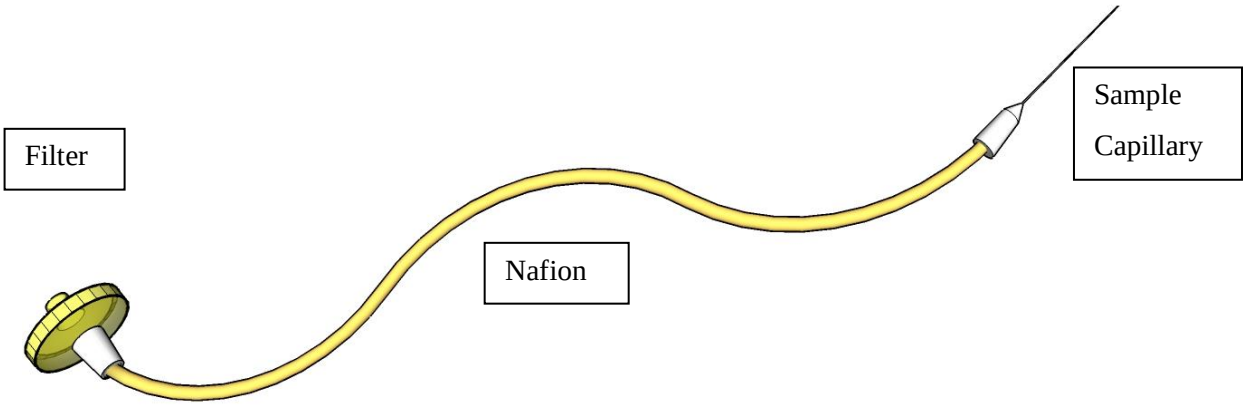


Figure 64 - Nafion tubing with sample capillary and filter in-situ

Analysis

Gas signal data were transferred from the Innocor to lab computer. An excel spreadsheet displayed the signal as an x-y plot with time. A stable concentration period was identified and mean, SD, Signal/noise ratio, min and max for that period were calculated.

Given that the standard deviation of the signal is very low, a t-test between dry and humid is not appropriate. Cohen's d was chosen to represent the effect size of the difference between dry and humid gas concentration. Cohen's d is defined as the difference between the means divided by a pooled standard deviation(Cohen 1992).

$$Cohen's\ d = \frac{Mean\ concentration\ (dry) - \ mean\ concentration\ (humid)}{Pooled\ standard\ deviation}$$

If the d value increases, this indicates that the overlap of values in the two groups (humid vs. dry) is less. Therefore, a d of 0.0 indicates complete overlap, while a d of 2 corresponds to less than 20% overlap of values around the mean. Increased Cohen's number indicates difference in concentration between dry and humidified. Cohen determined that an effect size (d) of 0.2 is small, 0.5 is medium and 0.8 is large.

Results

3 experiments were conducted, 2 with a humidity and temperature probe to confirm 100% relative humidity (RH) in the gas sample. Nafion lengths quoted include sample capillary and filter.

Experiment description	Nafion Lengths (cm)
Experiment 1 – 3 lengths of nafion	183
Devillbis nebuliser, room temperature	91.4
Dry gas RH <2%	32.0
Humid gas RH ≥95%	-
Experiment 2 – 5 lengths of nafion	65.4
Devillbis nebuliser, room temperature	52.5
Dry gas RH <2%	42.5
Humid gas RH ≥95%	34.0
	31.5 (different piece of tubing)
Experiment 3 – 4 lengths of nafion	182
Fischer and Paykel MR750 medical humidifier	92
Temperature 39^{o}C	34
	31.5 (different piece of tubing)

Table 25 - Nafion experiment design

A steady state of gas concentration was achieved with all lengths of nafion. Signal to noise ratio (standard deviation divided by mean) was maintained with all lengths and did not change with humidity.

Cohen's *d*, effect size measure, was small throughout, barely rising above 0.2 (Table 26). However, *d* appeared to increase exponentially at smaller lengths of nafion (<40cm) in multiple experiments using more than one piece of nafion (Figure 65). Log transformation of length vs. d plot (Figure 66) shows a significant negative correlation between nafion length and Cohen's d ($r^2 = 0.74$, p=0.0003).

	Dry			Humid			Cohen's *d*
Length (cm)	Selected sample time (s)	Mean SF_6 concentration	SD	Selected sample time (s)	Mean SF_6 concentration	SD	(humid vs. dry concentrations)
183.00	30.01	0.210	0.00038	30.01	0.210	0.00025	0.004
182.00	20.01	0.203	0.00017	20.01	0.203	0.00016	0.006
92.00	20.01	0.204	0.00016	20.01	0.203	0.00031	0.040
91.40	28.01	0.210	0.00026	27.01	0.210	0.00021	0.032
65.40	11.20	0.208	0.00028	6.01	0.208	0.00025	0.030
52.50	20.39	0.209	0.00027	20.45	0.208	0.00025	0.048
42.50	15.71	0.209	0.00019	15.01	0.208	0.00019	0.048
34.00	9.21	0.208	0.00024	10.01	0.207	0.00024	0.020
34.00	20.01	0.204	0.00028	20.01	0.204	0.00039	0.108
32.00	22.55	0.226	0.00025	23.67	0.224	0.00025	0.183
31.50	20.01	0.204	0.00020	20.01	0.203	0.00028	0.138
31.50	8.95	0.208	0.00022	11.01	0.207	0.00023	0.204

Table 26 - Cohen's d values at different nafion lengths. Cohen's d represents the degree of overlap of values, with lower values indicating increased degree of overlap.

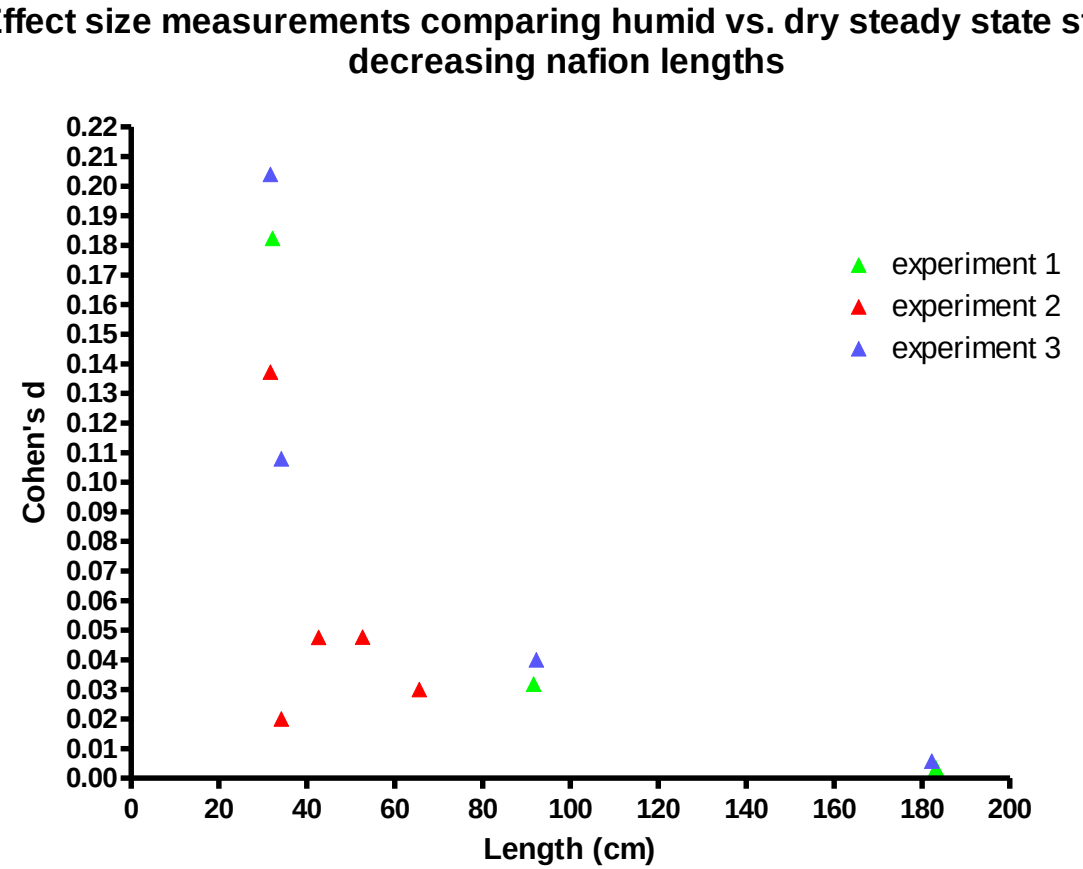


Figure 65 - length of nafion vs. Cohen's d value. At lower lengths of nafion, an exponential rise (less overlap) was seen.

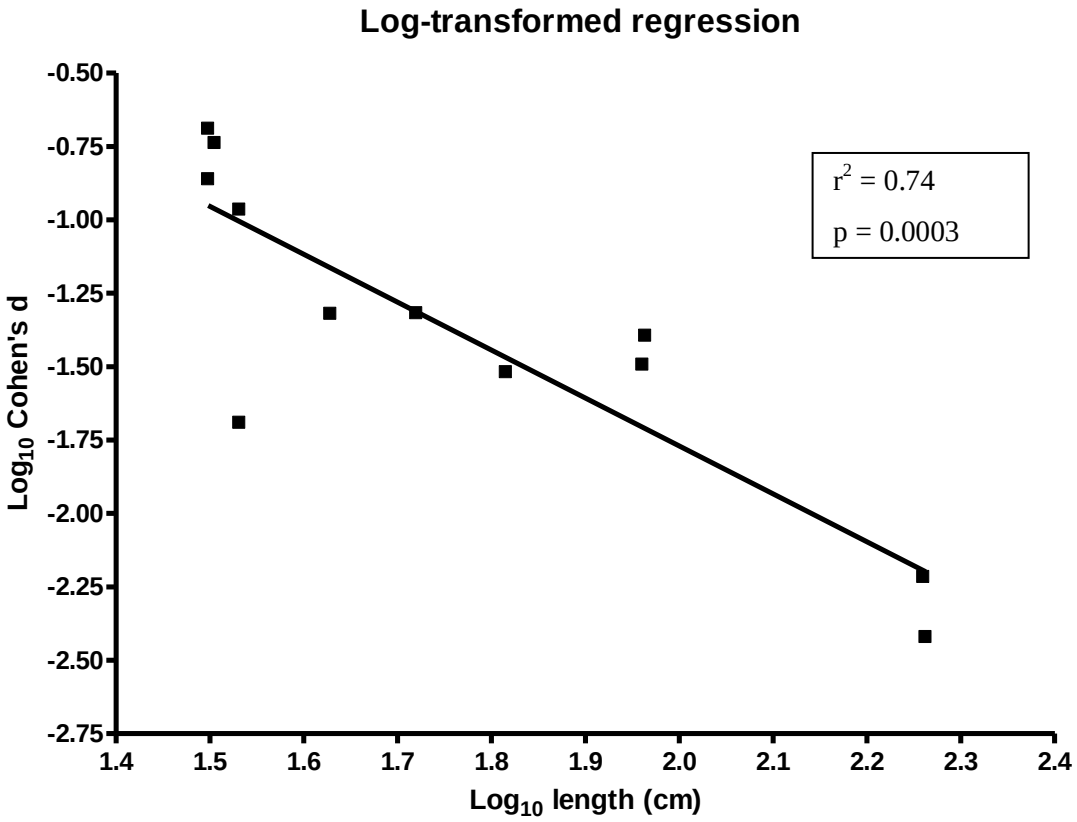


Figure 66 - log transformed nafion length vs. Cohen's d indicates relationship between length and degree of overlap of dry vs. humidified values.

Conclusions

According to previous publications, the likely shortest length of nafion able to equilibrate expired gas humidity to room conditions is 40cm. This has been confirmed in these experiments, where there is a significant correlation between nafion length and Cohen's d. Using lengths of nafion shorter than 40cm largely resulted in higher d values.

Shortening the nafion length is important in improving the performance of the Innocor gas analyser for infant washout studies. There is a practical minimal length beyond which the nafion would not connect the pneumotach with the Innocor. I have shown that, in a comparable environment to MBW measurements, nafion lengths greater than 40cm perform well in equilibrating humid gas and ensuring a stable gas concentration throughout. It is therefore appropriate to limit the nafion length to no less than 40cm for the infant Innocor setup.

Gas sample flow rate

The Innocor sample flow rate was measured to determine the amount of lost gas during MBW testing. During normal testing gas is sampled from the pneumotach during MBW testing and is not included in expired or reinspired volumes (i.e. lost). This is more important for washouts in young children and infants compared with older children and adults, as the gas sampling rate is large relative to the tidal volume. Each Innocor machine differs slightly and the documented gas sample flow rate according to the user manual is 120-130ml/min. Innovision have stated that this can be increased to improve signal response time but this may affect the stability of the gas signal and will also increase lost gas. Also, this needs to be done by Innovision technicians.

Aim

The documented sample flow rate from the official Innocor documentation is 120ml/min. To determine the actual sample flow rate in the RHSC Innocor to allow calculation of lost gas during washouts, the following experiment was conducted.

Method

Flow was measured through the pneumotach with one end blocked. The flow generated by the gas sample flow was detected by the flow transducer (Figure 67).

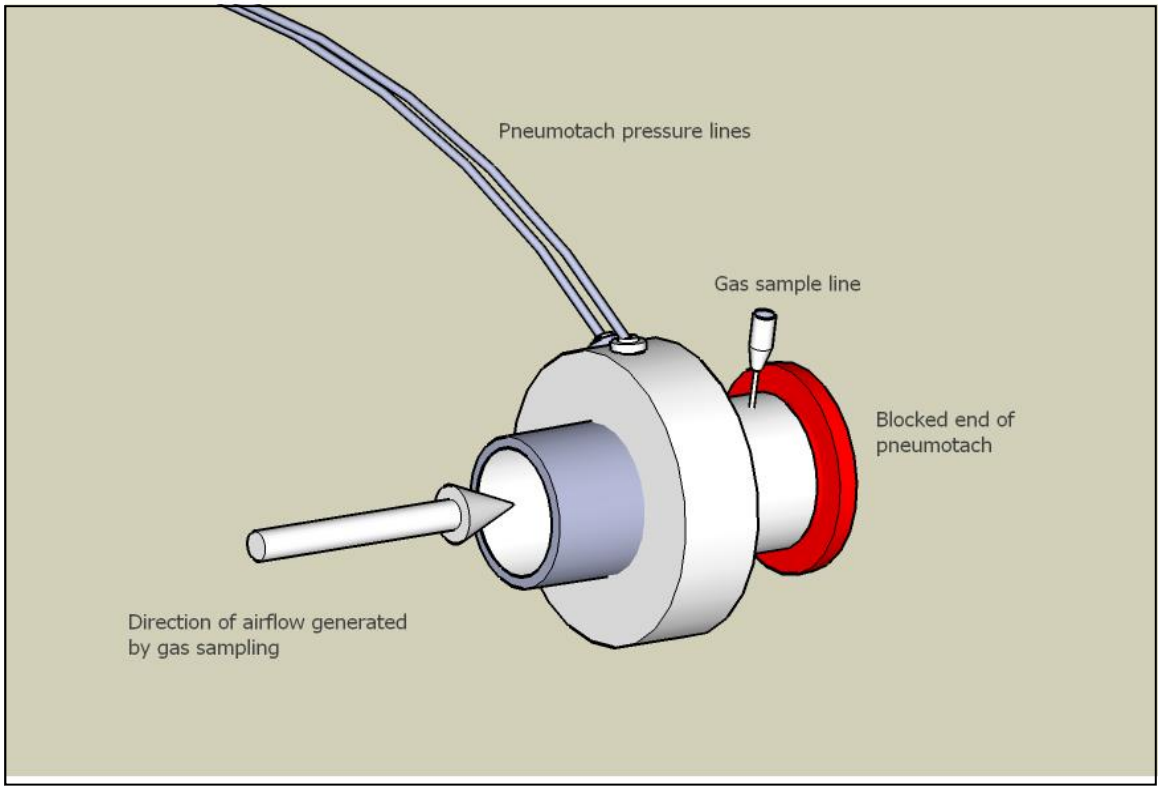


Figure 67 - schematic of flow transducer to measure sample flow rate. Red disc is the blocked pneumotach.

All flow pressure transducers have a baseline drift. This is due to slight inaccuracies in calibration and electrical signal drift over time. This baseline slope of the flow signal was accounted for by determining the intercept of a linear regression line through all the values. The slope of the regression fit is the flow drift of the pressure transducer. The negative offset is the constant sample flow rate

Results

Flow, at low rates (~2-3ml/s) was highly variable (

Figure 68). Mean(SD) flow in one experiment was -2.90 (0.65)ml/sec, coefficient of variation (%) was 22.5%.

A sample graph is shown here with flow measured over time just after a flow meter reset. The regression slope intersection on the y-axis determines the mean sample flow rate.

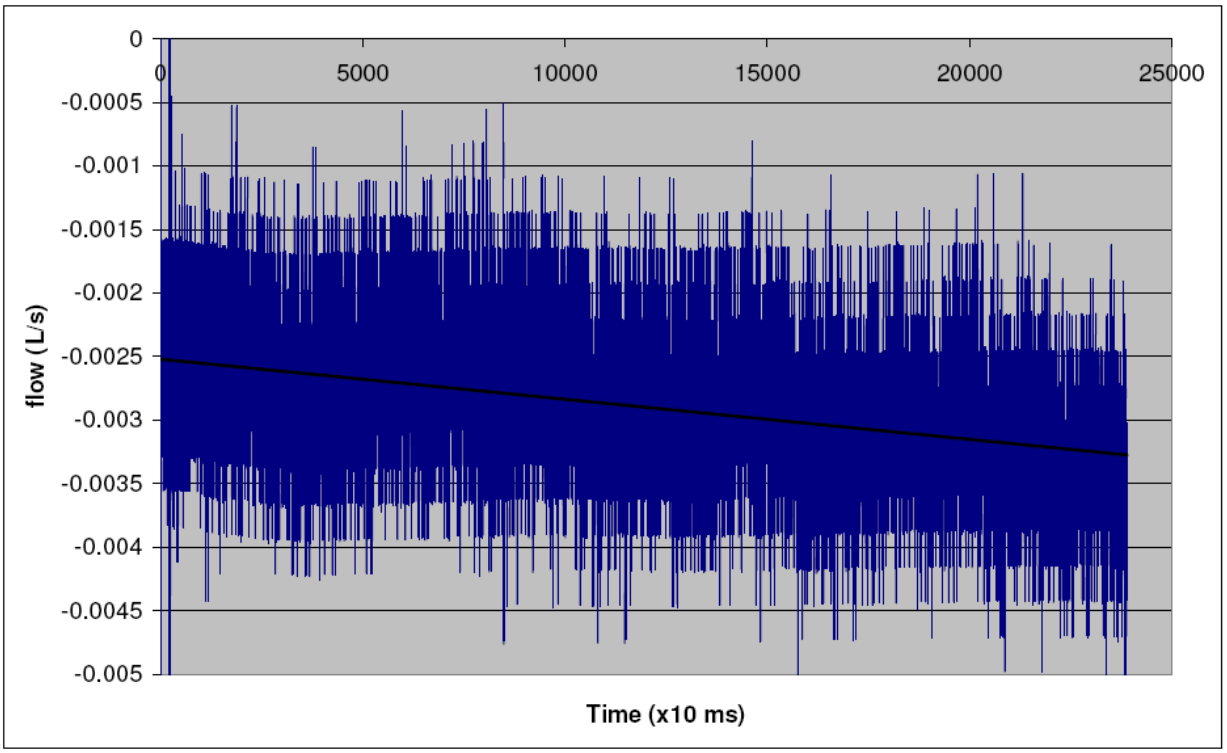


Figure 68 - Raw flow reading during gas sampling. Variability of real time measurements shown, with drift in flow shown by the black regression line. Intersection of the regression is the underlying sample flow rate.

The mean sample flow rate, measured over 3 experiments, is shown in Table 27

Experiment no.	Measurement time (seconds)	Sample Flow Rate (ml/min)
1	344	125.45
2	362	138.78
3	240	151.20
Mean		**138.48**

Table 27 - three sample flow rate experiments. Sample flow rate and mean value shown.

Conclusions

This basic experiment indicates the sample flow rate is highly variable and on average slightly higher than documented in the official Innovision documentation. It is expected this could have resulted in a faster response time, but this was not seen in practice. Other factors may of course contribute to this.

When considering infant washouts, approximately 2.3ml is lost from the system every second. As this is a large volume in proportion to tidal volumes seen in infants, this is a factor that will contribute to MBW expired and inspired gas volume inaccuracies.

Chapter summary and conclusions

Innocor has already been adapted for adults and older children with some technical adjustments. In previous experiments, conducted by Alex Horsley, the Innocor was altered by replacing the large dead space Pneumotach and gas switching system for a standard low dead space pneumotach and side-stream sample capillary needle. This setup was based on similar mass spectrometer equipment designed by Per Gustafsson. A comparison with the mass spectrometer system showed that with simple SF6 signal speeding, acceptable results were achieved, relevant to washouts conducted in children as young as 5 years old.

To summarise the issues discussed in this chapter:

- Standard speed of Innocor gas sample analysis is too slow for infant washouts based on mathematical modelling of gas concentration curves.

- A rise time of less than 100ms, as recommended in current literature, may still result in large errors in gas volume estimation at higher respiratory rates.
- The Innocor has extra internal steps, not needed for MBW, which can be bypassed.
- Simple hardware modifications can speed step concentration rise time to less than 100ms.
- The nafion tubing can be shortened without affecting its drying properties.
- The sample flow rate in Innocor is 138ml/min, which may lead to large errors if not compensated for.
- Current methods for measuring gas sample delay may not be adequate for infant equipment.

MBW in young children and infants requires detailed adherence to published guidelines. Attention must be paid to equipment dead space, re-inspired gas volume, gas analyser speed and acceptability of the test to the age group in question. Specifications for all of these are well documented, but it may be that some are not based on published experimental evidence, as studies in this field are few.

It is unlikely that the standard Innocor setup, even with a reduced equipment dead space, will be able to accurately estimate expired gas volume in subjects with higher respiratory rates and low tidal volume, due to slow signal rise time. This is indicated from mathematical gas concentration curves. Simple, in-house technical modifications have improved the signal rise time to a value that is acceptable to the most recent infant lung function guidelines. As a result of these modifications, the standard daily signal alignment calibration process needs to be revised.

These experiments were necessary to investigate the feasibility of further in-vitro and in-vivo experiments, performing washouts in subjects with small tidal volumes and fast respiratory rates. The Innocor has a number of advantages over other MBW devices and these modifications may mean it is of interest to other research groups interested in performing MBW in larger populations. While it appears from mathematical modelling that there may be 3-4% error in infant washouts, even with the modifications made, this may be acceptable given the variability of the MBW test in human subjects. The only way to assess the accuracy of the modified Innocor device is to perform a clinical comparison with a known lung volume or against the

accepted gold standard MBW device. A known volume can only be measured with a mechanical lung model and the next chapter presents this model study and describes a clinical comparison across the age range with an MBW mass spectrometer.

Learning points and contribution to thesis

This is the first time that the Innocor has been subjected to rigorous investigation in preparation for infant MBW. Since no published guidelines exist for such equipment, all aspects have been addressed from first principles. This does not answer whether Innocor is able to accurately measure breath-by-breath gas concentration in infant washouts. Only a clinical study will demonstrate this. This chapter serves to provide a modified Innocor with theoretical accuracy in young subjects.

Chapter 9 - Validation of modified Innocor using mechanical lung model and comparison with mass spectrometer

Introduction

As stated previously, Innocor has a number of advantages in terms of size, ease of operation, cost and portability compared with other available MBW devices. In addition, because it uses 40 times less gas, Innocor has an environmental advantage for larger, multi-centre studies. As MBW has gained prominence as a suitable infant lung function measurement, these factors have been examined in more detail. To make the transition from experimental research measurement to widely conducted research and clinical test, LCI (as well as other MBW indices) must be able to be conducted in clinical situations, and the devices be validated for use in the subjects in question. There are a number of published standards for infant lung function, covering a number of different techniques, such as MBW, forced oscillation manoeuvres, plethysmography and airway resistance(Bates et al. 2000, 'ATS/ERS Statement: Raised Volume Forced Expirations in Infants: Guidelines for Current Practice' 2005, Beardsmore et al. 2007, Beydon et al. 2007, Frey et al. 2000b, Frey et al. 2000a, Frey et al. 2001, Frey 2005, Gaultier et al. 1995, Sly et al. 2000, Stocks et al. 1989, Stocks and Quanjer 1995, Stocks et al. 2001). Many groups who conduct MBW in research situations contributed to these standards, increasing confidence that equipment used is suitable. Validation of new equipment and techniques should thus stem from evidence based standards. A criticism of published standards is that in order to validate equipment developed prior to publication of standards, statements made in these standards publications may simply justify current practice, rather than reflect evidence. A good example of this, discussed earlier in this thesis, is the statement made about the upper acceptable limit for signal rise time during breath by breath gas washout measurement. While 100ms is a reasonable number, there is little evidence to support this.

Technical specifications, specifically related to the analyser, limit use of Innocor to older children and adults. These limitations stem from published standards, rather

than clear evidence of inaccuracies from experimental data. This chapter describes the first known experiments to validate Innocor for use in younger age groups. This is therefore key to the overall message of the thesis, and is of interest to researchers in other fields, looking to perform physiological measurements in young children, with equipment that may or may not have been validated appropriately. The importance of not taking reported technical limitations for granted, and rigorously testing equipment against an appropriate standard, will become clear as the chapter progresses.

The mass spectrometer system, using the AMIS2000 quadrapole mass spectrometer (Innovision, Denmark) was initially designed in Professor Per Gustafsson's department, Goteborg, Sweden. A number of published studies have explored the use of this mass spectrometer equipment in children with lung disease. Many have been mentioned throughout this thesis. Over the past 10 years he has set up similar systems in other centres, most notably the Institute of Child Health, Great Ormond Street Children's Hospital, London. As a result of these collaborations, the mass spectrometer system is considered the Gold Standard device for MBW in all age groups.

Despite performing well in experimental and clinical studies in older children and adults, there are specific limitations to Innocor which are considered to invalidate its use in younger children (< 5yrs). This chapter describes a lung model experiment and a clinical study undertaken to answer whether modified Innocor, as described in chapter 8, performs as well as the currently accepted gold standard mass spectrometer device.

Importance of rigorous validation

The previous methods description (Chapter 8) has shown how technical gas analysis limitations contribute to error in calculated gas concentration and volume. The following factors were both important and modifiable, disrupting the sampled gas front, thereby increasing the signal rise time:

- Nafion tubing and connections
- Oxygen measurement (oxygraph) of gas sample, prior to photoacoustic analysis

In addition, technical methodological issues became apparent during initial experiments which were addressed to improve washout accuracy:

- Signal alignment and measurement of flow gas delay
- Sample flow rate measurement
- Compensation for sampled gas lost from volume calculations

I have proposed solutions to these errors with hardware and mathematical compensation. The results of these experiments indicate Innocor may still lead to unacceptable error in volume calculation in younger age groups. Rigorous testing, involving human subjects, appeared the only way to properly address this question.

Previous comparisons with a mass spectrometer

Two previous studies (discussed in detail later in this chapter) have tested alternative MBW systems in-vivo, performing direct comparisons between mass spectrometry (MS) and the novel device in adults and infants(Fuchs et al. 2006, Pillow et al. 2004). Overall, these studies show that different devices can give very similar results when measuring FRC. However, it is clear that there are technical differences between the devices. While the mass spectrometer is considered the gold standard against which other devices should be compared, it must be borne in mind that this reference device may have an unrecognised technical or methodological error.

Need for comparison in health and disease

An important factor in equipment validation is the importance of testing equipment on those on whom future studies will be conducted. This has not been done before in MBW device comparisons. After investigating and adapting Innocor using mathematical modelling, it was shown that error in estimated expired volume is dependent on length of breath and tidal volume (Chapter 8). Because washouts are prolonged in disease, particularly cystic fibrosis, washout error may be dependent on presence or absence of disease. Any comparison for validation should include those with respiratory disease, as well as a healthy group.

Lung model washout method

Planning Innocor validation

Experienced research groups

Innocor had been adapted in the early stages by my colleague Dr Alex Horsley, clinical research fellow in Edinburgh, with considerable input from Professor Per Gustafsson. Two visits were made by Dr Horsley to Professor Gustafsson's department in Goteborg, Sweden. Simple experiments were conducted using volume syringes as a basic lung model and with a human subject breathing at different rates. Some of these data are published in this important Thorax journal publication(A. R. Horsley et al. 2008).

Initial planning for a clinical validation of Innocor involved obtaining a mass spectrometer for use in Edinburgh. As the simple gas signal was all that was needed, with flow data collected by Innocor, a full MBW setup was not required.

The cost of a new respiratory mass spectrometer was prohibitive, but after researching local institutions, a device was found in Glasgow University department of physiology. This was a quadropole mass spectrometer, with similar specifications to the AMIS2000. It was in the department for exercise testing of humans and seemed a suitable device for comparison of gas concentration data with Innocor. We proposed to this department a loan of the device, for a cost, as they were not using it at that time.

An agreement in principle was made with the professor of that department. However, following an initial positive meeting, it came to light that the device required considerable servicing as it had been out of use for some time. Another group also planned to use it, meaning we would have to wait until they were finished. This was a frustrating disappointment as there were no other available devices in the local area. After a number of weeks of negotiation it became clear this project was not going to happen within a reasonable timescale.

During this planning stage, further separate links were made between our department in Edinburgh and 2 other research institutes experienced in infant and paediatric lung function research. The following is a description of their input to the validation project:

- Department of Paediatric Respiratory Medicine, University Children's Hospital of Bern, Switzerland

  This department was then led by Professor Urs Frey, who has since moved to another institute. Professor Frey was closely involved in publishing Infant Lung function ERS task force documents (Frey et al. 2001, Frey et al. 2000a, Frey et al. 2000b).

  Following a presentation made at the European Respiratory Society international conference in 2007, I was approached by Dr Phillip Latzin, a clinical researcher for Prof. Frey. He was interested in the work done using Innocor, and how it could be improved. An invitation was made for me to visit their department to compare Innocor with the ExhalyzerD device (Eco Medics, Switzerland), the device used for MBW in their department.

  In January 2008, I took the Innocor to Bern by train. Three days were spent assembling the equipment, looking at data analysis, and testing Innocor using an in-house mechanical lung model.

  While it proved more difficult than expected to get Innocor to work using an infant-sized pneumotach, much useful experience was gained from this visit. I was able to spend time discussing important issues with experts in signal processing and multiple breath washout. The greatest benefit of this visit was being introduced to a mechanical lung model. This simple device was invaluable to preliminary Innocor validation experiments. It will be described in detail later. The device was built by Reudi Isler, a support and research representative of Eco Medics. He was kind enough to lend me the lung model to take back to Edinburgh.

  Once in Edinburgh, I was able to redesign a similar version to keep and use for further lung model washouts. This redesigned version was built by Mr George Campbell, a medical technician in the Western General Hospital, Edinburgh.

- The Department of Paediatric Respiratory Medicine, Royal Brompton Hospital, London

  Following a presentation of data from chapter 4, at the British Thoracic Society Winter Meeting, London, Professor Andy Bush proposed a clinical study comparing his newly purchased Innocor and mass spectrometer devices.

  Professor Bush is a world expert in paediatric respiratory medicine, with a highly respected research profile. The opportunity to work with him and his research fellow, Samantha Irving, was good for the thesis project as well for my personal research development. This was also a timely solution to the problem of difficult access to a respiratory mass spectrometer. The design of the clinical study that follows is discussed in detail in the following pages.

Through previous contacts and interest from presentations made at research meetings, I was able to discuss work with international experts and refine the study that follows. This enhanced the thesis project, allowing basic validation work to become important to the wider research community, in the context of equipment validation for infant respiratory function measurements.

Study 1 – In-vitro validation of Innocor using a mechanical lung model

In February 2008, I visited the department of paediatric respiratory medicine in Bern Switzerland. In collaboration with the manufacturers of the ExhalyzerD MBW device (Eco Medics), a lung model was assembled to measure accuracy of Innocor measuring SF6 washout from a small volume compartment. The main aim of the lung model is to mimic small volumes seen in infants, but with controlled and variable specifications. Therefore, if the washout device in question can accurately measure known model volumes, it would be inferred that it could accurately measure infant volumes in-vivo.

Description of Bern lung model

The Bern lung model consists of a dual compartment Perspex box partially filled with water. The two compartments communicate below water line, meaning that as

one side is filled with air, the other side rises. The washout volume (equivalent to FRC) is varied by different volumes of water. The breaths are driven by a motorised syringe, therefore tidal volume is constant, with a variable breath rate depending on the speed of the motor. This setup is ideal for simple washout experiments, but does not have any of the complexity of the human respiratory tree, therefore ventilation heterogeneity is not measured. The compartment "FRC" is derived from the normal dilution equations described in chapter 2.

This lung model allows validation of a washout device by mimicking the lung, in that gas is washed in and out of the compartment with tidal volume exchange. The volume in the model is known, either by volume displacement or by placing a scale on the box, and knowing the internal dimensions to calculate the volume. The volume of the connection hub and pneumotach must also be measured. High accuracy is required to quantify washout precision and error. A similar version of this lung model has very recently been used to validate a nitrogen MBW system, however a temperature control was added(Singer et al. 2012).

Below are pictures of the Bern lung model (Figure 69-71).

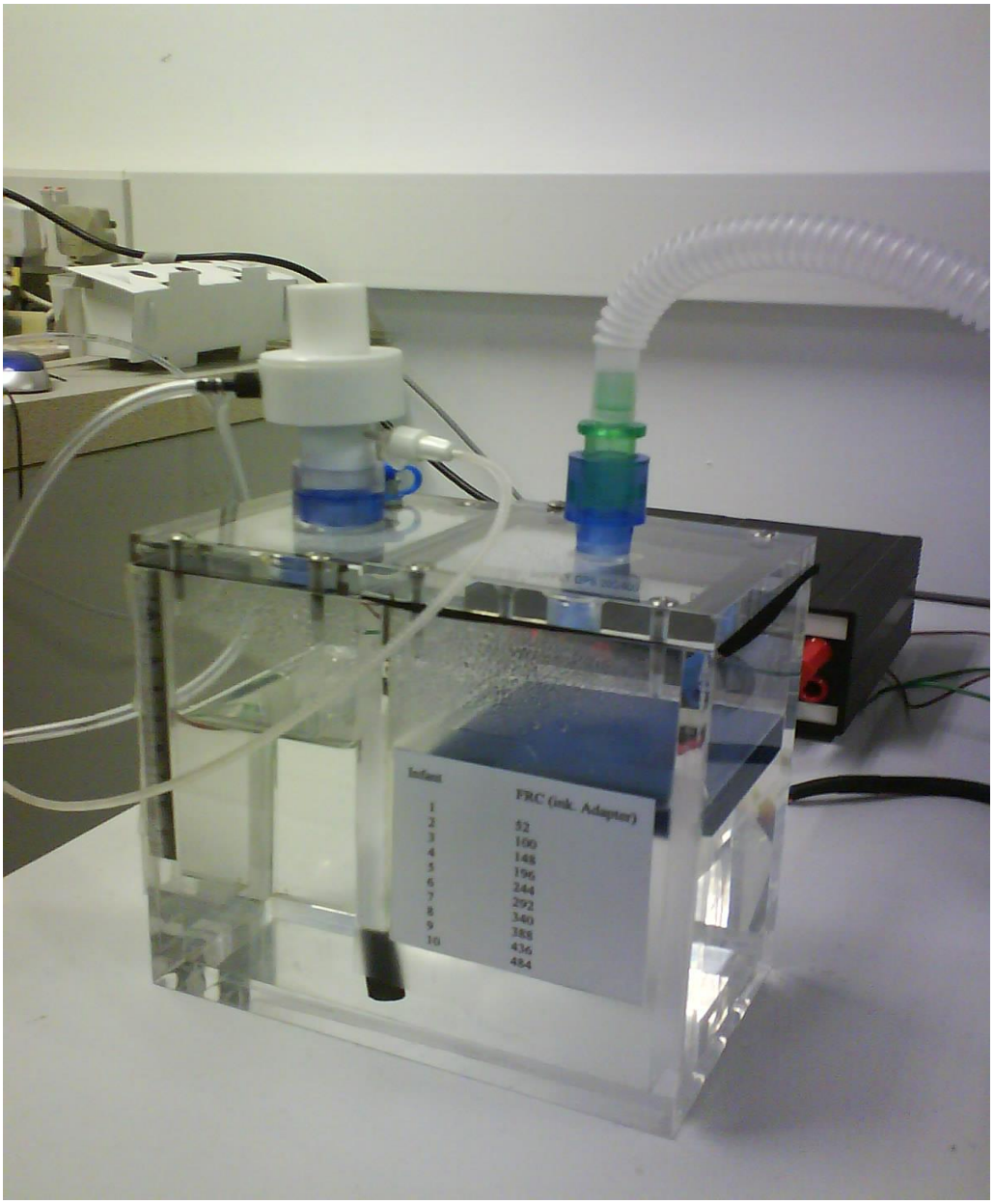


Figure 69 - lung model with dual compartments communicating below the water line. Left compartment is the variable "lung" volume and pneumotach with gas sample line set up for MBW. The ruler scale is stuck to the box, with pre-measured volumes written on the paper. Right compartment with blue foam to dampen waves has elephant tubing connected to the motorised syringe to deliver tidal volumes.

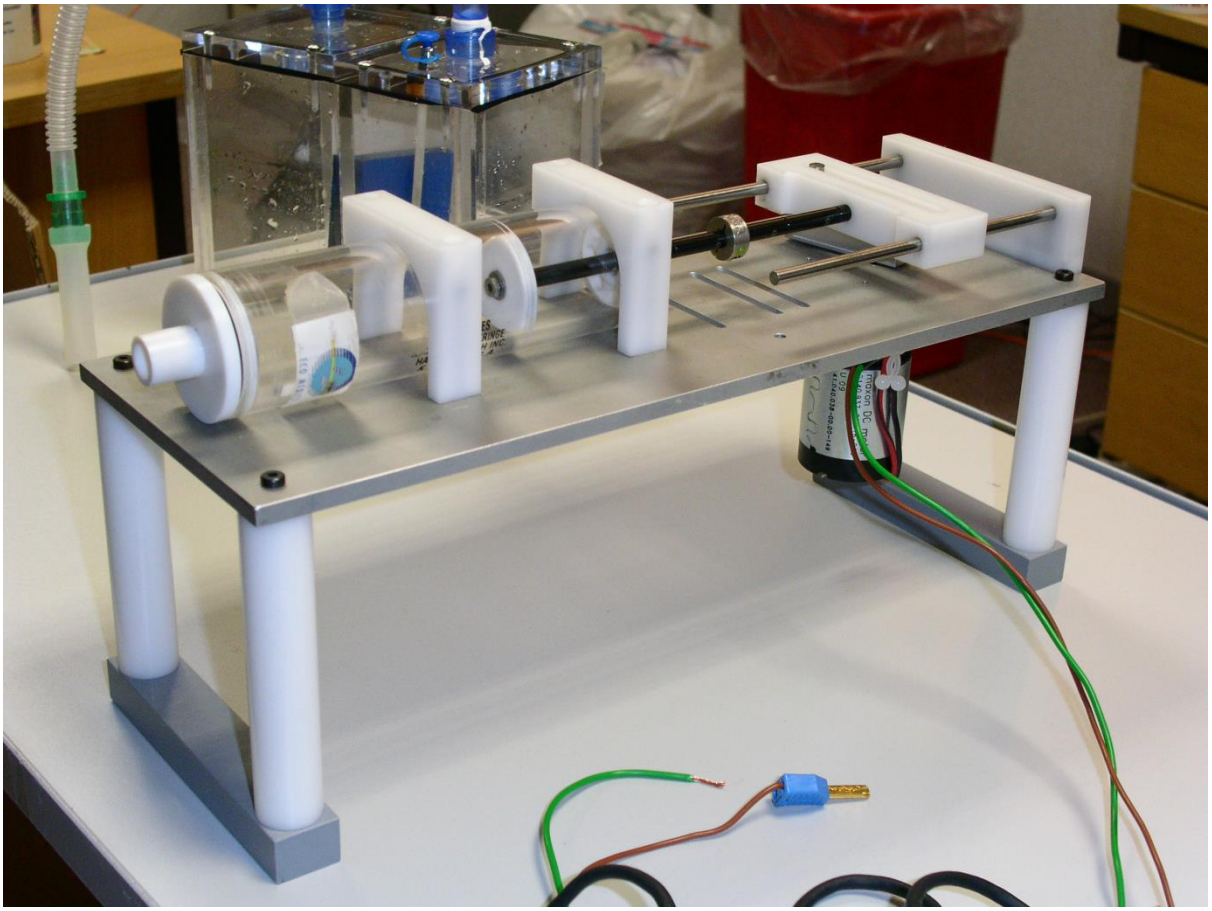


Figure 70 - motorised syringe. This syringe is fixed to the platform. A motor underneath the platform turns an arm, which pushes and pulls the syringe plunger. The volume on this setup is fixed. Speed (breath rate) is varied by the power supply voltage.

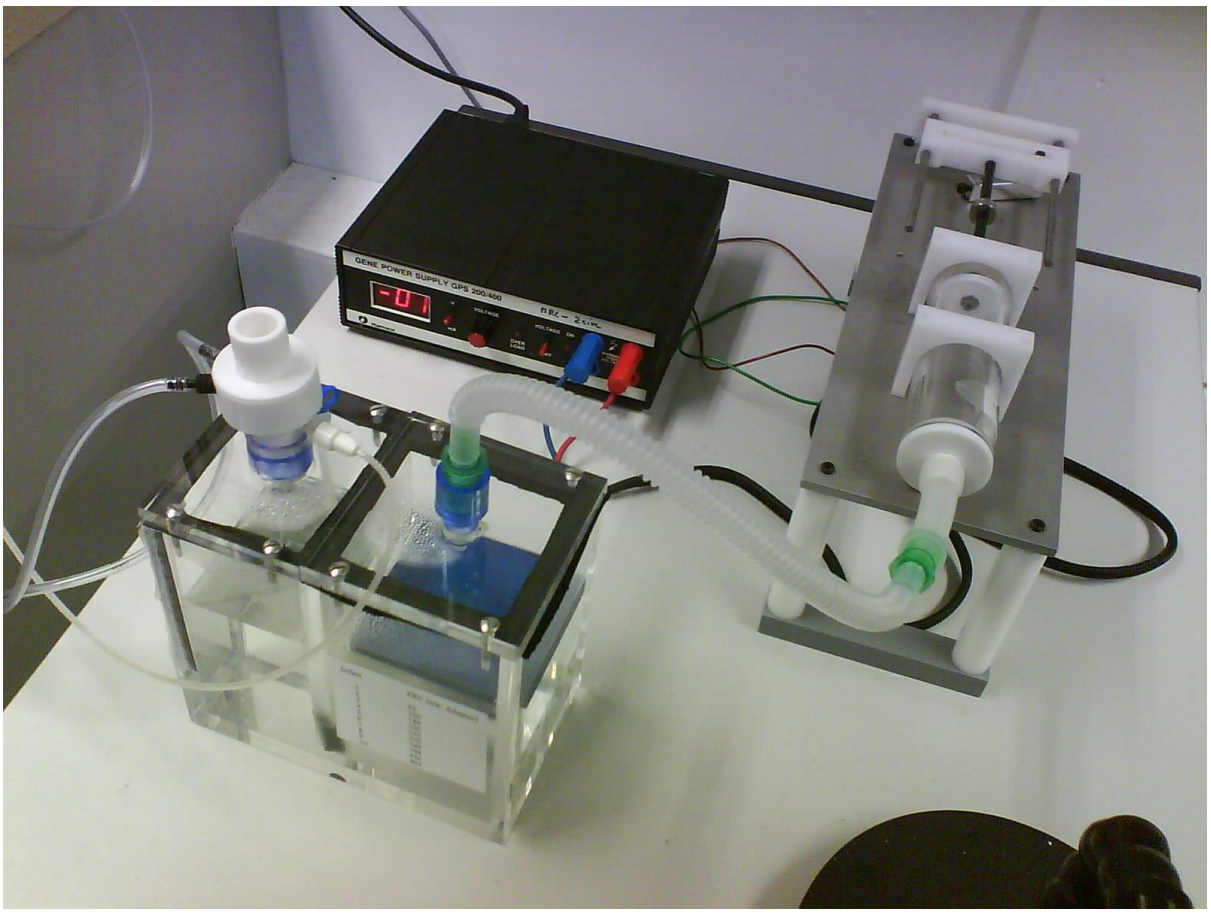


Figure 71 - the full lung model setup shows how the components connect. Power is varied using a DC power supply.

Description of Edinburgh lung model

Based on the design of the Bern lung model, a copy was developed for use in Edinburgh. The following slides (

Figure **72**) were made to instruct the technician:

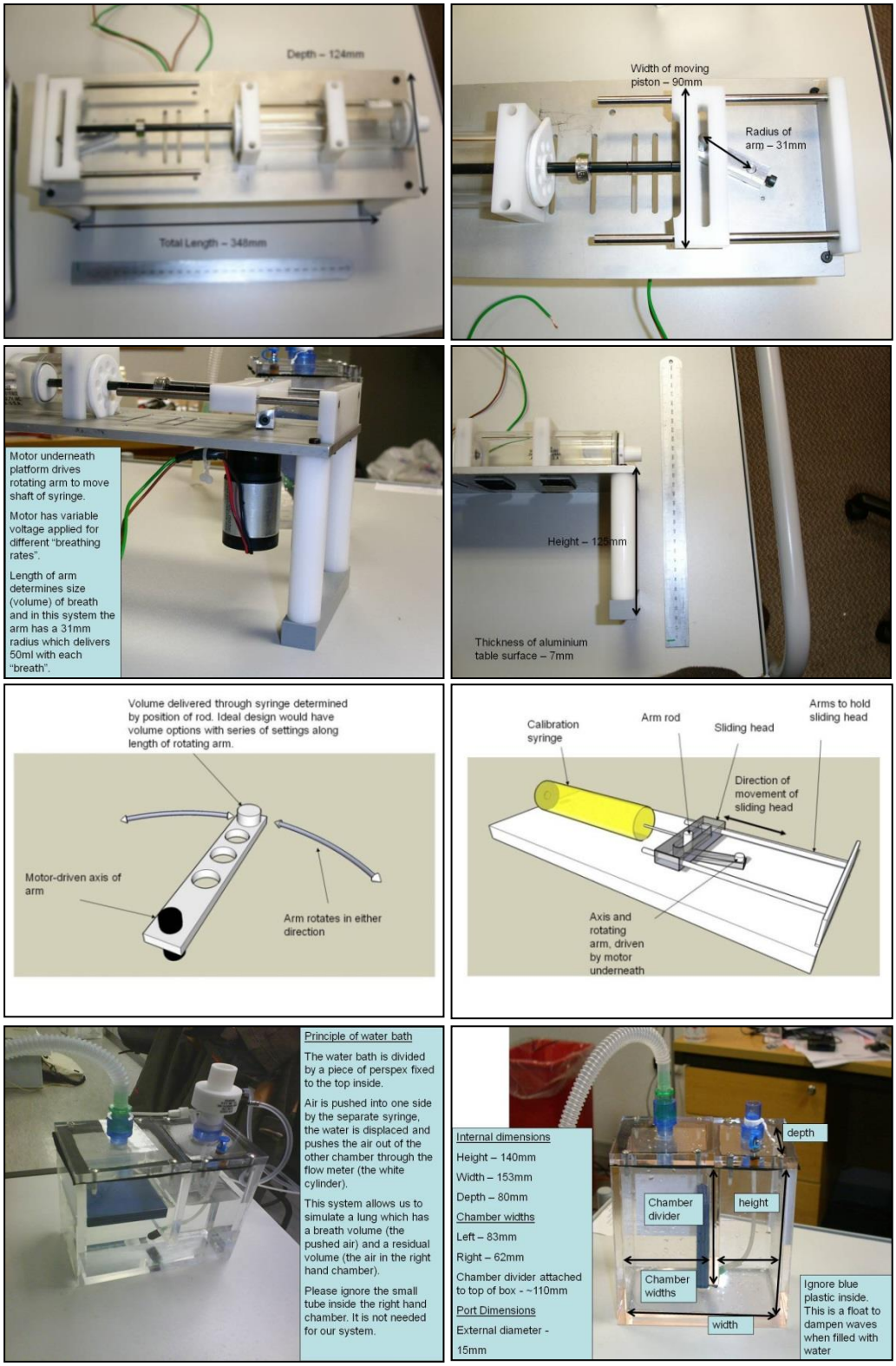


Figure 72 - specifications for copy lung model, presented to the Western General Hospital medical technician. The only change to the Edinburgh version of the lung model was to introduce variable tidal volumes by being able to attach the plunger at variable positions up the

rotating arm. It was only desirable to have variable tidal volume, rather than to be able to specify tidal volumes based on the length of the arm and the calibre of the syringe.

The resulting lung model is shown below. A higher torque is required to rotate the arm when the plunger is close to the axis, and the motor was of limited power. Because of this and the short length of the rotating arm, it was only possible to have one of 2 tidal volume options.

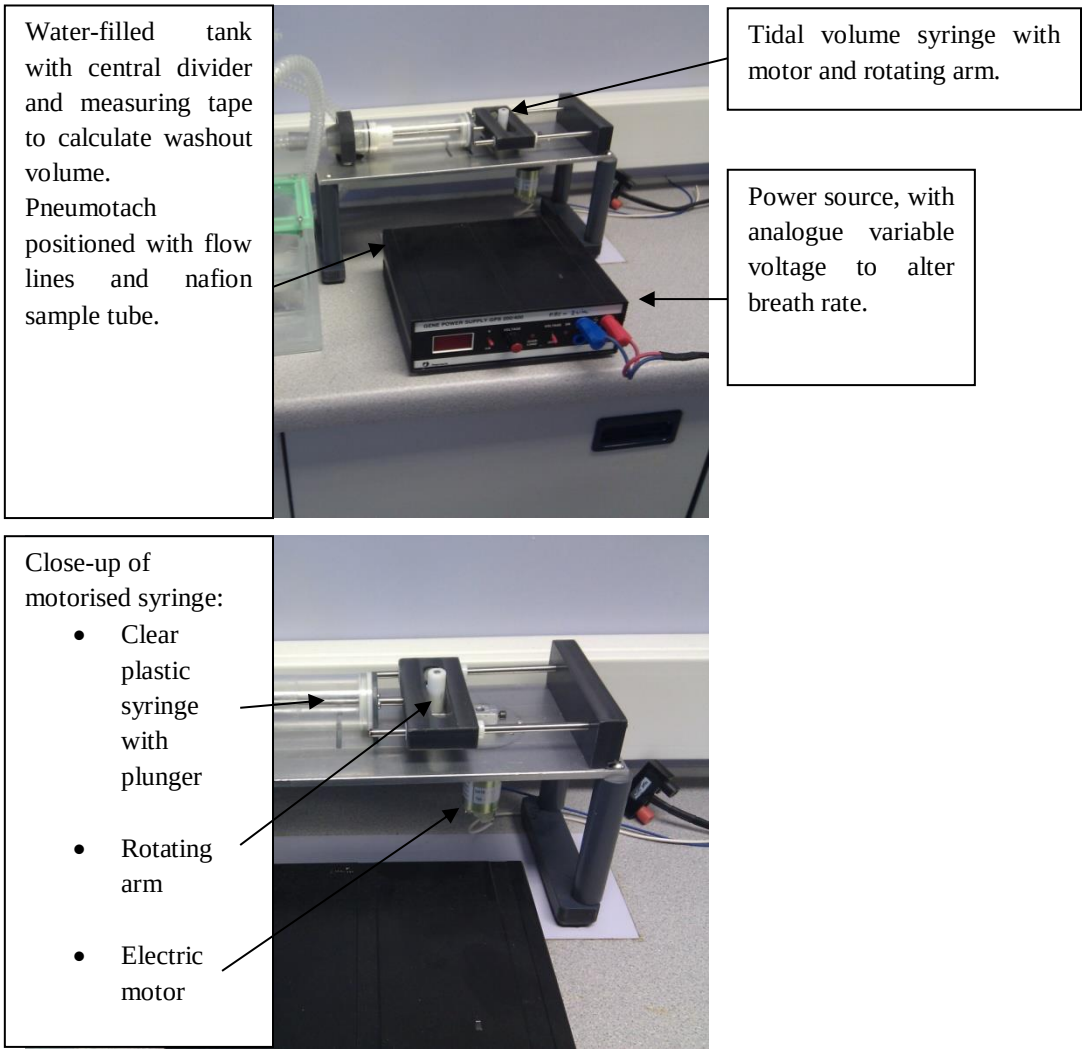


Figure 73 - detailed description of mechanical lung model.

Lung model variables

Washout volume (FRC) –

As previously described, the plastic box is partially filled with water, above the level of the divider. Thus, the washout volume can be varied. By knowing the internal diameters of the box, the volume in question can be measured. Alternatively, the volume was confirmed by filling the washout volume with water to the top.

Tidal volume –

The syringe is made from a clear plastic tube with a custom-built plunger. It was constructed in a similar fashion to a calibrated volume syringe, but does not deliver a set volume. It is not important what the exact volume is, however this must be determined to ensure this is appropriate to that expected in an infant. The tidal volume is varied by adjusting axis on rotating motor arm. There are 2 tidal volume settings.

Breath rate –

Infants have a faster respiratory rate than older subjects. Short tidal volume has been associated with increasing error in end-tidal expired gas concentration (Brunner and Westenskow 1988). For a lung model to mimic infant parameters, it must have a breath rate of approximately 60/min. The breath rate of the lung model was varied by adjusting speed of motor. A simple linear variable power pack supplied voltage within the specifications of the motor.

Using the Edinburgh lung model, the following experiments were performed

1. Measurement of lung model volumes
2. Pilot washouts to test adequacy of washout calculations at low lung volume, adjusting for BTPS, reinspired gas and lost sampled gas
3. Comparison of washout results between unmodified and rise time improved Innocor.
4. Effect of reducing lung model washout volume and tidal volume

Measurement of lung model volumes

It is important to determine the exact volume of the lung model components to compare washout results. This was done by filling the model with a volume of water, measured using scientific beakers and syringes. The volume of the lung model was

taken from the water surface at "expiration" (motorised syringe fully depressed) to the level of the sample capillary within the pneumotach. This was the washout volume or "FRC". An alternative approach to this is to measure the internal dimensions of the model and calculate the volume. While this seemed more mathematical and allowed a measuring tape to be fixed to the box to measure changes in volume, I was less certain about the accuracy of this as it assumed the tape was accurate and the box walls were absolutely perpendicular to each other. I opted to measure the volume directly.

The tidal volume was measured by the difference in the washout volume between full expiration and full inspiration. There are 2 tidal volume options, as the rotating arm could only allow two settings.

Pilot washouts to test adequacy of washout calculations at low lung volume, adjusting for BTPS, reinspired gas and lost sampled gas

Initial washouts to test the system were instructive. While it is easy to get accurate results (estimated washout FRC vs. known model volume) at larger lung volumes, it became difficult to get accurate results as the estimated volume was consistently 10% lower than the actual volume. The proposed reasons for this were:

- There is higher humidity within the lung model compared with room air, therefore, gas volumes should be adjusted for this.
- Re-inspired volume is over-estimated, resulting in a lower net expired volume for each breath, reducing the final FRC.
- Error in end-tidal concentration measurement.
- Sampled gas is lost from the system into PGA.

In retrospect it is easy to see the relative contributions of these factors to washout inaccuracy. Working through the problem at the time was challenging. There is an inherent physiological variability in MBW lung volume and ventilation heterogeneity in humans, which is conventionally tolerated up to approximately 10%. There is no variability in the lung model; therefore all error should be accounted for. Accuracy of the washout calculation is also only as accurate as the initial model lung washout

volume measurement. When the washout volume is as low as 100ml, a proportion of error can be attributed to this.

All measured gas volumes must account for the differences in humidity and temperature within the lung compared with room air that change the volume of measured expired and inspired gas. To standardise lung function tests, volumes are corrected to BTPS. The humidity within the box is higher than room air, and there should be an acknowledgement of this in the calculations.

Further analysis of this showed that humidity was higher in the box than air, only the reinspired gas portion is of importance to the calculations. This reinspired portion, residing within the pneumotach, is of the same humidity as within the box. It is not necessary to adjust for the humidity of the rest of inspiration, as the gas concentration of this part is zero, and doesn't contribute to the FRC calculations. BTPS was therefore not included in the final calculation.

The initial inaccuracy in Innocor measurements at low lung volumes are thought to occur from inaccurate estimation of changes in gas concentration due to slow signal rise and fall time. The adjustments made in chapter 8 aimed to resolve this inaccuracy but the analyser response time is still slower than the mass spectrometer (100ms vs. 40-60ms). It is feasible that even following the improvements, there may be under-estimation of expired gas concentration, or overestimation of re-inspired gas, resulting in low FRC. This is one possible explanation for the under-estimation of FRC in the initial experiments.

Another concern about slow rise time gas analysers is that with short expiratory times, seen in infants, there may be insufficient time for the gas concentration to plateau before the subject has started inspiring again(Brunner and Westenskow 1988). Therefore the end-tidal concentration – important for FRC and LCI calculations – will be under-estimated, artificially lowering the LCI, but in theory should not result in much difference in FRC, assuming the error at the beginning of the washout, is the same as at the end. Also, a lack of alveolar plateau can be observed during analysis. The lung model does not have the same breath concentration phases and reaches the maximum tidal concentration quicker than in a human breath. No premature falls in concentration were seen, as the breath time was always much longer than the rise time of the analyser (100ms).

Finally, a major concern about Innocor, raised during discussions with other researchers using alternative devices, was how much gas sample rate contributes to washout error. As discussed previously, Innocor samples gas at 2ml/second. This gas is lost from the equation as it is lost from both expiration and re-inspired portions. This is different from the bias applied to flow meter, which is compensated for during calibration. There is gas which is measured, on inspiration, which does not enter the lung, and gas which is measured on expiration, which does not exit the pneumotach. The proportion of this to the overall gas volume is small in larger subjects but is a large proportion of younger subjects' breath volumes.

Lost sample gas leads to under-estimation of FRC because re-inspired gas is over-estimated, as it is measured by the pneumotach and analyser, but doesn't reach the lung. The net expired SF6 (expired – inspired) is therefore greatly underestimated in younger patients, where this bias is large compared to the expired volume.

The solution to this problem had to be considered carefully. During flowmeter calibration, the calibration syringe is attached with the sample flow turned on. Therefore, the flowmeter is calibrated so that the bias created by the sample capillary is compensated for. For example, if 1 litre is pushed through the pneumotach with the sample capillary proximal to the screen mesh, slightly less than 1 litre will be measured. The calibration adjusts this up to 1litre. Similarly, if 1 litre is sucked back through, slightly more than 1 litre will be pulled as there is additional suction from the sample capillary. Again, the calibration adjusts for this. This calibration process means that even though there is a bias, the breath volume measured is the same as has come out or has been breathed by to the subject.

Unfortunately, this does not hold true for gas concentration. Calibration means underestimation of gas as it doesn't account for that which was removed from the equation. During re-inspiration, the flowmeter measures a volume of gas, integrated with concentration to calculate gas volume inspired. The flow meter is reading 1litre, when slightly less than that actually enters the subjects' lungs (over estimation of re-inspired gas). During expiration, the flowmeter reads 1 litre breathed out, but since this gas is removed from the equation anyway, it doesn't matter than it is partially sampled by the analyser.

Therefore, expired gas is accurately measured, but re-inspired gas, is over-estimated. Re-inspired gas is more important to the equation as the washout progresses, because in small subjects the PNT volume can be up to 20% of the breath volume. Therefore, approximately 20% of gas is re-inspired. If this is overestimated due to slow fall in gas concentration due to the analyser and over-estimation due to flowmeter error is unadjusted, the net expired volume can actually be negative.

After deliberation, I opted to reverse the bias generated during flow meter calculation. By adding the sample flow rate back to the pneumotach measurements, the true re-inspired gas concentration could be calculated. An ideal solution would be to adjust the flow only for the inspired portion of the washout, but this is technically difficult with the software I was using. It was very simple to generate a bias to both expired and inspired flow prior to analysis, reversing the calibration bias, but providing an accurate measure of re-inspired gas. Expired gas will therefore be over-estimated, but re-inspired gas, crucial to accurate washout measurements will be accurately measured. The adjustment above proposed to make the re-inspired concentration accurate, and slightly over-estimate the expired concentration to compensate for the slow fall in the gas analyser still over-estimating expired gas concentration.

This resolution is acceptable for FRC calculation, as absolute flow is not important. During LCI calculation, absolute flow is vital for measuring the cumulative expired volume during the washout. Therefore, the decision was made to analyse the FRC and CEV separately, before and after adjusting the flow readings, so that the most accurate measurements were made. This is relevant during the clinical comparison in human subjects.

Therefore, the only major influence on accuracy of lung model washouts, was the sampled “lost” gas. The analyser speed (100ms) may still be contributing error, but the empirical over-adjustment of expired gas is likely to compensate for this.

Comparison of washout results between unmodified and rise time improved Innocor.

The major alteration to Innocor was speeding the rise time from 160ms to 97ms. It was hoped that this improvement would be adequate to allow washouts to be performed in young children and infants. A comparison was made, before and after Innocor modification, quantifying to some extent the effect of speeding rise time on washout accuracy.

Using the lung model, 3 washouts were performed on the factory standard Innocor with 2 washouts performed on the rise time speeded version. The same flowmeter was used for both sets of washouts, calibrated for low flow and small tidal volume. With the rise time speeded device, analysis was done on data adjusted for sample gas flow. The sample capillary was positioned proximal to the flowmeter mesh. The standard Innocor setup had no alterations made to the standard washout setup.

Only a small number of washouts were recorded because of multiple adjustments to the analysis algorithm to accommodate the new setup. This was not to firmly quantify the relationship between analyser speed and washout accuracy at low volumes, because there are multiple factors involved, apart from analyser speed.

Effect of reducing lung model washout volume and tidal volume

Final experiments were performed using only the rise time modified Innocor. The setup is as described in chapter 8.

The aim of these experiments was to perform multiple runs of washouts using the lung model, varying washout volume and tidal volume to look at Innocor accuracy at these volumes. Values were chosen to mimic an infant, although the limits of the model were reached with a respiratory rate of 50, as the motor would not drive the arm any quicker. All washouts were therefore performed at a rate of 40 -50/min. While it is recognised infants often demonstrate periodic breathing, with periods quicker than 60/min, it was not possible to mimic this with the equipment I had.

A series of washouts was then performed on separate days. Setup of the lung model involved filling the plastic tank with water to a marked point, with the motorised syringe at full “expiration”. The model was then filled to the brim with a volume of water measured with scientific beakers and clinical standard syringes. The model

was emptied and refilled to the original fill mark. Thus the washout volume was determined. Different lung volumes were chosen to reflect those seen in infants and young children (Table 29). Some washouts were performed with different tidal volume, again to reflect breath volumes seen in infants.

It was noticed that there was a drift in the water level after each washout. This was presumably due to slight leak in the system due to the motorised syringe. The seal was re-established and the washouts restarted. The level of the water on a taped ruler was noted so that the internal dimensions of the tank could be used to determine the change in washout volume after each run.

The collected washout data were adjusted to correct for the lost sampled gas, then analysed using Professor Per Gustafsson's software. At that time, the software version was not able to detect breaths with low flow, such as those generated by the lung model. Therefore, there was no way to automatically detect breaths, and the markers had to be moved manually for each breath. This was very time-consuming but allowed accurate analysis. Since then, a software upgrade has been written which allows variable breath detection thresholds. Now lung model and infant washouts can be analysed automatically.

The mean FRC was determined for each set of washouts, and compared with the actual lung model volume. % error was calculated by comparing the difference between measured and actual volume, divided by actual lung model volume. Mean values for each set of washouts was plotted against average difference, and 95% limits of agreement calculated to show effect of lung volume on washout accuracy.

Lung model washout results

1. Compare original vs. modified

Washout results were compared from the un-modified Innocor and the rise time modified version. Lung model parameters are shown (Table 28). The unmodified Innocor was setup with the PNT sample capillary positioned distal to the flow pressure lines, as per the standard MBW setup for older children and adults. This renders the post-capillary dead space less than 5ml, and re-inspired gas is unaccounted for. The modified Innocor setup positioned the PNT with the sample

capillary proximal to the flow pressure lines, which requires re-inspired gas to be taken into account (post-capillary dead space 8ml).

Washout volume (FRC) results from each Innocor version were compared with the expected lung model volume (219ml). In both cases, a tidal volume of 50ml was used, with a breath rate of 40/minute. There was a mean over-estimation of 19% using the factory standard version. This over estimation was 1.2% when using the modified setup. Full results are shown in Table 28. This confirmed the basic hypothesis - that modifications improve washout volume estimation compared with the factory standard version with a longer rise time.

	Factory standard	Rise time modified
Breath rate	40	40
Lung model FRC (ml)	219	219
Number of washouts performed	2	2
10 – 90% rise time (ms)	168	97
Flow gas delay (ms)	1496	450
Gas sample flow rate (ml/s)	2	2
Mean(SD) measured FRC (ml)	260.7(3.27)	221.6(2.74)
Mean(SD) error in FRC (%)	19.03 (1.49)	1.18 (2.25)

Table 28 - comparison of unaltered factory standard Innocor vs. the rise time modified version. Mean (standard deviation) FRC values are shown. Rise time, flow gas delay and gas sample flow rate are taken from earlier experiments (chapter 8).

2. Compare series of washouts at different lung volumes and tidal volumes

Following the basic comparison of modified vs. unmodified Innocor, a series of washouts were performed using only the modified version. Sequences of washouts were performed on 7 separate days at various lung model volumes and tidal volumes. At each time, multiple washouts were performed, the number shown in the results table (Table 29). The experiments have been ordered by lung model volume and breath (tidal) volume for simplicity. These are the final satisfactory washouts once technical issues regarding model leaks, signal alignment and lost gas compensation had been resolved. In all of these washouts, no modifications were made to the equipment or analysis method. All washouts and analysis were completed as per the relevant sections of earlier chapters (2 and 8).

Experiment	Number of washouts	Breath rate (min^{-1})	Tidal volume (ml)	Lung model FRC (ml)	Mean (SD) Estimated FRC (ml)	Mean (SD) Absolute error (ml)	Mean (SD) relative error (%)
1	14	50	58	162.0	159.8 (2.6)	-2.17 (2.2)	-1.3 (1.6)
2	6	50	58	139.5	138.3 (2.0)	-0.01 (0.8)	-0.0 (0.6)
3	6	50	58	118.0	118.1 (0.9)	0.06 (0.9)	0.1 (0.7)
4	6	50	58	101.0	100.9 (0.7)	-0.09 (0.7)	-0.1 (0.7)
5	6	50	28	142.5	141.9 (1.9)	-0.58 (1.9)	-0.4 (1.4)
6	6	50	28	114.0	113.1 (2.2)	-0.89 (2.2)	-0.8 (1.9)
7	8	50	28	98.0	98.3 (3.5)	0.30 (3.6)	0.3 (3.6)

Table 29 - Series of lung model washouts using variable tidal volume and FRC settings. Mean error is calculated from the difference in estimated minus known lung volume.

Overall, there was no significant mean error when estimated FRC was compared against actual lung model volume (p=0.08). Table 29 demonstrates that mean error does not change at smaller model volumes. Plotting expected FRC against difference (Figure 74) shows that at a small volume of 98ml, approximately that of a newborn infant, there was a slight increase in variability (Coefficient of variation, CV 6%) compared with the other volumes (CV 1.4%). 95% limits of agreement based on a pooled standard deviation are shown (dotted lines). It is obvious that in the smallest volumes, variability was significantly higher. It is important to emphasise that this graph shows proportional differences. Mean(SD) absolute differences (ml) at the

smallest volumes were less than 0.5ml. There is also no evidence of bias at low lung volumes, as would be seen in the scenario of under or over estimation of expired gas due to limitations of the Innocor gas analyser. There is an appearance of a trend towards over-estimating of smaller lung volumes and under-estimating of larger lung volumes. While there is no statistical difference in error when comparing pairs of experiments in turn, experiment 1, with the largest lung volume comes close to a statistically significant difference in error when compared with some of the other experiment errors (p=0.06). This visual trend could be due to experimental error or drift in the lung model volume. Importantly, error in the gas signal would result in under-estimation of smaller lung volumes with lower tidal volumes.

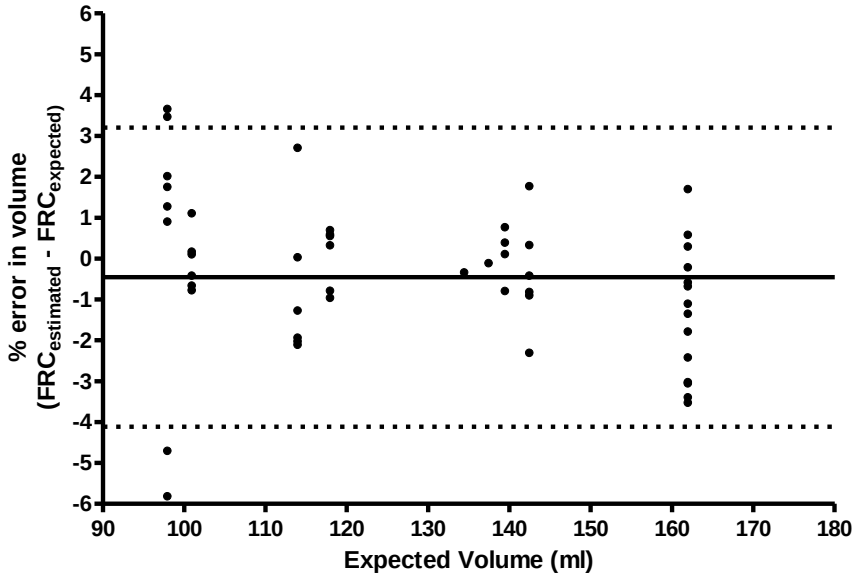


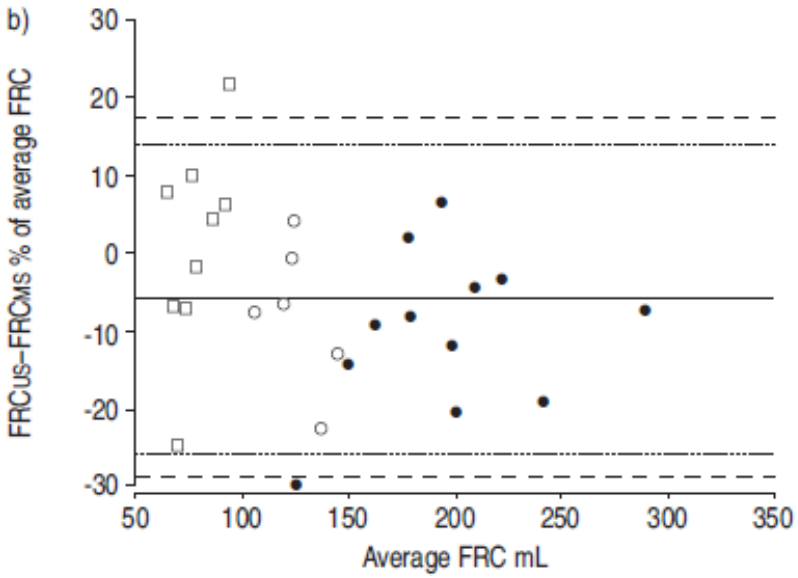
Figure 74 - Plot of expected FRC vs. % difference. Dotted lines indicate 95% limits of agreement(Bland and Altman 1986)

Innocor / mass spectrometer comparison

As a result of the above lung model work, a clinical comparison of Innocor against mass spectrometer was undertaken. Because of links with Professor Bush's research department, access was available to both an Innocor and AMIS 2000 respiratory mass spectrometer.

Initial protocol development had been started in Edinburgh prior to this link with Professor Bush. We had established the principles of device comparison. A literature search resulted in two relevant studies comparing multiple breath washout devices.

Pillow et al conducted a comparison study of an early version of the Ecomedics ultrasonic molar mass technology flow meter vs. mass spectrometer(Pillow et al. 2004). 23 healthy infants (mean gestation 39.7 weeks) performed multiple breath washout 2-3 times on each device in turn to obtain an average of technically acceptable washouts. While there was a strong correlation between FRC values measured on both devices, there was a mean(SD) under-estimation of FRC on the ultrasound device compared to the mass spectrometer of 5.7 (1.0)%. This was statistically significant, although the differences were small. There was a wide scatter of differences (95% CI 1.0 – 10.4%), explained by the inherent physiological, technical and methodological variability. This study was concerned only with gas washouts and volume calculations in infants.



b) Bland-Altman plot of the % difference in FRC obtained using the mass spectrometer and ultrasonic flow meter plotted against the average FRC. Solid line indicates mean difference while the dashed line indicates 95% limits of agreement (mean difference 1.96SD). The dotted line demarcates 20% of the mean difference between the two techniques. □ FRCMS with Rendell-Baker 1 Mask and FRCUS with Laerdal 0/1 Mask; ○

Figure 75 - extract from Pillow et al 2004.

In another study, Fuchs et al aimed to compare a different ultrasound molar mass device (Spiroson, ndd medical technologies, Switzerland) with mass spectrometer(Fuchs et al. 2006). This prototype device was altered during the study so that molar mass gas concentration was taken from a side-stream sampled technique, rather than mainstream analysis (for technical reasons, as the beginning of the washout could not be determined from the mainstream signal). Another side-stream sample tube was sited to directly measure CO2, as this needed to be subtracted from the molar mass signal. Finally, the mass spectrometer sample capillary was positioned in the same flow meter as the molar mass, so that simultaneous gas concentration measurements could be made during the same washout. This has the advantage of eliminating inter-test variability. This study was conducted in 14 healthy adults, with 5 subjects' results subsequently discarded because of technical issues. Intra-subject variability was low (<5%). There was a mean (95%CI) difference (mass spectrometer – ultrasound flow meter) of 0.124L (0.08 to 1.7), which amounts to a small but statistically significant mean difference of 2.8%.

These studies indicate important principles for any comparison of devices for validation. Firstly, there are complexities in such studies. The implication of the text is that Fuchs et al had to substantially change the ultrasound equipment to get comparable results with the mass spectrometer. Such changes to hardware and analysis may make equipment complex and prone to further unknown errors. The population was limited to healthy adults, therefore it is difficult to draw conclusions on how useful this equipment is in younger children or infants. The multiple side stream sampling may generate a large flow bias, requiring careful compensation. In addition, Pillow et al studied a well-controlled population of sleeping infants. Even in this situation, there is still a wide inter-device variability up to +/-20%, indicating physiological variability is high. In comparison, the Fuchs study eliminates physiological variability between devices with simultaneous gas sampling from a single flow meter, resulting in differences of up to 300ml (10%) between devices (numbers approximate as not explicitly stated in text). The graph of FRC difference against mean (Bland-Altman plot) is shown below, with LCI results for discussion later.

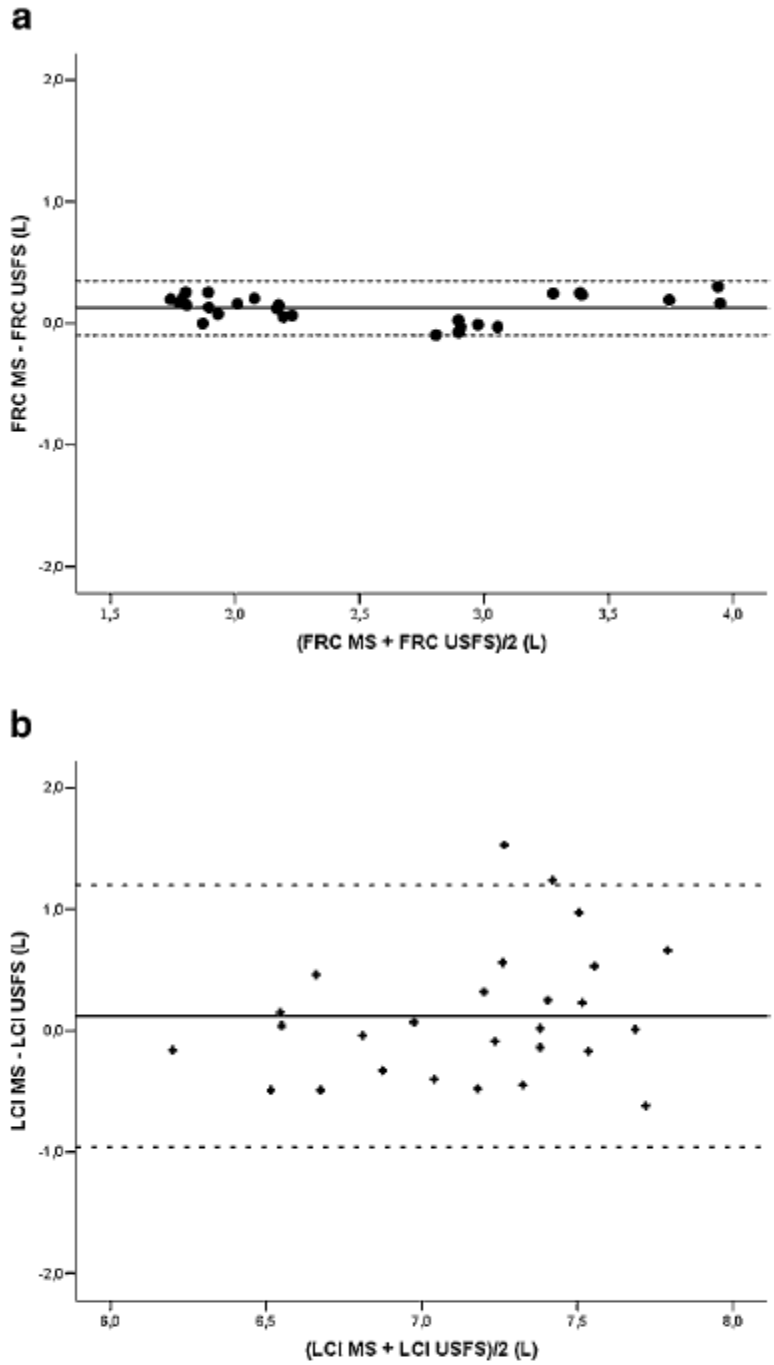


Fig. 5. Agreement between (a) FRC and (b) LCI when measured with mass spectrometry (MS) and sidestream ultrasonic flow sensor (USFS): The black line represents the mean difference of all measurements. The dotted lines represent plus and minus two standard deviations of this mean difference (i.e., 95% limits of agreement).

Figure 76 - extract from Fuchs et al. 2006

Methods

Originally, the Innocor – mass spectrometer comparison, comprised of simultaneous sampling of gas to both devices from the same pneumotach. This was decided when planning to use the quadropole mass spectrometer from Glasgow University. When changing to using the London equipment, a discussion was initiated with Professor Gustafsson, whose mass spectrometer setup was to be used. An important difference between the devices is the concentration of SF6 used (4% for mass spectrometer, compared with 0.2% for Innocor). One solution was to compromise the Innocor by measuring 1% (within its calibrated range) and tolerating the increased MS noise. This is unsatisfactory, as changing both sets of equipment reduces the clinical applicability of the study in the future. Innocor cannot be calibrated easily to measure 4% SF6 without extensive expert input thereby raising the question of whether conclusions from that protocol could be applied to the Innocor when measuring 0.2%. A decision was therefore taken to perform sequential randomised measurements, with order determined by random number. Healthy volunteers and children with cystic fibrosis were selected to perform up to 3 washouts on each device. The intention was to have subjects over a wide age-range, including young children. This was the first time subjects had been tested using the modified Innocor. Whilst the pneumotach was of standard construction, there was a degree of caution amongst the research group about such a comparison without knowing whether the device was accurate in older children and adults. Therefore, adults were recruited first, and then children of descending age. Children with cystic fibrosis were recruited, as device errors may be more apparent with prolonged washouts. Adults and older children with CF were not recruited as many were at that time involved in another clinical study (UKCFGT "Run-in Study").

Inclusion Criteria

Healthy volunteer patients

(a) 4 age groups

adult - >16yrs

child – 5-16 years

young child – 2-5 years

infant – 1-24 months

(b) No history of respiratory disease

(c) No significant medical history

(d) Non-smoker

Cystic Fibrosis patients

(a) 3 age groups

child – 5-9 years

young child – 2-5 years

infant – 1-24 months

(b) Confirmed diagnosis of cystic fibrosis (positive sweat test or genetic testing)

(c) Clinically stable – no recent exacerbations (requiring antibiotics) in past 4 weeks.

(d) $FEV_1 \geq 40\%$ predicted

(e)

<u>Subject Exclusion Criteria</u>

For Child volunteers

(a) Any past history of: recurrent wheezing episodes, pneumonia, cystic fibrosis pertussis or tuberculosis.

(b) Previous diagnosis of asthma or taking anti-asthma medication.

(c) Previous hospitalisation for respiratory infection.

(d) Born before 34 weeks gestation.

(e) Neuromuscular weakness or bone disease likely to affect respiration.

(f) Congenital cardiac defects requiring treatment.

(g) Previous adverse reaction to sedating agent used during testing

For adult volunteers

-As above for children, plus:

(h) Any ongoing or recurrent respiratory symptoms, such as cough, wheeze, breathlessness or nocturnal waking.

(i) Current smoker (within last 6months).

(j) Ex-smoker with >10pack yrs smoking history.

(k) Any other co-morbidity requiring ongoing treatment or medical review.

For CF paediatric patients

(a) $FEV_1 < 40\%$

(b) Recent chest exacerbation (in past 4 weeks)

(c) Born before 34 weeks gestation.

(d) Neuromuscular weakness or bone disease likely to affect respiration.

(e) Congenital cardiac defects requiring treatment.

(f) Previous adverse reaction to sedating agent (if required for testing).

Subject Withdrawal Criteria

(a) Respiratory symptoms 7 days prior to attending (healthy volunteers)

(b) Patients/parents will be able to withdraw at any point during the testing if they wish.

Ethical approval

Prior to being unable to get access to a mass spectrometer, we planned to conduct this study in Edinburgh. At that time the expectation was that the youngest patients would require sedation. Following guidance from others performing infant lung function studies (Institute of Child Health, University College, London), a sedation protocol was drawn up. This would have been possible in Edinburgh, as there is a fully staffed clinical research facility. This was not available in the Royal Brompton at that time, so for ethical reasons the decision was made not to sedate. We were then only able to select those children who would be likely to sit still, or sleep naturally during the test. The most difficult group is from 6 months to 2 years. This still allowed recruitment of the youngest infants, those most of interest to this study.

Ethical approval was initially sought from the Lothian Research Ethics Committee for a study based in Edinburgh. Once the study was moved to London, a transfer and approval was received from the Royal Brompton and Harefield Research Ethics Committee (REC 07/S1102/40). A learning point for this thesis as a whole is that research ethics committee processes are complex, and require good communication. A decision from one committee does not mean approval from another. The forms are often not easily transferred from one centre to another as it is not a truly online system. It took a number of months before ethical approval was received in London. It would have been simpler to re-apply with a new application, rather than transfer.

Paediatric clinical research in the Royal Brompton Hospital, London

It was a great advantage to this project to have access to both a respiratory mass spectrometer, set up by Professor Gustafsson, and Innocor in the same department. Mrs Sam Irving was employed to conduct such studies in London by Professor Bush. Sam has a background in engineering, making her ideal for operating the mass spectrometer. In addition, she attended a mass spectrometer training course at Innovision's headquarters in Denmark. At the time of the study Sam had experience of conducting MBW in teenagers and older children. She had received training in using the Innocor from me and Clare Saunders, a clinical physiologist conducting MBW for the CF Gene Therapy Consortium in London. The AMIS 2000 was installed and set up by Prof Gustafsson in April 2008. At that time a period of training was started to prepare for the comparison study. I produced 3 documents, with the help of Sam Irving and Prof Gustafsson. One gives details on conducting MBW using the mass spectrometer equipment. The others describe the hardware modifications for Innocor, and important techniques for determining the flow gas delay. These are included in the thesis as appendices.

There were drawbacks to performing the study in London. The lack of a proper research facility meant families had to attend a smaller room which was not exclusively set aside for clinical work. Also, recruitment of younger patients proved very difficult. Despite the large population, access to the Royal Brompton Hospital from outwith central London is very time-consuming. Asking families to come for

this validation study required persuasion, and was usually those with a staff connection to the department or attending with a sibling for another reason.

Statistical analysis

Mean results from the individual washouts were obtained, and compared between devices.

Clinical results from the Innocor device in each subject were compared with mass spectrometer using Students paired t-test of average FRC and LCI measurements. Variability of between-device results were compared with intra-test coefficient of variation (CV%) on each device. Modified Bland-Altman plots (mean of device results vs. % difference) were used to show evidence of Innocor bias. Prism (Graphpad software, USA) was used for statistical analysis.

Raw data from mass spectrometer and Innocor were analysed by independent researchers. Sam Irving analysed data from the mass spectrometer blinded to the Innocor results, analysed by me. When finalised, the results from the two devices were compared.

Innocor / mass spectrometer comparison results

16 Healthy volunteers (median [range] age 11 [3-49] years) and 9 children with cystic fibrosis (median [range] age 7.4 [0.4-11] years) were recruited. Unfortunately, in 3 young children, only one washout was possible on each device due to poor subject tolerance; these have all been included in the analysis. 12 individual washouts (10%) were removed due to poor test quality (Evidence of leak or erratic breathing pattern, resulting in FRC >10% higher or lower than the other washouts). Mean FRC and LCI were measured using the modified Innocor in healthy and CF groups, comparing with results from the mass spectrometer.

	Health Volunteer			Cystic Fibrosis		
N	16			9		
Median Age (yrs) [Range]	11.7 [3 – 49]			7.4 [0.4 – 11]		
	Mass Spec	Innocor	Mean [95% CI] difference	Mass Spec	Innocor	Mean [95% CI] difference
Mean (SD) FRC (L) [Range]	1.56 (0.9) [0.48 – 3.40]	1.46 (0.9) [0.54 – 3.40]	-0.10 [-0.22 to 0.02]	0.86 (0.6) [0.11 – 1.75]	0.87 (0.6) [0.15 – 1.72]	0.01 [-0.05 to 0.07]
Mean (SD) CEV (L) [Range]	11.10 (5.8) [3.61 – 22.07]	9.90 (5.6) [3.69 – 21.91]	**-1.21*** [-2.16 to -0.26]	8.62 (6.0) [1.03 – 21.24]	9.23 (6.1) [1.68 – 19.88]	0.61 [-0.78 to 2.00]
Mean (SD) LCI [Range]	7.46 (0.8) [6.50 – 9.33]	6.85 (0.6) [6.21 – 8.64]	**-0.61†** [-1.03 to -0.19]	10.4 (1.5) [7.96 – 12.30]	10.66 (2.2) [7.03 – 14.25]	0.26 [-0.73 to 1.26]
Mean (SD) Vt (L) [Range]	0.57 (0.2) [0.23 – 0.96]	0.46 (0.2) [0.17 – 0.86]	**-0.11†** [-0.18 to -0.04]	0.31 (0.1) [0.10 – 0.51]	0.29 (0.1) [0.10 – 0.48]	-0.02 [-0.06 to 0.02]

Table 30 - Comparison of results between devices in 2 clinical groups. Mean difference between devices indicating significant differences. Mean differences non-significant (p>0.05), except * p<0.05, † p<0.01. FRC, functional residual capacity, CEV, Cumulative Expired Volume during washout, LCI, Lung Clearance Index, Vt, average washout tidal volume.

There was no mean difference in FRC in healthy controls. The modified Innocor gave a slightly lower LCI than mass spectrometer (mean [95%CI] difference -0.6, [-1.0 to -0.2], p=0.007), and a lower CEV (mean [95% CI] difference -1.2L [-2.2 to -0.3], p=0.02) and Vt (mean [95% CI] difference -0.1L [-0.2 to -0.04], p=0.005). No significant mean differences were seen between devices in children with CF (table 2). All subjects with CF had a raised LCI, defined by previous published ranges as >7.5 (A. R. Horsley et al. 2008, Gustafsson et al. 2003a), detected by the mass spectrometer washouts. To investigate the above differences in healthy controls, the within-device coefficient of variation (CV%) of the 2 or 3 washouts on each device was compared with the between-device CV% and found to be comparable (table 3). In addition, between device differences in LCI were associated with differences in CEV (p=0.005), suggesting that observed differences between devices are likely to be due to physiological variability rather than Innocor inaccuracies. There was no correlation between differences in LCI and differences in Vt between devices (p=0.24), suggesting change in relaxed tidal volume did not influence ventilation heterogeneity.

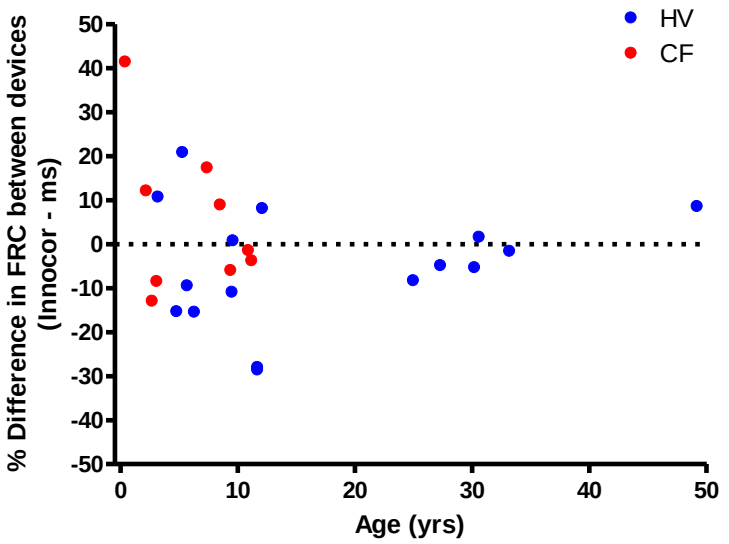
	Mass Spectrometer	Innocor	Between-device
Healthy volunteers (n=14)			
Mean (SD) CV% in FRC	9.36 (6.82)	5.92 (3.71)	8.36 (6.97)
Mean (SD) CV% in LCI	8.12 (6.74)	5.08 (2.78)	6.80 (6.38)
Cystic Fibrosis (n=9)			
Mean (SD) CV% in FRC	6.06 (8.68)	6.63 (5.29)	9.15 (9.45)
Mean (SD) CV% in LCI	8.95 (6.83)	5.19 (5.02)	6.08 (5.66)

Table 31 - comparison of within- and between- device coefficient of variation (CV%) in health volunteers and children with CF

Effect of age on washout results

It was expected that in the youngest subjects (<5yrs), the modified Innocor might under-estimate expired gas volume, due to slow rise time effects. There is no evidence, using the modified Innocor, of overall FRC or LCI bias in younger patients, and FRC is not under-estimated (Figure 77 and 78). The youngest child (4.8 months), demonstrated a high CV between devices (24.2%), but also had a high CV% within each set of washouts (mass spectrometer 16.6, Innocor 17.6).

Figure 79 and 80 illustrate the effect of magnitude of FRC and LCI on difference between devices. In those with a higher LCI, there is some evidence of over-estimation of LCI measured with Innocor. This may suggest that the devices cannot be used interchangeably. This trend is not evident in FRC.



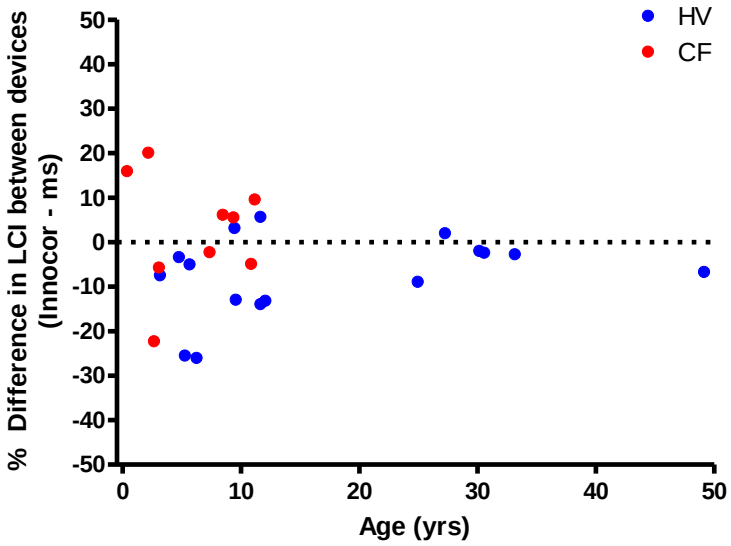


Figure 77 and 78 - between device differences in FRC and LCI by age (healthy volunteer – blue, children with CF – red).

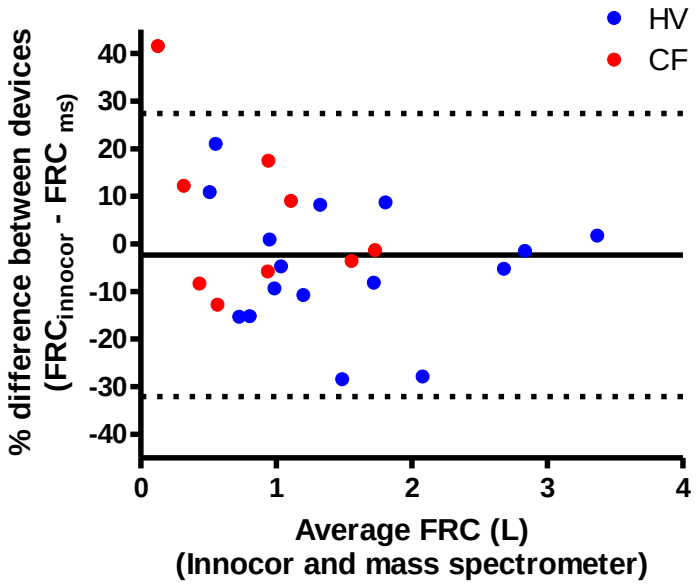


Figure 79 - comparison of average FRC between devices vs. %difference. Dotted lines indicate 95% limits of agreement (healthy volunteer – blue, children with CF – red).

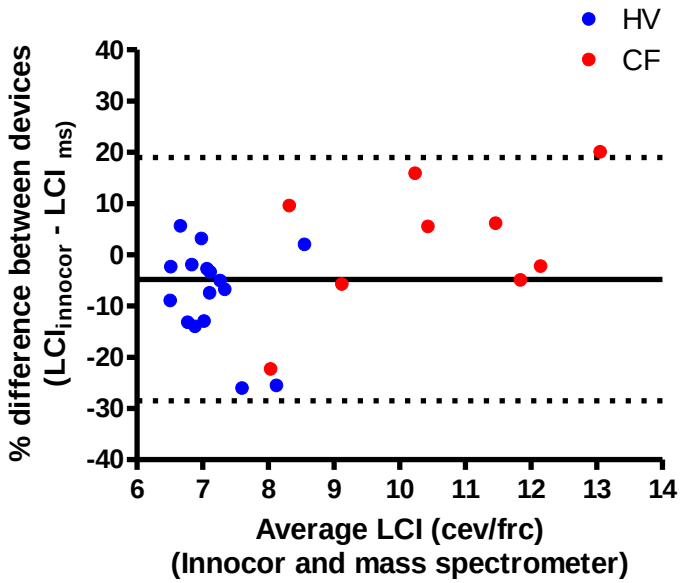


Figure 80 - comparison of average LCI between devices vs. %difference. Dotted lines indicate 95% limits of agreement (healthy volunteer – blue, children with CF – red).

Conclusions

Following hardware modifications, and testing using a lung model, this study aimed ultimately to compare Innocor against the current gold standard device in young children and infants. This is a culmination of 2 years of preparation, and is important to the wider MBW research community as well as to this thesis. Innocor is a compact, portable device which, if accurate in younger children, may be very useful in multi-site research studies. These arguments have been well made previously.

Modifications to improve gas signal rise time led to improvements in volume estimation using a basic lung model. When performing multiple washouts at low lung volumes there was no evidence of bias. Variability was higher, raising the question of how accurate signal alignment, breath detection, volume-concentration integration and lost gas compensation is. The volumes concerned, however, are very small (less than 6ml) and could be considered to be insignificant, within the normal within-test variability of the measurement (normally around 5%).

The new Innocor rise time is just under 100ms. Available recommendations state that the optimal rise time should not exceed 100ms and, despite this not being firmly based on evidence, it is reasonable to be able to adhere to this standard(Beydon et al. 2007). It was convenient that modifications improved the rise time to just lower than the recommended value, and results show that with this rise time, Innocor accurately estimates small washout volumes at faster respiratory rates.

To reach the stage where a lung model could be used to assess Innocor, careful consideration was applied to technical issues, including signal alignment, breath detection and lost gas compensation. Adjusting results for BTPS, flow bias and signal alignment can alter results greatly. Care must be taken to justify each adjustment, as additive errors may make results look more accurate than they actually are.

There are great advantages in using a device which measures raw signal rather than deriving gas concentration from more complex calculations. Ultrasound measurement of gas concentration (molar mass) is a complex technique, as fluctuations in oxygen, CO2, humidity and temperature alter the molar mass signal. The visit to the Bern group showed that slight adjustments to BTPS especially, has large effects on the washout pattern. The algorithm used is based on making the

washout trace look like a standard washout trace. No adjustments are made to the Innocor gas concentration signal, and all other adjustments to flow and integrated volume are derived from first principles.

Drawbacks to this study are that only 2 washouts were performed when comparing the factory standard to the modified Innocor. More washouts could have been performed, but time limitations (setting up lung model and equipment), combined with convincing initial results, led to moving to performing series of washouts only using the modified Innocor.

A further limitation to this study is the basic lung model used. While this design was obtained from experienced colleagues, it is clear that this model only mimics a lung in terms of volume, and not in terms of temperature, humidity or gas mixing. Only limited conclusions can be made from such a lung model, and a clinical comparison was planned as a result of these limitations.

Finally, this study contains a number of novel calculations. There is no published evidence of compensating for lost gas in multiple breath washout. The calculations have not been verified, and results obtained could be accurate numerically, but obtained from conflicting additive errors. Results may not be so accurate in different age groups or in the presence of disease, where washout duration is longer. Care has been taken to justify each calculation, but a lung model study where LCI can artificially be prolonged would be ideal. This thesis has not addressed whether a lung model can be created with in-homogenous gas mixing. Most importantly, this study provides a hypothesis for a clinical study of Innocor use in young children.

The clinical comparison is the first to compare Innocor and mass spectrometer MBW devices in humans. Despite a slower 10-90% rise time in the Innocor, there is no evidence of bias in FRC measurements. Variability between devices is comparable to the within-device variability on each device. Innocor, in fact demonstrates less variability than mass spectrometer. There is a suggestion of a trend towards a greater difference in LCI measurements as LCI increases (Figure 80). This may suggest that results measured on different devices are not interchangeable in children with disease.

Even in the youngest subjects (down to 4 months of age) there is no evidence of underestimation of FRC, which would be expected if the gas signal was the source of

the error. Also, in children with CF, where washouts are prolonged due to lung disease, there is no increase in variability and no evidence of bias. This indicates that the modified Innocor, with a rise time of <100ms and infant appropriate PNT and patient interface, is suitable for washouts in younger ages.

Study limitations

While the aim of the study was to recruit children in descending ages, including young infants, this has not fully been achieved. It is disappointing not to report larger numbers of infants, as they are relatively simple to test, and represent that most extreme group in terms of volume (except preterm infants). The factors that confounded this aim were the location of the study, at a central London location with difficult access. Families of young children attending for other reasons did not have time to stay for another lung function study. Sam Irving found it extremely difficult to identify and recruit significant numbers. Technical complications from Innocor and mass spectrometer increased these difficulties, resulting in some results being discarded, or visits cancelled.

While Sam Irving was competent to conduct studies, dealing with young children was a relatively new experience for her. To conduct the study in Edinburgh, with a paediatric nurse present would have been ideal, but not possible as I have already explained.

The limitations to the study stem from this. Only five children under 5 years completed the study. Only one infant was recruited. Of these younger children, only one washout was possible in 2 subjects. This greatly limits the conclusions of the study, even though the lung model study reinforces the conclusion that volume estimation is accurate at low lung volumes.

Time did not allow a similar lung model study using the mass spectrometer. There were a number of demands on the testing room in London, and my visits were occupied with resolving technical difficulties and testing patients. This lung model study may have been instructive but is contrary to the hypothesis that mass spectrometer is the gold standard device.

FRC measurement appears highly accurate, with no significant difference between devices. There are differences in LCI, CEV and Vt, however, which require

discussion. To assess why these measurements are different between devices, while FRC appears accurate, Table 32, below, indicates the effects of various errors on FRC and LCI. If FRC is unaffected, it is the cumulative expired volume that is primarily different. If FRC is affected, LCI is dependent on this and will also be different. The theoretical basis for these descriptions is in previous chapters.

		Primary effects
Gas analyser error	Underestimation of expired volume due to slow 10-90% rise time	FRC, LCI
Flow meter error	Incorrect linearisation or calibration leading to volume inaccuracy	FRC, LCI
Physiological variability	"natural" changes in lung volume over time due to changes in central breathing control. Affected by changes in flow meter resistance and equipment dead space.	LCI (FRC to some extent)
Poor subject compliance	Irregular breathing pattern, sighing, laughing etc. causing leaks or washout rate changes	LCI (FRC to some extent)
Operator error	Incomplete washin of SF6, incorrect washout detachment timing and premature washout termination	FRC, LCI
Analysis differences	Differences in processing of data such as breath detection thresholds and subtraction of dead space.	FRC, LCI

Table 32 - contribution of MBW errors to final results

These data indicate that for LCI to be affected, independent of FRC, it is mainly physiological variability and poor subject compliance that are implicated, as any hardware error is likely to affect FRC. It is clearly not the case that physiological variability and subject compliance do not affect FRC measurements. There is a higher within test variability than is demonstrated in other studies in this thesis.

FRC is influenced by tidal volume, in that a subject can breathe more or less deeply. Depth of breathing is regulated by central chemoreceptors and depends on blood O2 and CO2 levels(West 2005). The level of distraction can also influence this, in that a well-distracted subject is likely to breath naturally, less aware of the apparatus and slightly increased work of breathing required to ventilate through the flow meter.

An important difference in equipment in this study could have been overlooked. The flow meter used in Innocor for older children and adults was the Hans Rudolph (3500 or 3719). The mass spectrometer equipment used a size 00 or 01 Fleisch pneumotach. While the resistance of each is acceptable within the normal range of flow, the resistance is not necessarily the same. A small difference in resistance may have altered tidal volume and thereby influenced FRC or CEV. It is feasible that differences in resistance may have affected CEV disproportionately, resulting in the outcomes seen. In addition, poor washout quality in some instances is a factor.

It is clear from this study, as well as other similar published work, that device comparison is a complicated validation step. There is tension between testing each device as it is normally used, and wanting to control for all of the many variables. This project seeks to balance these opposing forces, but is a victim to some extent of the two devices having significant differences which may have contributed to the physiological variability. Also, as the test is relatively long, subjects may have had some fatigue, or simply became bored.

The most accurate way to compare devices is using a mechanical lung. Unlike the simple device used for this study, the same group that loaned me that version have now marketed a larger, more complex lung model for the purpose of device development. This should encourage manufacturers to build alternative devices with refined technology. This lung model is similar to the simple Perspex box, but is submerged in a water bath to control temperature. CO2 is added to the washout

volume, and breaths are driven by a volume regulated mechanical ventilator. This enhanced device is expensive, but variables can more accurately be controlled.

Learning points and contribution to thesis

This chapter is central to the theme of Innocor validation. Clinical studies conducted prior to hardware modifications were unable to answer the question of whether Innocor could be used in younger children and infants.

To some extent the chapter is successful in confirming that Innocor, with specific modifications can measure gas concentration accurately even in young subjects. This project proved to be more difficult to conduct than expected. This was mainly because of having to move the study to London, with another person performing measurements.

The results were difficult to interpret as physiological variability and washout quality may have prevented true differences between Innocor and Mass Spectrometer being elicited. The large scatter of differences about the mean are initially concerning, but with further analysis show that FRC is measured accurately, whereas other measurements are subject to bias and higher variability.

The main aim of this thesis is to validate Innocor for use in children and infants by providing sources of error in measurements. The conclusion of this is that because of high inter-test variability a direct comparison such as was performed is not practical for device validation. Further use of a lung model would have provided better evidence of performance.

With more time and better access to equipment, a full lung model series of experiments would have been the ideal study to perform. It was not clear at the start how difficult this would have been to perform. The delays due to obtaining equipment, getting ethical approval and difficulties in recruiting adequate numbers of children were the main barriers. A lung model, once set up, would be able to accurately compare washout results between Innocor and mass spectrometer with known and reproducible lung volumes.

While a lung model study is ideal to compare the gas analyser and flow meter, this does not bring Innocor, and other washout devices for that matter, closer to the clinical domain. Only by recognising and quantifying sources of error in real patients

with lung disease as well as healthy controls, can the true utility of clinical washout devices be assessed. This study would suggest that washouts performed by a relatively inexperienced, although fully trained, operator vary considerably. This variability can be up to 40% in younger patients. This reduces the confidence of measurements performed between devices in the same patient.

Chapter 10 Thesis summary and future directions

The overall aims of this thesis were as follows:

1. Show that MBW indices are a suitable selection for detecting and tracking lung disease in CF and other paediatric airway diseases.
2. Describe the MBW technique and modified Innocor equipment in detail.
3. Demonstrate through a series of clinical studies the use of Innocor in health and disease.
4. Evaluate suitability of Innocor equipment in younger children (<5 years) and demonstrate validity.

Upon commencing this thesis project in 2006, there was emerging experience of MBW in children, particularly from Professor Gustafsson's group, and associated researchers in London. Professor Frey's group in Switzerland have also achieved important milestones in the use of MBW indices in young children and infants. Now this thesis is complete, the field of paediatric MBW has not changed greatly. Many groups are still validating equipment and performing observations studies. Whilst it is very likely that data will be presented in the near future involving longitudinal observations in larger groups, there are only a very small number of therapeutic studies involving LCI as an outcome measure in children (Fuchs et al. 2010, Amin et al. 2010, Amin et al. 2011). This lack of progress in expanding use of MBW in paediatric populations is indicative of the lack of spread of use outside the limited centres that have pioneered the technique. The state of the art document, currently in press, should provide greater standardisation of equipment and technique to allow other centres to use MBW in larger therapeutic studies.

Since starting this thesis, there has been some progress by Innovision in the development of dedicated MBW equipment. Unfortunately this equipment did not use any of the validation experience from Edinburgh, and while it is marketed for children as young as 3 years, there are no validation data to support this. Ecomedics, manufacturers of the molar mass MBW device, are currently marketing a nitrogen washout system, in conjunction with Professor Gustafsson. While this may provide a much simpler solution to the complexities of SF6 washout, there is still concern

about the validity of nitrogen washout with 100% O2 in infants. A new sequence of validation studies will presumably emerge in the near future.

The adaptation of Innocor began because of the need for a cheaper, portable alternative to mass spectrometry in UKCFGT consortium clinical studies. Further validation in younger age groups continued because of the appeal of Innocor as a commercially suitable device and interest shown at international meetings. The clinical studies presented in this thesis show that Innocor performs well compared with similar devices, and fulfils the aims of the thesis.

The detailed description of methods and technical specifications are documented to demonstrate Innocor MBW technology is based on available evidence and peer reviewed recommendations. The modifications described in chapter 8 were done with careful consideration of the recommendations and appropriately improved the specifications without harming precision or accuracy. The setup described in chapter 8 is feasible for larger research and clinical studies. Commercial development would address the need for manual alterations to the hardware and software limitations. The problems experienced in parts of North America in accessing large volumes of SF6 are not addressed in this thesis but would currently limit use of the modified Innocor there.

Data from healthy paediatric populations and those with CF and asthma show that Innocor produces similar results to other equipment. Innocor is able to distinguish disease from health, and detects subtle responses to acute changes such as bronchodilation and posture. Innocor LCI was measured over time in children with CF and may be more appropriate as a measure of disease progression than FEV_1 given its ease of use and measurement of a different aspect of airways disease.

Innocor was used in multiple locations in the department in RHSC, Edinburgh. There were no analyser accuracy concerns in the areas where it was used. Software crashes happened occasionally, but were easily resolved (computer reboot). In comparison, the mass spectrometer is difficult to operate and does not tolerate being transported. In addition, a regular service is required to maintain the accuracy of the analyser, which incurs expense. Unexpected problems can result in the equipment being unavailable until expert unscheduled servicing takes place. This was not the case with Innocor.

All of the above findings show that Innocor is suitable for MBW in children, as was shown in adult studies published by Dr Alex Horsley in our department. The studies in this thesis go further to investigate Innocor MBW in children and cover important issues of test standardisation and effect of interventions (bronchodilation, physiotherapy and posture) on lung function in children with measureable lung disease. Innocor was previously untested in children and limitations to the original Innocor setup were addressed with novel adaptations. The resulting equipment was further validated for use in younger children. It is these novel adaptations and validation experiments that are most important to the thesis. The lung model experiments, in particular, demonstrate that the photoacoustic analyser is able to accurately estimate infant lung volumes at faster respiratory rates. The reduction in analyser rise time, along with improvements in gas sampling lines contributed most to the improvements in signal response.

A further strength of this thesis is the adherence to published infant lung function standards. While many modifications were novel, use of published standards adds to the validity of the findings. It is convenient that such standards were being improved just prior to the thesis commencing, but this reflects the desire amongst the paediatric respiratory research community for progress in lung function use in the youngest patients.

The weaknesses of this thesis are, firstly, that effects of interventions on lung function were not able to be linked with effects on different domains of disease (structure, inflammation, infection). It was not possible given limitations in funding, staffing and time to organise this type of study. In addition, limitations in numbers tested severely hampered the conclusions of the second section of the thesis (chapter 9). On reflection, greater numbers of infant subjects would have been more conclusive but, given the variability of the test in unsedated infants, it may still have proved difficult to draw meaningful conclusions as to the accuracy of Innocor vs. mass spectrometer. The recent publication of the use of a more sophisticated lung model shows this type of MBW equipment validation may be superior to a clinical comparison in infants(Singer et al. 2012).

Another weakness is in sequencing of assays in the studies. Whilst all studies were conducted in a uniform way, performing spirometry prior to exhaled NO may have altered the usefulness of this assay. In addition, there were multiple assays performed as part of the CFGTC "run-in" study. While I reported only the lung function, airway clearance manoeuvres and sputum expectoration, as well as exhaled breath condensate measurement may have altered the LCI results. Taking these tests in isolation and comparing with other data may have reduced the validity of the results.

A final weakness is to do with MBW protocol. MBW is a long test, and efforts to shorten it with fewer washouts would be welcome. A concern with performing 2 washouts is that data quality may suffer. Throughout this thesis 3 washouts were performed unless the patient was unable (i.e. infants only). A proportion of washouts were discarded if the FRC or LCI values were too variable according to pre-defined parameters. In the run-in study, where 34 children performed measurements on 4 separate occasions, 12% of washouts were discarded. This is less than the 30% of washouts discarded during the study in asthmatic children and healthy controls, possibly relating to the different age-group and the larger proportion of children naïve to lung function. Examining the change in values over the 3 washouts, there is no significant difference comparing LCI 1 with LCI 2 or 3 in the sequence at any one visit. There is no evidence of a trend in those with higher LCI values (more advanced lung disease), even though test variability is often higher (Figure 81). There is currently no evidence to reduce the number of washouts from 3 to 2, as this might result in more subjects being excluded from analysis, given that there was no evidence of washout 3 being more reliable than 2 or 1 for example. Given that relying on only 1 washout is unacceptable, 3 washouts serves as a reasonable standard based on the studies presented here.

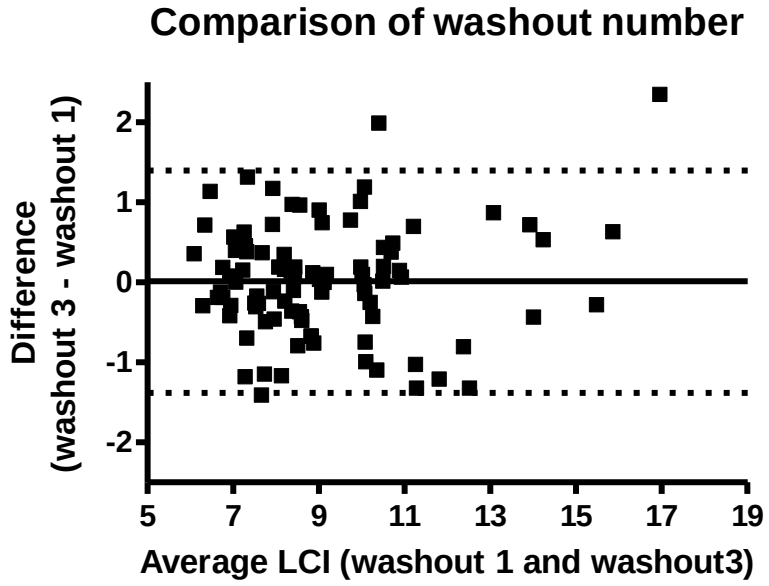


Figure 81 - Bland Altman plot of average LCI from washout 1 and 3 vs difference in children with CF recruited for chapter 7. Shows uniform variability across disease severity.

Future directions

Innocor shows great promise throughout all age groups. It is frustrating for me not to be able to be more positive about its use in infants. Future studies, particularly lung model studies may provide the necessary validation data to begin Innocor use in younger children. With full validation Innocor may be more widely used. The UKCFGTC has adopted its use in currently ongoing gene therapy trials. This indicates the Innocor has a utility for such multi-centre studies.

Wider use of Innocor requires commercial development. Innovision have made progress with commercialising Innocor for MBW. Unfortunately this version involves a re-breathing washin manoeuvre which, as a significant change to the test protocol, was not validated publicly. A recent paper suggests this method causes large changes in tidal volume from washin to washout, increasing test variability(Pittman et al. 2012). An alternative commercial setup could retain the open-circuit washin manoeuvre, with the speeded Innocor. This would obviously require the external gas supply, which is bulky. This would be an important future direction.

Finally, Innocor allows larger clinical studies to be performed in multiple centres. With the advent of novel treatments for CF, Innocor may be able to access the necessary patient numbers for studies of class-specific agents such as VX-770(Ramsey et al. 2011). These new agents suggest that CFTR function can be restored to levels that normalise sweat chloride and improve lung function (FEV_1) and weight. For these to be used in infants, repeatable assays such as LCI may be key to demonstrating efficacy in this age group. The number of subjects is greatly limited; therefore a single centre trial may not be ideal. Evidence that lung function is abnormal at 3 months of age in screened infants(Hoo et al. 2012) should increase the interest in finding sensitive repeatable assays with which to test these novel therapies in young children to halt, or even reverse early lung damage.

Final conclusion

The central aim of this thesis was to investigate how appropriate Innocor is in larger research studies and clinical use. From a device capable of measuring LCI in adults, Innocor has been adapted, validated and used in 5 moderately sized paediatric studies with important outcomes relevant to the wider research community. Each study has contributed to the main thesis aims, but also generated important results which allow further research work to be continued in Edinburgh.

On starting the thesis project there was no respiratory research lab in the RHSC, Edinburgh. Because of the CFGTC funding, and as a result of the studies in this thesis, there is now a programme of paediatric respiratory physiology research. While Innocor cannot yet be used as a clinical device, further research work is ongoing which may provide important new data on the natural history of lung diseases such as CF and asthma, the effect of interventions and disease severity on measureable lung disease, and the efficacy of novel treatments such as Gene Therapy for CF.

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List of appendices

1. Original Innocor MBW manual (author: Alex Horsley)
2. Manual flow gas delay measurement instructions
3. Innocor rise time shortening description and hardware adaptation manual
4. Mass spectrometer standard operating procedure