The development of normative values of LCI in healthy school-children using the SF_6 (Innocor) Multiple Breath Washout technique

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List of Abbreviations

Abbreviation	Description
BHR	Bronchial hyper-reactivity
CDI	Convection-dependent inhomogeneity
C_{end}	End tidal marker gas concentration at the end of a washout
CEV	Cumulative Expired Volume
CF	Cystic Fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CI	Confidence interval
C_{init}	End tidal marker gas concentration at the start of a washout
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
CV	Coefficient of Variation
DCDI	Diffusion-convection-interaction-dependent inhomogeneity
ENaC	Epithelial sodium channel
ERV	Expiratory reserve volume
FEF	Forced Expiratory Flow
FER	Forced expiratory ratio
FEV_1	Forced Expiratory Volume in 1 second
FRC	Functional Residual Capacity

FVC	Forced Vital Capacity
GOLD	Global Initiative for Chronic Obstructive Lung Disease
IC	Inspiratory capacity
IRV	Inspiratory reserve volume
LCI	Lung Clearance Index
MBNW	Multiple Breath Nitrogen Washout
MBW	Multiple Breath Washout
mmHg	A millimeter of mercury (is a manometric unit of pressure)
MS	Mass Spectrometer
MVV	Maximum voluntary ventilation
NICE	The National Institute for Health and Care Excellence
NRDS	Neonatal respiratory distress syndrome
P_{alv}	Aalveolar pressure
P_{atm}	Aatmospheric pressure
PE	Pulmonary embolism
PEF	Peak expiratory flow
PEFR	Peak expiratory flow rate
Pip	Intrapleural pressure
Raw	Airways Resistance
RV	Residual Volume

RV/TLC	Ratio of Residual Volume to Total Lung Capacity
RVRTC	Raised-volume rapid thoracoabdominal compression
S_{acin}	A measure of ventilation heterogeneity due to diffusion-convection interaction in the lung compartment defined by the acinar airways
SBW	Single Breath Washout
S_{cond}	A measure of convective ventilation heterogeneity in the lung compartment defined by the conducting airways
SD	Standard Deviation
SEM	Standard Error of the Mean
SF_6	Sulphur Hexafluoride
Slll	Phase III (alveolar) slope
SnIII	Normalised phase III (alveolar) slope
TLC	Total Lung Capacity
TO	Lung Volume Turnover
TV	Tidal Volume
V/Q	Ventilation / Perfusion ratio
VC	Vital capacity
VD	Dead space
Vexp	Volume expired
WHO	World Health Organization

Dedication

I dedicated this work for Allah, may Allah accept it for the sake of his majesty.

I would like to dedicate this work for my parents, may Allah bless them.

Last but not least, I would like to dedicate this thesis for the person whom I started this journey for, whom I walked the journey with, and whom without her I would never achieved it. This is for you my angel.

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Abstract

Background Some respiratory diseases are believed to start early in life, yet manifest later as lung damage progresses. Early pathological changes are usually missed on traditional methods of lung function assessment. Therefore, multiple breath washout (MBW) may be of value in clinical practice as it is known to be more sensitive for early airway changes. This study aims to identify normative MBW outcome measures, particularly the lung clearance index (LCI), for the SF_6-based Innocor MBW device as well as explore the effect of demographic and environmental factors on MBW measures in the paediatric population.

Methods A cross-sectional study was carried out on a sample of healthy children (aged 9-15 years; n=64) recruited from two schools in Stoke-on-Trent. Three runs of MBW testing were performed on each child in one sitting using the SF_6-based Innocor device. A questionnaire was filled prior to testing by each child's parent/guardian to assess the demographic and environmental factors of the study population.

Results Sixty-two out of the 64 recruited subjects had valid measures, which were further analysed. The mean LCI was found to be 6.18 (SD=0.3). Gender, BMI, activity level, social deprivation, pollution level, and age were not found to have a significant effect on the outcome measures. The 95% limits of agreement was 0.96 and this equate to 15.5% of the normative value. The mean time for the three measurements was 17 minutes and 36 seconds (min = 13 minutes and 20 seconds and max = 26 minutes).

Conclusion This study has suggested a normative LCI value for healthy individuals, particularly those aged 5 to 40 years. It also demonstrated the possibility of taking MBW testing from the research to the community setting using the portable Innocor MBW device. LCI may prove to be a valuable lung function assessment measure in the future as it is reliable, repeatable, sensitive, stable across age groups, and shows low variability among healthy individuals.

1 Introduction

The burden of respiratory diseases can be seen as a significant global problem (Murray and Lopez, 1997). Acute and chronic respiratory diseases are prevalent worldwide and can affect all age groups. Respiratory disease is considered a leading cause of mortality and may be a cause of premature death (Dodge et al., 2007). Chronic Obstructive Pulmonary Disease [COPD] and asthma are two of the five most threatening respiratory diseases worldwide (Bateman et al., 2008; Pauwels et al., 2001). They are considered leading causes of disability and death (World Health Organization, 2008). According to the World Health Organization, 64 million people worldwide have COPD (World Health Organization, 2017b). About three million (3.7 million) deaths every year are attributed to COPD, which means COPD contributes to 5% of all deaths around the world (World Health Organization, 2017b). Statistics have also shown that there are more than 235 million people that suffer from asthma which, unlike COPD, is commonly seen in children (World Health Organization, 2017a).

In the UK, respiratory diseases are the fifth most common cause of death and, due to an increase in incidence, is expected to be the fourth major cause of death by 2030 (World Health Organization, 2008). COPD in the UK is found to affect more than three million people (Raluy-Callado et al., 2015).

In Kuwait, the burden of respiratory disease is increasing (Khadadah, 2013; Masjedi et al., 2018). Currently, it is estimated that chronic respiratory diseases are responsible for 2% of total deaths in Kuwait (Miniño et al., 2011). Kuwait was found to have the highest prevalence of asthma amongst the eastern Mediterranean countries (25.9%) (Khadadah,

2013; Masjedi et al., 2018). Chronic obstructive pulmonary disease (COPD) prevalence is also high in Kuwait (7.1%) (Masjedi et al., 2018).

The increased incidence of asthma and COPD all over the world may be related to the increased exposure to risk factors such as smoking, pollutants and early life respiratory problems; the interaction of such risk factors may play a critical role in the development of lung disease later in life (Devereux, 2006). Further research in this area is thus urgently required.

In Kuwait there is a rise in the prevalence of smoking and this could partly explained by the low levels of public awareness about lung disease risk factors, from both smoking and air pollution (Masjedi et al., 2018) . In Kuwait, it was found that tobacco smoking is the sixth most significant risk factor associated with disease burden in the population (World Health Organization, 2011) yet smoking continues to increase within the population in Kuwait. In 2010, nineteen percent of the Kuwaiti population were found to be smokers (World Health Organization, 2010). The increasing prevalence of smoking is leading to a rise in mortality and morbidity secondary to lung disease, a decrease in the quality of life of affected individuals and consequently an increase in health costs (Shahab et al., 2006).

Several other factors have been associated with the onset of early pathological changes in the lung, the most important of which are genetic predisposition, prematurity, method of childbirth, and prenatal and early-life environmental exposures such as smoking, viruses and allergens (Kouzouna et al., 2016). These factors act on the lung during a critical phase of its development, changing lung structure and metabolism and possibly inducing

abnormal responses to harmful agents, all of which eventually lead to chronic respiratory disease later on in life (Kouzouna et al., 2016).

Maternal smoking during pregnancy, for example, has been thought to cause early harmful effects on the lung by causing in utero dysregulation of cytokine production and by interacting with asthma susceptibility genes (Hanrahan et al., 1992). Prematurity causes reduced lung airway calibre and exposes the neonate to hyperoxia, which causes exacerbated inflammatory responses in the developing lung (Rona et al., 1993). These early changes are thought to mark the onset of chronic respiratory disease in the growing individual (Carraro et al., 2014). It is thus important to understand key factors that contribute to the increasing incidence of lung diseases so as to ascertain how to identify susceptibility to these diseases and utilize the most effective methods of early detection.

Chronic respiratory illness is believed to begin early in human life (Martinez, 2009). Even before clinical symptoms develop, pathological changes in the lung are already taking place at an early age (Martinez, 2009). If we are able to detect these early changes, then it may be possible to intervene appropriately and reduce the risk of the problem becoming chronic and irreversible. Further, it may also be possible to identify factors that may exacerbate the pathological changes and take steps to educate patients on how to avoid these exacerbating factors. Unfortunately, however, the medical field is still lacking in approved diagnostic methods that are effective, efficient and practical in detecting early and subclinical chronic respiratory disease.

For example, spirometry, the most commonly used method of diagnosing chronic respiratory disease (Pauwels et al., 2001), has been known for its insensitivity to small

airway obstruction and is therefore unable to detect the early changes that occur in the small airways of the lung. This causes a delay in diagnosis of chronic respiratory diseases and increases the number of early undetected cases in the population, who may benefit from early intervention. Instead of waiting until, for example, spirometry results of a patient are abnormal indicating that considerable deterioration of lung function has occurred and pathology has already reached a level significant enough to affect the large airways, research needs to find better tests of lung function that can pick up changes much earlier, when pathology is confined to the small airways and patients are still asymptomatic. This creates a need for research that aims to find methods of measurement of lung function that can be used in communities as screening tests, which, ideally, should be able to detect respiratory problems in early life such as in infancy or preschool.

Respiratory screening tests will help discover the disease at its early stages and detect those who are susceptible to developing lung disease in order to control the disease before complications appear, delay its progression, and as a result, improve quality of life. Ideally, the testing method should be feasible for use for young children and newborn infants as understanding respiratory function in the very early stages of human life may give us insight on factors that affect normal lung development in the foetus as well as detect early abnormalities that may suggest future respiratory disease in individuals. Therefore, the focus of clinical research is directed towards finding a valid, simple, reliable, and feasible way of evaluating lung function that is more sensitive than diagnostic modalities currently used in medical practice.

Efforts worldwide are being exerted to control the impact of chronic respiratory diseases on societies by improving health services and treatment. For example, the world's

international respiratory societies (which include the American Thoracic Society, American College of Chest Physicians, Asian Pacific Society of Respiratory, Asociacion Latinoamericana del Torax, European Respiratory Society, International Union against Tuberculosis and Lung Disease, and Pan African Thoracic Society) have kept regular meetings to set common goals to deal with the impact of respiratory diseases in their societies. Their main focus was to find methods that help control disease incidence and improve management (Ferkol and Schraufnagel, 2014). Countries that recognise the impact of respiratory disease on their populations have also started supporting educational and research programmes to in an effort to minimise the burden of respiratory disease.

In this research study, we investigate the clinical applicability and reliability of an established method of testing lung function known as multiple breath washout (MBW), which has not yet been approved for routine clinical practice. This method offers an advantage over the traditional pulmonary function tests and imaging modalities in that it is a very safe test and is sensitive to changes in the small airways, which may help detect subclinical chronic respiratory disease in children; these characteristics may make it feasible to use this method as a screening modality in the pediatric population. We further investigate the effect of several demographic and environmental factors on MBW outcome measures in children, assessing susceptibility to chronic respiratory disease based on such factors. This may help us identify which children are at an increased risk for chronic respiratory disease, and thus direct screening to a certain group of susceptible individuals rather than the entire population. We also established normative values for MBW outcome measures for the pediatric age group.

In order to conduct this study, it is crucial for one to have a good understanding of normal lung development, the normal physiology of breathing and ventilation, the pathophysiology of chronic respiratory diseases, and the mechanisms by which currently-used lung function testing modalities assess lung function as well as the limitations and disadvantages of these lung function tests. The aim of the next chapter is to present a general background in these aspects of lung physiology and current methods of lung function assessment.

2 Background

In this chapter, the aim is to briefly describe the normal anatomy, development, and physiology of the respiratory system, as well as the pathophysiology of the respiratory diseases relevant to the understanding of our study and testing method, which are bronchial asthma, cystic fibrosis, and chronic obstructive pulmonary disease. The relevance of these diseases to our study is the fact that they express changes in the small airways before involving the larger airways, and can thus be detected and monitored by the MBW method, the lung function assessment method of interest in our study. The chapter then goes on to explain the standard lung function tests available today in medical practice (spirometry and computed tomography (CT) imaging), the mechanisms by which these tests assess lung function, and their limitations. We also explain the mechanisms by which single and multiple breath washout tests assess lung function and the advantages that have made these methods of great interest to researchers in the past decade. Understanding the basics will facilitate and is crucial for the understanding of our research study and its significance.

2.1 The development of the respiratory system

2.1.1 Antenatal lung development

Lung development in the fetus is divided into four chronological stages and is illustrated in Figure 1.

1. The embryonic period (0–6 weeks of gestation): In the embryonic period and specifically by the end of the fourth week of gestation, the lung appears as the ventral bud of the esophagus. Lobar and segmental portions of the airway tree develop, forming high columnar epithelium tubes. By the end of the embryonic period (by week six), an adult pattern of central vascular and airway structures develop (Ward et al., 2010).

2. The pseudoglandular period (6–16 weeks of gestation): In this stage and just after the seventh week, the lung appears as a small primitive gland, giving this period its name. During this stage, the conducting airways develop. The airway branches into twenty generations until they reach the level of the acinus (the term for a respiratory zone of the lung). By the end of this stage (by week 16), vascular development is complete, and the level of terminal bronchioles is reached (Rackley and Stripp, 2012; Ward et al., 2010).

3. The canalicular period (16–24 weeks of gestation): The pulmonary parenchyma starts to develop in this stage. At this point, a proliferation of blood vessels and a capillary network form around air sacs. Surfactant secretion also begins during this time (at 24 weeks) (Smith et al., 2010b; Ward et al., 2010).

4. The saccular period (24–40 weeks of gestation): The last stage is when the blood vessels grow, respiratory tissue expands, and new arteries form. By the 26th week, the maturation of airway wall structure is achieved (Hislop et al., 1986; Smith et al., 2010a). The first actual appearance of the alveoli happens at around the 30^{th} week of gestation. Approximately half of the total number of adult alveoli may be reached at the end of full-term pregnancy (Hislop et al., 1986; Langston, 1984).

Some, however, believe that the majority of alveoli (90%) develop after birth (J. Gordon Betts et al., 2014; Ward et al., 2010). Microvascular maturation, which is considered the final stage of lung development, happens after the alveolar development.

2.1.2 Postnatal lung development

Lung volume increases throughout childhood (Figure 1); during the first seven years of life, the growth rate of the lung reaches tenfold and continues until the age of 18 years (Burri, 2006). The increase in the number of airways stops at birth, while airway size continues to grow. Alveoli, however, continue to increase after birth in both number and size (Hislop et al., 1986; Smith et al., 2010a). The number of alveoli significantly increases in the late phase of the last trimester of pregnancy, followed by a rapid multiplication of alveoli until the age of 2 (Ward et al., 2010). The speed of alveolar multiplication decreases after the age of four and it stops at the age of 8. The number of existing alveoli normally varies in the adult lung, which makes alveolar number difficult to interpret and unreliable in determining pathology. Alveolar dimension/size is another point of interest. It seems to show small changes during the first years of life but it continues to increase until the age of 22 (Zeman and Bennett, 2006). In one study, increase in alveolar size was suggested to take place between 2 and 20 years (Merkus et al., 1996). Overall, postnatal lung growth is rapid, especially within the first year of life (Ward et al., 2010).

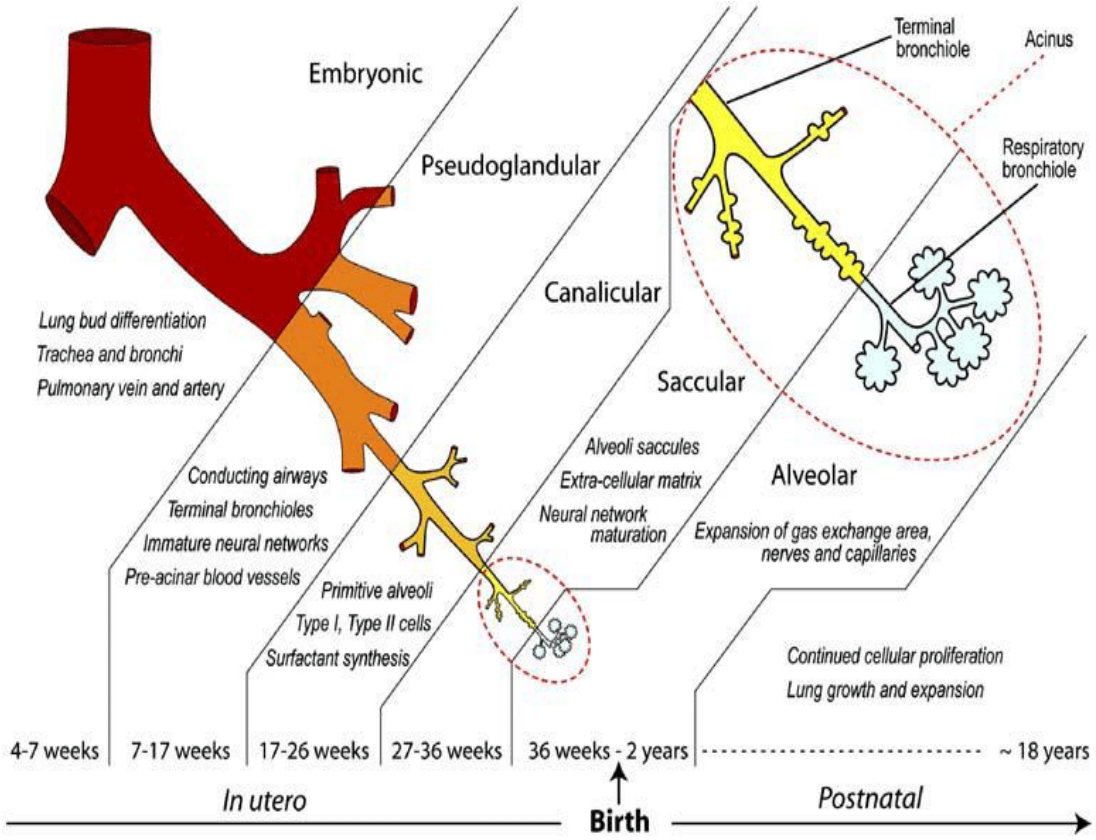


Figure 1 The development of the respiratory system

This is an illustration of the development of the respiratory system divided in stages starting from fetal life (antenatal development) and progressing to postnatal development (Kajekar, 2007).

2.2 Lung function

2.2.1 The large and small airways

The main function of the airways is to deliver air to the gas exchanging units of the lung, the alveoli. Lung airways are comprised of approximately 23 generations of branching tubes that extend from the trachea to the visceral pleura and are classified as large and small airways (McNulty and Usmani, 2014). The large airways, which start just terminal to the trachea, are termed bronchi and are cartilaginous conducting airways with a wall containing smooth muscle and connective tissue. These large airways contribute the majority of airway resistance in the lung. The small airways, on the other hand, are

termed bronchioles and are non-cartilaginous conducting airways with a fibromuscular wall and a diameter of less than 2 millimeter. At the end of the last bronchiolar divisions, the airway lining is interrupted by alveoli, the site of blood-gas exchange in the lung; these distal respiratory bronchioles that directly connect with alveolar ducts and sacs form the respiratory lung unit termed the acinus (Figure 2) (Jeffery, 1998).

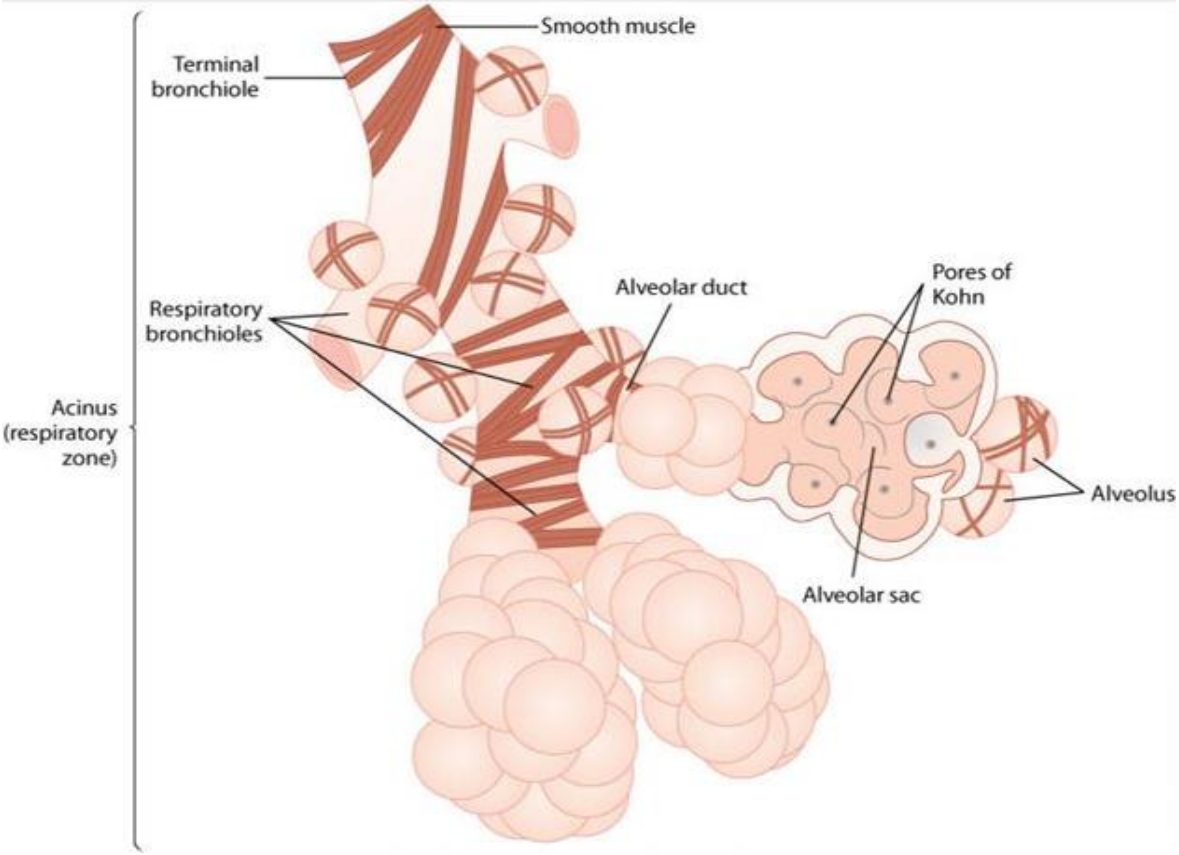


Figure 2 The anatomy of acinus

This is the functional respiratory unit of the lung, which includes many alveoli; each terminal bronchiole gives rise to an acinus (Beachey, 2017).

The small airways contribute to 10% of total lung airway resistance, while the larger airways constitute 90% of total airway resistance (Macklem, 1998). As a result, small airway obstruction may have a minor influence on total lung function, but may more profoundly affect gas exchange, particularly beyond the point of obstruction (at the alveolar level). In such cases, mechanical properties of the lung may not be affected, but ventilation distribution may be.

An important physiological difference between small and large airways involves the velocity and behavior of gas flow in these airways. In the small airways, due to the division of flow over larger cross-sections, gas flow is laminar, independent of gas density, and flows at a lower velocity compared to gas in the large airways, where flow is turbulent and density dependent. The laminar flow in small airways diminishes the impact of gas density on small airway resistance; however, in large airways, resistance is influenced by changes in gas density. Airway resistance was found to increase in large airways when a very dense gas was inhaled compared to airway resistance measured during inhalation of air, but no change was found in small airway resistance (Macklem, 1998). As a result, the effect of gas density on airway resistance can be used to determine at what level (small or large airways) airway obstruction is present in lungs with obstructive respiratory disease.

Another physiological difference between small and large airways is concerned with the low surface tension of the small airway lining. Unlike large airways, small airways have no cartilaginous support nor mucous glands and are lined with a surfactant, which protects these airways from collapsing, particularly at low air volumes in the lung (i.e. during expiration) (Macklem, 1998). Diseases that increase surface tension in the small airway lining (due to abnormal existence of mucus or inflammatory infiltrates, hypertrophy of smooth muscle lining the airways, or increase in airway wall thickness) lead to a collapse of these airways, trapping gas distally in the lung and precluding ventilation in the respiratory units distal to the obstruction (Macklem, 1998).

2.2.2 Ventilation

Ventilation is the process of breathing, in which air moves into and out of the lungs (inspiration and expiration, respectively). It is driven by an interaction between atmospheric pressure (P_{atm}), alveolar pressure (P_{alv}), and intra-pleural pressure (P_{ip}).

The process of ventilation is dependent on the difference between air pressure in the atmosphere and air pressure in the lung. Contraction and relaxation of the diaphragm and intercostal muscles are responsible for most of the pressure changes that drive the process of inspiration and expiration, causing air to either flow in or out of the lung. Thoracic wall compliance, the ability of the thoracic wall to stretch, is an additional factor that affects the breathing process. The expansion of the thoracic cavity directly influences the capacity of the lungs to expand. Therefore, decreasing the thoracic wall compliance, such as in some diseases of the pleura, will limit the expansion of the thorax and consequently restrict the lungs to expand and fill with air.

During inspiration, the diaphragm contracts, moving inferiorly towards the abdominal cavity, along with the intercostal muscles. This increases the size of the thoracic cavity and leads to lung expansion, which consequently decreases air pressure in the lung compared to atmospheric pressure; as a result, air flows from the atmosphere (the area of higher pressure) into the lung (the area of lower pressure) until equilibrium is reached; this is in consistency with the basic laws of physics. During expiration, the opposite occurs; as the diaphragm and intercostal muscles relax, elasticity of lung tissue causes the lung to recoil and decrease in size and volume, which causes air pressure in the lung to increase higher than atmospheric pressure, expelling the air out of the lung. Normal

expiration is a passive process, which means no energy is exerted in pushing air out of the lungs. Alveolar and intra-pleural pressure values are described in relation to atmospheric pressure; therefore, negative alveolar or intra-pleural pressure is lower than the atmospheric pressure and positive alveolar or intra-pleural pressure is greater than atmospheric pressure.

Alveolar pressure changes during the different phases of breathing, as was described above. Because the alveoli are connected to the atmosphere through the airways, the interpulmonary pressure of the alveoli always equalizes with the atmospheric pressure. Intrapleural pressure is the pressure of the air within the pleural cavity, which is the space between the visceral and parietal pleurae. Like alveolar pressure, intrapleural pressure also changes during the different phases of breathing. However, due to certain characteristics of the lungs, intrapleural pressure is always lower than the alveolar and atmospheric pressures, measuring approximately -4 mmHg throughout the breathing cycle. Transpulmonary pressure is the difference between the intrapleural and alveolar pressures, and it determines the size of the lungs.

2.2.3 Gas exchange

It is in the lungs where the process of gas exchange takes place, in which red blood cells exchange the carbon dioxide brought from the body with oxygen from the air (Maynard, 2006). The process of gas exchange occurs at the tissue level in the alveoli across the thin (0.5 µm) alveolar-capillary membrane. Exchange of gases is directed by the difference of the partial pressure across this membrane, in other words, diffusion (Ward et al., 2010),

where gas molecules diffuse passively from the medium with higher concentration to that of lower concentration.

Gases are brought to respiratory tissues from the atmosphere by means of two mechanisms: convection and diffusion. Convection is the process by which air molecules are transferred from the atmosphere into the lungs. Diffusion is the process of moving the respiratory gases across tissue membranes. Diffusion is driven by differences in partial pressure of gases across the alveolar-capillary membrane from the area of higher concentration to that of lower concentration. Therefore, when the partial pressure of O_2 in the alveoli is higher than in the bloodstream (deoxygenated blood), O_2 will diffuse from the alveolar space through arterial capillaries and bind with haemoglobin in the blood; blood will consequently be oxygenated. On the other hand, CO_2 will diffuse from the blood in alveolar capillaries, where there is higher concentration of CO_2 (partial pressure CO_2), through the thin capillary membrane into the alveolar space, from where it will be eliminated from the body through expiration.

The quality of ventilation depends on the quality of gas exchange. The function of body tissues is maintained as long as the gas concentration in the alveolar gas exchange unit is in balance. A dysfunction in the gas exchange process reflects a failure in the respiratory system. One of the available methods used currently in the medical field to assess ventilation quality is arterial and venous blood gas analyses, which measure the amount of gases (O_2 and CO_2) into the blood as well as blood pH levels.

2.2.4 Respiratory volumes and capacities

Respiratory volumes represent the different volumes of air moved by or associated with the lungs at a given point in the respiratory cycle and described as: tidal volume (TV), residual volume (RV), inspiratory reserve (IRV), and expiratory reserve (ERV) (Figure 3). Tidal volume is the amount of air that normally enters the lungs during normal breathing (about 500 mL) (Wanger et al., 2005a). Expiratory reserve volume is the amount of air one can forcefully exhale past a normal tidal expiration (up to 1200 mL for men) (Wanger et al., 2005a). Inspiratory reserve volume is produced by a deep inhalation, past a tidal inspiration. This is the extra volume that can be brought into the lungs during a forced inspiration (Wanger et al., 2005a). Residual volume is the air left in the lungs when one exhales as much air as possible. The RV makes breathing easier by preventing alveolar collapse (Wanger et al., 2005a).

Respiratory capacities are combinations of two or more respiratory volumes (Figure 3). Total lung capacity (TLC) is the sum of all the lung volumes (TV, ERV, IRV, and RV); it represents the total amount of air an individual can hold in the lungs after a forceful inhalation (6000 mL air for men; about 4200 mL for women) (Leith and Brown, 1999). Vital capacity (VC) is the amount of air a person can move into or out of his/her lungs, and is the sum of all of the volumes except RV (TV, ERV and IRV), which is normally between 4000 and 5000 mL (Wanger et al., 2005a). Inspiratory capacity (IC) is the maximum amount of air that can be inhaled past a normal tidal expiration, corresponding to the sum of the TV and IRV (Leith and Brown, 1999). Functional residual capacity (FRC) is the amount of air that remains in the lung after a normal tidal expiration (Leith and Brown, 1999); it is the sum of expiratory reserve volume and RV.

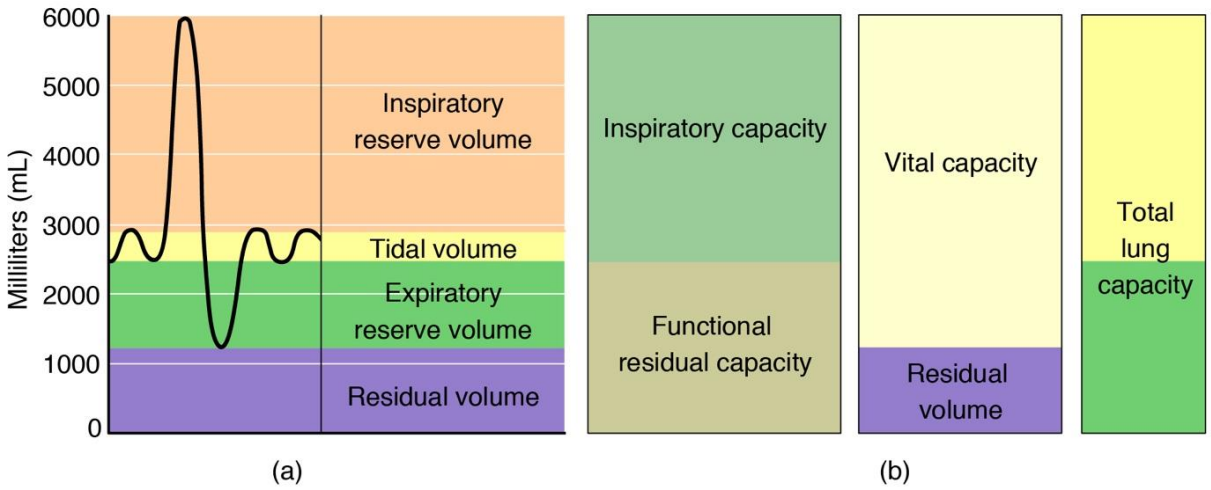


Figure 3 Respiratory volumes and capacities
This illustrated (a) respiratory volumes: inspiratory reserve volume (IRV), tidal volume (TV), expiratory reserve volume (ERV) and residual volume (RV), and (b) illustrates the different combinations of volumes that result in respiratory capacities: VC = IRV + TV+ERV , IC = TLC – FRC, ERV = FRC – RV, IRV = TLC - (FRC + VT) (J. Gordon Betts et al., 2014).

Besides respiratory volumes, the respiratory system also contains anatomical dead space, which is the air that is present in the airway but never reaches the alveoli and, therefore, never participates in gas exchange. Alveolar dead space, on the other hand, represents the air that reaches the alveoli but does not participate in gas exchange due to alveolar malfunction as a consequence of disease or abnormal blood flow. The total dead space refers to the anatomical and alveolar dead space together, which represents the total amount of air in the respiratory system that is not being used in the gas exchange process (Macklem,1998).

2.3 Ventilation distribution and homogeneity

In a normal individual, inhaled gas is distributed evenly (homogeneously) throughout the lung, and the mixing and turnover of alveolar gas is relatively rapid. However, when airway obstruction is present, gas distribution tends to become uneven (inhomogeneous) and the mixing and turnover of the gases takes longer.

Ventilation inhomogeneity occurs primarily when one of three mechanisms exists: (1) convection-dependent inhomogeneity (CDI) in the zone of proximal airways to terminal bronchioles, (2) diffusion-limitation-related inhomogeneity which is infrequent, or (3) diffusion-convection-interaction-dependent inhomogeneity (DCDI), which involves alteration in the interaction between convection and diffusion at the intermediate zone (acinar entrance) (Robinson et al., 2013b). Assessment of ventilation inhomogeneity can therefore be used as a valid measure of lung disease. SBW as well as MBW testing, the method of lung function assessment studied in this research, rely on measuring ventilation inhomogeneity in the lung and will be further explained later in this chapter.

2.4 Risk factors for lung disease

In the literature, the association between exposure to risk factors in early life and the lung function of an individual in early childhood and later on in adulthood has been comprehensively reviewed (Kouzouna et al., 2016). Exposure to risk factors in early life may cause immediate or long term negative effects on the developing lung, leading to abnormal growth of lung structures, such as the airways and lung tissues; this results in abnormal lung functioning due to low compliance, low lung volume and increased airway resistance (Kouzouna et al., 2016). This dysfunction progresses to manifest as respiratory disease. A malfunctioning lung may be susceptible to recurrent infections and numerous pathologic risk factors that may cause permanent damage of the lung tissues and chronic illness.

A systematic review conducted in 2016 (Kouzouna et al., 2016) was aimed at evaluating the early life factors that are associated with subsequently reduced lung function. It revealed the results of 25 studies that investigated the associations between exposure to a total of 74 early life risk factors and lung function. The authors of this systematic review supported the notion that in the early stages of life, a person is susceptible to exposure to some factors such as: early infection, bronchial hyper-reactivity (BHR) / airway lability, a diagnosis of asthma, wheeze, family history of atopy or asthma, respiratory symptoms and prematurity / low birth weight all of which may negatively influence the lung function later in life. The negative effect on lung function was represented by prospected forced expiratory volume in 1 second (FEV_1) deterioration, where FEV_1 represents the amount of forced expired air a person can exhale in one second.

Lungs may be susceptible to several pathologic risk factors that increase the risk of chronic respiratory disease and can be classified into two categories: those that present in the antenatal period and those that present in the postnatal period.

Antenatal risk factors include maternal smoking, which is found to affect airway growth and therefore leads to lung function abnormalities; a reduction in the maximum expiratory flow of functional residual capacity and static compliance and conductance were found in infants of mothers who smoked during their pregnancy (Goksor et al., 2007). Infants who are exposed to antenatal smoking were also found to be more susceptible to lower respiratory infections, asthma, and wheezing (DiFranza et al., 2004). The rate of chronic respiratory disease was seen to be higher in infants whose mothers had smoked during pregnancy than those who had smoked after giving birth (Greenough, 2000). Maternal exposure to secondhand smoking during pregnancy or after birth may also be a leading cause of acute chest illness in young children (Strachan and Cook, 1997). Second-hand smoking during pregnancy was also found to increase the risk of developing childhood asthma (Hollams et al., 2014). Lung function has been found to be negatively affected at school age for children of mothers who smoked during pregnancy (Strachan and Cook, 1997). This effect is limited to the early childhood period, as the variation decreases with age. This proposed effect of maternal and second-hand smoking may have a more profound impact on future respiratory health and thus requires further investigation.

Additional antenatal risk factors for diminished lung function and chronic lung disease found in the literature are low birth weight and prematurity (Beyer et al., 2009; Greenough, 2000). Prematurity is associated with lung function abnormalities, such as low lung compliance, low lung volume, elevated airway resistance, and decreased

functional residual capacity (Hjalmarson and Sandberg, 2002). Prematurity affects lung function mainly as a consequence of incomplete lung growth and maturation. The adverse effects of prematurity on the lungs has both an immediate and long-term effect on respiratory function. Neonatal respiratory distress syndrome (NRDS), which is a hyaline membrane disease, and bronchopulmonary dysplasia (a long-term consequence of NRDS) are prematurity-related respiratory conditions that predispose to chronic respiratory disease, such as asthma and COPD (Stocks and Sonnappa, 2013).

Chest infection and viral illness in early childhood are considered postnatal risk factors associated with a subsequent decrease in lung function. The literature shows lower predicted FEV_1 values in children exposed to early childhood infection. Viral infections before the age of 3, such as respiratory syncytial virus infection, rhinovirus infection, and bronchiolitis, are related to reduced lung function (Guilbert et al., 2011). Other postnatal risk factors that may affect lung function in children are obesity and socioeconomic status. According to the WHO, 80% of chronic respiratory disease deaths occur in low and middle income countries (Abegunde et al., 2007). This may be a consequence of poor healthcare (Abegunde et al., 2007). Environmental pollution may also predispose to chronic respiratory disease.

Asthma has been associated with early risk factor exposure (Kouzouna et al., 2016). Some studies link lung function reduction to abnormalities of the lung secondary to congenital or immunological causes since birth (Postma et al., 2015). On the other hand, COPD appears later in life (40-50 year olds); however, exposure to early risk factors was still found to be associated with the development of COPD in adults (Phelan et al., 2002). Persistently low lung function as well as early exposure to heavy smoking was found to

increase the risk of COPD development (Svanes et al., 2010a). Moreover, severe asthma in childhood was found to be associated with COPD in older age (Phelan et al., 2002).

The effect of early-life risk factors is complex, as each risk factor needs to be independently investigated. Accordingly, the effect of risk factors may be considered as a single group entity in our study. Some are health factors, such as viral infections or prematurity, some are social factors, such as social deprivation, and some are environmental factors like exposure to tobacco smoke, all of which are considerably important.

2.5 Lung diseases

Lung diseases vary in their nature and in their time of onset. Some disease conditions start early in life and manifest early, such as cystic fibrosis (CF). Some diseases start early in life but reveal later, such as COPD. Other diseases affect people anytime in life, such as bronchial asthma. These three diseases were found common amongst the living population and they have the characteristics of starting in the small airways of the lung and progressing to involve the larger airways.

2.5.1 Cystic fibrosis

Definition

Cystic fibrosis (CF) is a disease that affects the respiratory system. It is an autosomal recessive genetic disease affecting the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7 that manifests clinically in early childhood. Caucasian people have 1:2500 of the autosomal recessive trait, making it the most common genetic cause of morbidity and mortality in this population (Spitz and Coran, 2013).

Prevalence

In the European Union, 1 in 2000-3000 newborns were reported to be affect by CF. In the USA, an incidence of 1 in 3500 newborns was reported. In Asia, CF is severely underdiagnosed, but existing evidence indicates that prevalence is rare (World Health Organization, 2019).

Pathogenesis

The main characteristics of this disease are obstruction of the lung airways due to increased viscosity and subsequent stasis of epithelial mucus as well as exocrine pancreatic insufficiency. The CFTR mutation stops regulating epithelial chloride channels, and thus ionic transport is impaired; mucus as a consequence becomes

dehydrated, causing the abnormally increased viscosity of mucus secretions. This negatively affects the action of cilia and impairs its function, causing persistent mucus existence in areas of the body where it should be cleared, such as the lung. Consequently, bronchial and lung infection become frequent. The persistent bronchopulmonary infections cause bronchiectasis, resulting in permanent damage and dysfunction of the lung (Cotes et al., 2009).

Symptoms

Initially, the patient develops recurrent symptoms of cough and respiratory infections, despite the normal results of initial lung examinations and investigations. The disease is usually discovered as it progresses and further lung damage occurs. This delay in diagnosis may lead to the patient losing the opportunity to reserve his/her lung function. Other symptoms involve the digestive system and include foul-smelling greasy stool (steatorrhea), intestinal blockage, and poor weight gain and growth.

Diagnosis

Lung damage that is caused by CF can be demonstrated as changes in lung structure. Such changes can be observed on computed tomography (CT) imaging as abnormal dilatation of the bronchi, bronchial wall thickening and gas trapping (Sly et al., 2009). Spirometry, the most commonly used method for testing lung function, unfortunately, is unable to detect the early changes that occur in CF and requires patient cooperation, which cannot be achieved in the very young (Gustafsson et al., 2008). Sweat chloride and

sputum testing are other diagnostic tests that are undertaken when there is clinical suspicion for CF.

Monitoring

In recent years, the mean age of death of CF patients has increased to 39 years in the UK (Bell et al., 2011). This has been achieved due to advances in monitoring of disease progression, guiding appropriate treatment and care. The main method of monitoring CF today is by serial CT of the lung, as it can detect early signs of progression before development of symptoms. The risk of radiation exposure, however, is a concern especially in children. This has created an interest in discovering new safer methods of lung function assessment, such as MBW testing (Lum et al., 2007).

2.5.2 Asthma

Definition

Asthma is a chronic inflammatory disorder of the airways where there is an abnormal response towards particular allergens. Asthma is considered a burden on a personal and economical level globally. There are three types of asthma classified according to cause. The first is extrinsic asthma, which is caused by an external trigger and leads to an allergic response. This is a symptomatic type that appears in childhood and is affected by different factors. They may develop IgE antibody to a particular antigen. The second type is intrinsic asthma, in which no external cause is recognized. It is believed to be occur

secondary to chronic or recurrent infections. This type appears in adults, and it is IgE-independent. The third type is a combination of the first and the second (Beachey, 2013).

Prevalence

The prevalence of asthma is approximated to 300 million worldwide, and the mortality rate increases in spite of the improvement of health services and care (Braman, 2006). According to WHO, annual death rate of asthma is estimated to be 250,000 worldwide (World Health Organization, 2019). The prevalence of clinical asthma is found to be ≥ 10.1% of the UK population, while it is estimated to be 7.6-10.0% in Kuwait (Khadadah, 2013).

Pathogenesis

Asthma is characterised by airway narrowing, impeded airflow, nonspecific airway hyper-responsiveness, and increased mucosal inflammation and recruitment of inflammatory cells. Asthmatic patients have a hyper-reactive response to certain allergens. Factors such as dust, tobacco smoke, exhaust fumes, emotional stress, viral infections and exercise can provoke the abnormal reaction of the airways. In asthmatic patients, the immune system reacts to specific materials (allergens) causing the airway epithelia to be injured. The mucus membrane becomes swollen producing a thick sticky mucus. This abnormal reaction is accompanied by contraction of the smooth muscles of the bronchi, which minimizes the airway diameter and therefore increases the resistance to air flow. The recurrent attacks may initiate long term damage to the airway that leads to

formation of fibrous tissue at a lower epithelial layer, which causes mucus glands to increase (Beachey, 2013).

Symptoms

Symptoms of asthma are mainly shortness of breath, wheezing, which is more obvious in children, chest tightness of pain, and frequent cough.

Diagnosis

An asthma attack is accompanied with wheezing, indicating tightness or constriction of the bronchi. The asthmatic patient suffers breathlessness (dyspnea) attacks which vary in their intensity and often happen after exercise or physical activity. Severe attacks may start with a dry cough and chest tightness and progress due to bronchospasm, possibly leading to respiratory failure and death. Assessment of asthma is mainly achieved using the spirometer (Beachey, 2013).

Monitoring and treatment

The intervention programs that have been established for asthma are found to improve healthcare and show a remarkable reduction in hospitalization and death rate. These programs are being implemented in communities all over the world with the common goal of controlling asthma in the community. For example, education programs targeted

to patients and families of patients with asthma as well as school staff have been constructed to increase awareness about asthma, increase recognition of the signs and symptoms of an asthma attack, and how to address such an attack. Personalized instructions and recommendations are given also given as a part of some intervention programs to patients through home or clinic visits for discussing ways of reduction of asthma triggers based on a child's skin allergy test. Undiagnosed and undertreated cases of asthma are primary factors of morbidity and mortality in this disease (To et al., 2012).

Treatment of asthma depends on the relief of symptoms. Rest, relaxation, bronchodilators and avoidance of causative factors comprise the management of asthma attacks. The method of treatment that is used for long term maintenance of asthma as well as control of asthma attacks are anti-inflammatory drugs (corticosteroids) and bronchodilators. The latter work on relaxing the smooth muscles of constricted airways as an emergency relief. Genetic factors, atmospheric pollution and maternal smoking during pregnancy are risk factors for raised IgE levels. Consequently, asthma and airway hyper-responsiveness may develop.

Clinically, the process of dealing with and controlling asthma depends on observing and monitoring of lung function and symptoms. Although lung function may appear normal and well controlled clinically, inflammation of the airways may exist (Sont et al., 1996). The most popularly used method in assessing the severity of asthma is spirometry, in addition to assessment of symptoms. Nitric oxide (NO) is another method used as a non-invasive marker in the assessment of airway inflammation in asthma. The level of exhaled NO is found to reflect airway inflammation in an asthmatic patient (Jatakanon et al., 1998). However, NO level decreases when using inhaled corticosteroids in the treatment

of asthmatic patients and is therefore not helpful in these particular group (Kharitonov et al., 1996).

2.5.3 Chronic obstructive pulmonary disease (COPD)

Definition

COPD is defined as an incompletely reversible airway obstruction that is demonstrated by a ratio of post-bronchodilator forced expiratory volume in one second to forced vital capacity (FEV_1/FVC) of less than 70%. This definition is based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (Mannino et al., 2006). Other definitions exist for COPD according to how the disease is classified. Symptomatically, it is defined as chronic bronchitis and, anatomically, as emphysema. The latest definition however is airway obstruction, which follows a physiological disease classification (Snider, 2003).

Prevalence

COPD represents a significant healthcare burden and the forth leading cause of premature death worldwide (Calverley et al., 2003). The prevalence was estimated to be 328 million COPD incidence worldwide (Quaderi and Hurst, 2018). In addition to the tremendous costs of investigating and managing the disease, there is a further cost burden due to disability and impaired quality of life (Ferrer et al., 1997) .

Pathogenesis

The pulmonary defect that occurs in COPD is noted to appear clinically in the fourth decade of life. The onset of the disease, however, may happen in early life in some cases. COPD is characterized by incompletely reversible lung damage, progressive airway obstruction, and persistent inflammation of the airways (Halbert et al., 2006). COPD patients have been found to have increased inflammatory cells in the small airways correlating to the severity of airflow obstruction (Turato et al., 2002).

The prevalence of COPD is related to some factors. Smoking is the most significant associated risk factor. Prevalence of COPD was previously considered significantly higher in males, but a rise in smoking rates in females is causing prevalence to rise in the female population. COPD incidence is also noticed to increase with age. Exposure to inhaled substances such as occupational smoke or dust and ambient air pollution are probable risk factors that may need further investigation.

Symptoms

The main symptoms of COPD are shortness of breath, wheezing, chest tightness, and chronic cough.

Diagnosis

COPD starts in the small airways and therefore early detection of the disease may help control its progression and delay the irreversible damage that occurs with time (Svanes et al., 2010b). This fact may highlight the importance of the investigation of this disease at its early stages.

Currently, diagnosis of COPD is achieved by spirometry results that show a post-bronchodilator FEV_1/FVC ratio of less than 70%, which signifies irreversible or partially reversible airway obstruction, which characterizes COPD and distinguishes it from asthma; in asthma, airway obstruction is reversed by bronchodilator therapy, normalizing the FEV_1/FVC ratio.

Small airway obstruction is one of the three major phenotypes of COPD, but is not represented by the FEV_1/FVC ratio, as this ratio assesses for larger airway obstruction. CT imaging can detect small airway changes in COPD; however, it is not routinely performed on COPD patients as patients present for assessment after the disease progresses to affect the larger airways, manifests as symptoms, and is detectable by spirometry.

Monitoring and treatment

FEV_1/FVC ratio is a measure of airway resistance and is thus found to elucidate only large airway obstruction. It is incapable of demonstrating the diversity of the symptoms and quality of life in COPD due to the notion that the primary location of the disease pathology is the small airways and lung parenchyma (Ward et al., 2010). In addition to the pathological and physiological changes in lung airways, radiographic assessment indicates loss of the airways or size differences. Galban and Colleagues (2012) found that no apparent defects appeared on radiographic images of patients with less severe disease although disease was present in sites of the lung (Galbán et al., 2012). All of the preceding information emphasises that the irreversible damage to the airways significant enough to cause symptoms, which is termed emphysema, is a late stage of the disease, while the illness potentially commences in the changes of small airways, where investigation should be directed but currently limited (Postma et al., 2015).

Detecting changes in the small airways in early stages of COPD is considered a challenge, especially if a non-invasive technique is required. It has been found that small airway disease marks the beginning stage of COPD. Therefore, the demand for a feasible method that is sensitive to the early changes in the small airways of the lungs at an early stage is greatly needed.

2.6 Measurement of respiratory function

Lung function measurement is used to determine the abnormality of the respiratory system and detect the site of defect. Measuring lung function also assists in monitoring the progression of disease, specifies its severity, and determines the effectiveness of treatment. Lung diseases are mainly found to cause damage to either the airways or the alveoli. Consequently, either lung volumes or lung capacities will be affected (Ward et al., 2010).

Available testing methods enable measuring the different variables that are required to evaluate lung function. Measured parameters could be divided into four categories: lung volume, ventilation parameters, diffusion capacity and expiratory flow rate (Beachey, 2013).

A variety of modalities are available to measure lung function. Some of these modalities measure lung volume, such as spirometry, plethysmography, and multiple-breath washout (MBW). Computed tomography (CT) is another method that is used to demonstrate lung defects by imaging of lung structure. Comparison of the most important methods of lung function assessment (plethysmography, spirometry, CT, and MBW) is summarized in Table 2 in section 2.6.3.17 of this chapter.

2.6.1 Spirometry

Spirometry is a non-invasive lung function testing tool that measures lung capacities, dynamic volumes and flows (Miller et al., 2005). It is considered the most common tool for lung function testing, as it is a simple and feasible technique that measures how efficiently and rapidly the lungs fill and empty with air during inhalation and exhalation, respectively (Beachey, 2013). Spirometry a Latin word which means Spiro- to breathe and meter- to measure. It was invented in the 1840's by an English surgeon named John Hutchinson. The device was a calibrated bucket placed upside down in water. The volume of exhaled air from fully inflated lungs could accurately be measured by exhaling into a tube leading into the bucket. Around 1950 obstructive (limited flow rate) and restrictive diagnosis were determined vital capacity measured restriction. In 1950 measurement of air volume during a given time frame was introduced i.e. FEV_1 (Spriggs, 1978). Spirometry is not only used for diagnostic purposes; it is also used to follow up the progression of respiratory disease and accordingly evaluate treatment plans (Pierce, 2005).

Spirometers are categorized into two types depending on the method of technology that is used: volumetric and flow-measuring spirometers. The volumetric type is designed to measure the immediate amount of air the person can breathe in and out of the lung. It shows this amount through a volume-time graph called a spirogram (Figure 3.a). The second type of spirometer is designed to measure the speed of air movement during inhalation and exhalation. Flow-measuring spirometers are usually smaller in size than the volumetric ones and they provide a flow-volume graph (Figure 3.b) (de Jongh, 2008).

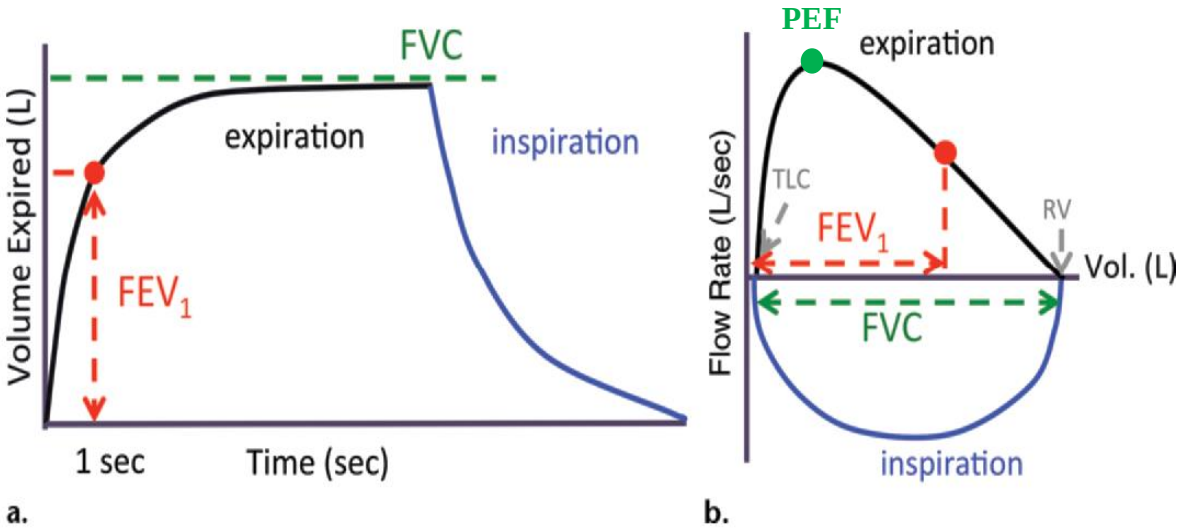


Figure 3 Graphical representation of spirometric measurements.

This illustrated (a) Volume-time plot records cumulative volume expired over time. The red dot represents the forced volume expired in one second (FEV_1), (b) The flow-volume loop is an alternative graphical representation of spirometry that plots expired flow rate over volume of air expired. The green dot represents the peak expiratory flow (PEF) (Tseng et al., 2017).

The measurement process

During spirometry an individual starts by making a tight seal around the spirometer mouth piece. The individual then takes a voluntary deep breath that fills the lungs to total capacity and breathes out as quickly and forcefully as possible until all the maximum amount of air is expelled out of the lungs. The result of this measure is demonstrated through a graphic display of expired and inspired volume of air against time, as a spirogram or a flow-volume curve. Severity of lung disease is measured and quantified by measurements of inspired and expired lung volumes in spirometry.

The outcome measures

The main outcome measures of the spirometer are: FEV_1, which is the amount of forced air a person can breathe out in 1 second, when volume is plotted against time; and the FVC (Miller et al., 2005), the total amount of air a person can breathe out of his lungs. Forced expiratory ratio (FER) is the ratio of FEV_1 to FVC, which shows how much air the individual can expire in the first second out of the total expired air (Miller et al., 2005).

$$FER = FEV_1/FVC$$

A healthy person is expected to force out between 0.75 and 0.90 (75%-90%) of his total FVC (Ward et al., 2010). This ratio may be higher in healthy children.

Spirometry can be used to measure other outcome measures such as peak expiratory flow (PEF), which represents the a person's maximum speed of expiration. It may be equivalent to the forced vital capacity (FVC). Maximum voluntary ventilation (MVV) is another value that can be measured by the spirometer and it is the maximum amount of air that a person can breathe in and out within one minute (de Jongh, 2008) (Figure 4).

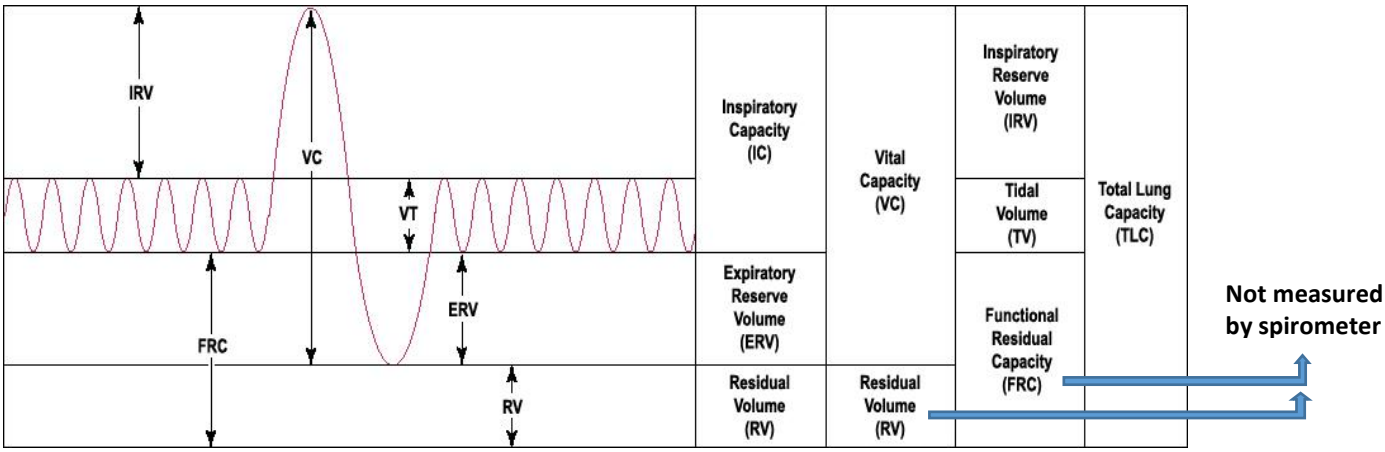


Figure 4 Output of a spirometer

This illustrating the outcome measures of the spirometry; note that FRC and RV are not measured by spirometry (J. Gordon Betts et al., 2014).

The interpretation of the outcome results (FEV_1, FVC, PEFR) of spirometry depends on comparing the measured value with normative reference values in conditions using the same testing protocol. Obtaining a value that is out of the range of the reference limits indicates an abnormality of ventilation (Pierce, 2005).

The clinical usefulness of spirometric measures

Spirometry has a role in diagnosis, monitoring and evaluating the role of the pulmonary system. It helps identify lung abnormalities by measuring airflow during expiration and inspiration. It measures the amount and the time that is taken to exhale and produces from this several outcome measures. The outcome measures help to distinguish between obstructive and restrictive respiratory diseases (Miller et al., 2005). It helps evaluate respiratory signs, symptoms and abnormal laboratory tests imaging modalities, such as chest radiographs. It also plays a critical role in the assessment of lung conditions for smokers and the people exposed to environmental hazards.

In obstructive diseases, such as COPD or acute asthma, FER value is < 0.70. In asthmatics, FEV_1 and FVC are found to increase after bronchodilators (Johannessen et al., 2006). Spirometry assists in evaluating reversibility of obstructed airways. The reversibility of airways can be demonstrated by an increase in FEV_1 or FVC ≥ 12% and ≥ 200 mL in response to bronchodilators, which reflects bronchodilator effect on airflow obstruction (Pellegrino et al., 2005). A significant response to bronchodilators reversible airway obstruction rather than COPD.

FEV_1 is used clinically to determine the progression of COPD by indicating the stage and severity of the disease. Depending on the value of FEV_1, COPD is divided into four stages:

- Stage 1: mild ➔ FEV_1 ≥ predicted value, FEV_1/FVC <70%
- Stage 2: moderate ➔ FEV_1 between 50-79 % of predicted value, FEV_1/FVC <70%
- Stage 3: severe ➔ FEV_1 between 30-49% of predicted value, FEV_1/FVC <70%
- Stage 4: Very severe ➔ FEV_1 <30% of predicted value, FEV_1/FVC <50% with chronic respiratory failure (Hajiro et al., 2000).

Restrictive diseases such as lung fibrosis, on the other hand, cause a reduction of both FEV_1 and FVC while the FER is normal or high. Therefore, FER is not considered helpful in making a diagnosis of restricted lung disease (Wang et al., 2009).

Table 1 compares spirometry finding in obstructive vs restrictive lung diseases.

Table 1 Obstructive vs restrictive lung diseases

Obstructive lung disease	**Element of comparison**	**Restrictive lung disease**
Limitation of airflow due to partial or complete obstruction	**Definition**	Reduced expansion of the lung companied by decreased TLC
↓ 70%	**FER (FEV_1/FVC)**	Normal or ↑
Marked ↓	**FEV_1**	Normal or slight ↓
Normal or ↓	**FVC**	↓

This illustrating the comparison between obstructive and restrictive lung diseases showing the difference of the spirometry outcome measures

The limitations of spirometry

Spirometry is a valid and easily applicable method; it is the gold standard lung function testing method in many respiratory conditions, yet it has its disadvantages. It requires active participation and cooperation of the individual. Consequently, applying this method may present as a challenge for the young population, mainly preschool-aged children and infants, or for unconscious subjects. The Rapid Thoracoabdominal Compression (RTC) technique has been adapted to be used in infants where eliciting a maximal expiratory effort is challenging (Merkus et al., 2005). Using this technique requires the infant to be sedated and dressed in a plastic jacket that wraps around the chest and abdomen with a mask over the mouth and the nose. Then, external pressure is applied on the chest and abdomen by inflating the jacket at the end of inspiration, and expiratory flow and volume are subsequently measured. The measured FRC through this technique is unstable and highly variable, affecting reproducibility of measurements; this impacts the sensitivity of the test to airway changes and poses a major limitation for this technique. Additionally, this technique demands providing specialised equipment in

pulmonary function clinics which is considered a further disadvantage (Zaoutis and Chiang, 2007).

One more limitation of the spirometer is that FEV_1 is appropriate to be performed on adults, but when taking children's measurements, FEV0.75 or FEV0.5 values are found to be more suitable or a better measurement of lung function (Pesant et al., 2007). Moreover, FEV_1 is a changing parameter in time, with individual variation of up to 5% within a day and possibly up to 12% from week to week (Ellemunter et al., 2010); this is the situation among the adult population, but a chance of error is expected to be even higher among the paediatric population, affecting the reliability of FEV_1 measurements in this age group.

Although spirometry is the most commonly used tool for lung function testing, spirometry is found to be insensitive to early changes in the pulmonary system, particularly the peripheral airways. Therefore, it is unable to detect abnormalities in the airway at early stages (Lum et al., 2007). An estimation of 75% of airway obstruction found to occur before spirometry can detect the abnormal changes (Burgel et al., 2013).

2.6.2 Plethysmography

Plethysmography is a well-established method of pulmonary function testing. The idea of the plethysmography was first described in 1956 by DuBois et al who presented the method as a rapid and practical measurement of lung volumes that depended on measuring the change in the volume of the gas when it was compressed (DuBois et al., 1956). It simply measures the amount of air the lungs can hold. The plethysmography

technique is based on Boyle's law, which states that "under isothermal conditions, when a constant mass of gas is compressed or decompressed, the gas volume decreases or increases and gas pressure changes such that the product of volume and pressure at any given moment is constant" (Coates and Burton, 1997; DuBois et al., 1956). The amount of air held in the lungs, which is represented as the TLC may be most accurately measured by plethysmography. It assists the physician particularly in identifying whether the problem is caused by lung structure damage or due to loss of expansion ability.

Figure 5 The body plethysmography in a clinic setting

(Bersten, 1999)

Plethysmography helps in evaluation of airway obstruction and aids in diagnosis of respiratory conditions. Additionally, it contributes in monitoring disease progression and treatment response (Marin, 2004).

Plethysmography uses tidal breathing to measure airway resistance and functional residual capacity (FRC). It depends on the idea of measuring the change in pressure while breathing. This technique follows the same principle in infants as is followed in adults. For infants, however, it requires small and sensitive equipment to accommodate for the difference between the body and lung size of the baby. This technique is computerized, which facilitates calibration and monitoring of a baby's sudden unwanted events such as apneas (Marin, 2004). Figure 5 depicts the plethysmography machine used in clinical practice today.

The measurement process

During plethysmography, the individual is expected to sit in an airtight chamber with a mouth piece and nose clip. The procedure requires breathing normally and quietly (tidal breathing) followed by a series of soft panting at a frequency of 0.5-1.0 Hz. Pants with higher or lower than this range lead to error. The pants are taken against a closed shutter while the pressure is measured in the sealed chamber and lung volume is measured. Afterwards, the shutter is unlocked allowing the individual to breathe out the air and empty the lung. A full inhale follows to fill the lung with air once more (Beachey, 2013).

The outcome measures

FRC and RV are two outcomes that can be measured by using the plethysmography technique. Both outcomes are found to be increased in some diseases, such as asthma, bronchitis and emphysema. This is explained by the increased resistance in the airways

causing an increase in trapped air, which is manifests as an increase in residual air volume and capacity. This technique allows measurement of TLC and allows detection of severe airflow obstruction (Coates and Burton, 1997) (Figure 6).

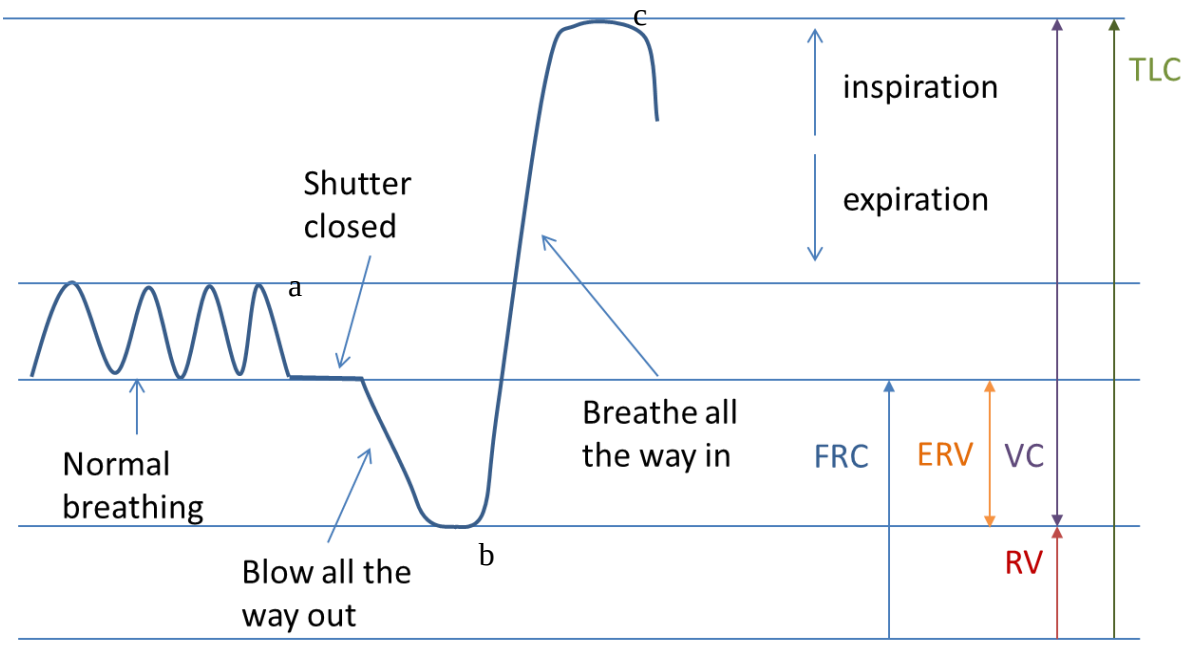


Figure 6 Volume tracing for body plethysmographic measurement of static lung volumes.

This illustrating the outcome of body plethysmography (a) when the shutter is closed FRC is measured, followed by a full exhalation all the way to (b) where RV is measured; then a full inhalation is taken to reach (c) TLC, which is measured (bronchiectasis toolbox, 2019).

The clinical usefulness of the measures

In restricted lung disease, there is a decrease in TLC, FRC, RV and VC. Usually RV is the earliest affected value in restricted lung disease; therefore, it is used to measure airway resistance. In obstructive disease, FRC and TLC will increase as the lung reacts to keep the airways open by reducing lung recoil, in an effort to maximise lung volume (Barisione and Pellegrino, 2015). Plethysmography is used to confirm a restrictive component of lung disease that can only be suggested by spirometry. Body

plethysmography is often considered to be an accurate and repeatable measurement of TLC, and can thus be of diagnostic value in restrictive lung disease when combined with spirometry (Barisione and Pellegrino, 2015).

The limitations of plethysmography

The plethysmography technique may present a few disadvantages. Chambers may cause anxiety, discomfort, and claustrophobia for some people. It might be inappropriate for very obese patients or those with skeletal deformities. Dizziness, light-headedness, and shortness of breath may result from this test due to the build-up of heat, pressure, and carbon dioxide. Despite the simplicity of its principle, the usage of this equipment exists in highly specialized centers and requires trained staff. Plethysmography is expensive and requires precise performance which makes it a difficult routine procedure to perform for assessment of lung function (Marin, 2004).

2.6.3 Computed tomography (CT)

Computed tomography (CT) is another method used to assess lung abnormalities that provides visualisation of structural lung defects through imaging. CT imaging was invented by British engineer Godfery Hounsfield and South-African-born physicist Allan Cormack of Tufts University back in 1972, and they were later awarded the Nobel Prize for their great contribution to medicine and science. CT was first performed on humans in 1974 and was limited to imaging of the head only. Later in 1976, whole body CT imaging was made available, and by the 1980s, it became a widely-used method of imaging in the

medical field (Beckmann, 2006). Over the years, this imaging modality underwent great improvements in speed, patient comfort, and resolution. Computed tomography is a technique that is highly sensitive to small lung abnormalities. It can estimate lung tissue and air volumes as well as estimate lung volumes occupied by changes in density (Wanger et al., 2005b). It is considered an important tool for detecting lung diseases in early stages. This technique is considered to be noninvasive and accurate. It is a non-painful and relatively quick imaging procedure (Tiddens et al., 2010), though it exposes the patient to the risk of ionizing radiation.

The clinical usefulness of the measures

CT is an accurate measuring technique in the field of respiratory diseases. It has been evaluated in different studies and for various conditions. It is found most useful in detecting lung cancer at early stages when there is a high cure rate. When combined with angiography, CT has also been found to play a vital role in the diagnosis of acute pulmonary embolism (PE). The results of the study showed that CT is sensitive and specific in diagnosing PE compared to ventilation-perfusion lung scans (V-P scans) and other diagnostic modalities (Sodhi et al., 2010).

The measurement process

CT is a technique that depends on taking computerized cross sectional images of an organ in several directions (three dimensions) and the outcome image can be displayed on a computer screen, printed as a high-resolution image, or copied to a CD or DVD**.** As this

method depends on exposure to radiation, the dose is associated to the individual's size and the level of software technology being used by the machine.

The CT imaging process resembles the usual x-ray examination, in that it depends on differences in absorbance of body tissues to x-ray radiation. The difference in CT scanning, however, lies in that the patient in CT scanners are exposed to numerous rotating x-ray beams that are received on the opposite side of the beam by electronic x-ray detectors. The software combines these multidirectional images to form an image of excellent resolution for assessment of disease. In other occasions, the examination table may rotate itself in the scanning process to create a spiral path, known as a spiral CT scan which have added resolution. During the scan, the individual is requested to lie down on the examination table, usually in a supine position. The usage of straps and pillows may help stabilize the patient during the examination. Movement during the scanning should be kept to a minimum. The procedure usually takes 30-60 minutes in total, including the time of preparation of the patient.

The outcome measures

CT scans provide a detailed image of the lung and thoracic cage volume. The high resolution computerized tomography (HRCT) scan is preferred in lung conditions as it provides great detail of the anatomical structure of the lung in order to clearly identify and locate small airway and emphysematous changes (Cotes et al., 2009) (Figure 7).

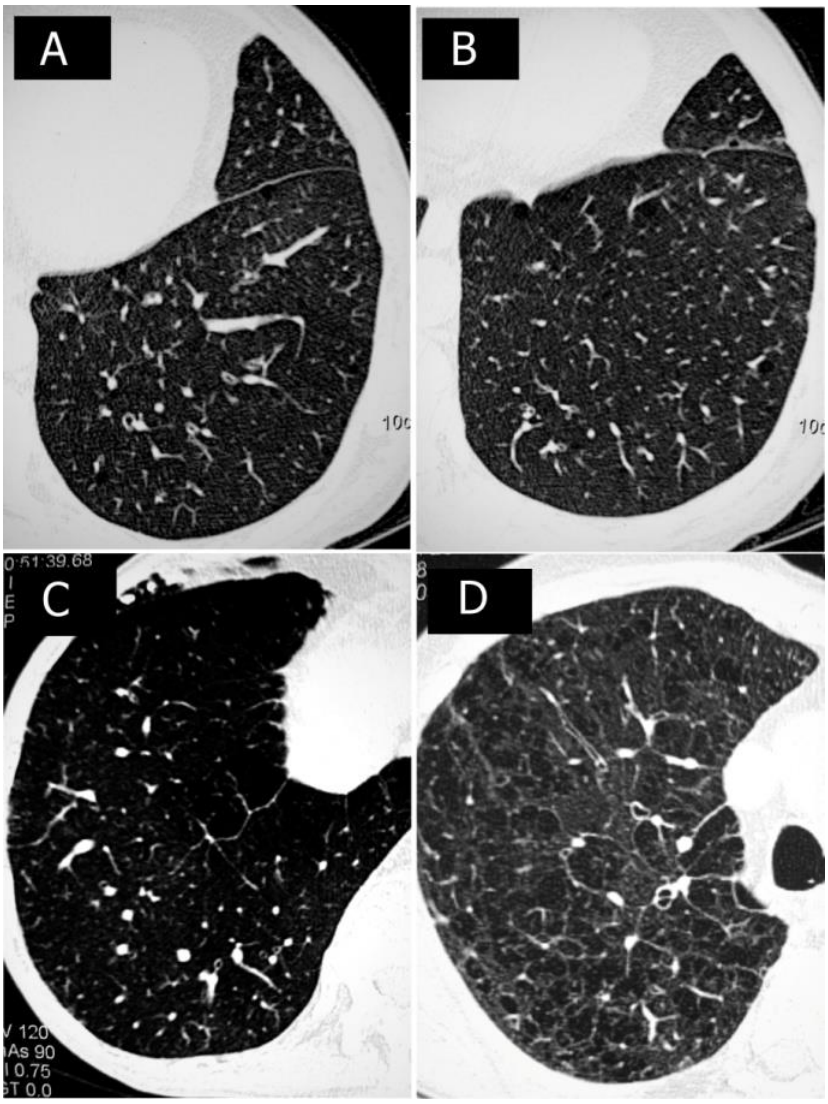


Figure 7 HRCT axial cuts in a COPD patient.

This showing HRCT axial cuts in a COPD patient in which direct visualization of the small airways in (A-C) demonstrate air filled ring like structures and in (D)demonstrate air filled branching tubular structures. Identify the reference please (Gupta, 2011).

The limitations of CT

CT has its disadvantages. Although it is an accurate lung structure testing method, it exposes the patient to an ionizing radiation dose, which may pose a health risk to the patient when frequently repeated (Cooper and MacLean, 2006). Consequently, it cannot be considered a routine method for measuring lung function.

It is a time-consuming procedure, especially for younger children. CT was found to be an inappropriate method for subjects with limited ability to cooperate. Therefore, it is a challenging method in the case of young children or infants. In such cases when the scan is essential to make a diagnosis, sedation or general anesthesia may be administered, which exposes the subject to further risks besides radiation. These disadvantages make CT an inappropriate method for routine assessment of pulmonary disease, and is reserved for selected cases and conditions.

2.6.4 Inert gas washout

Two methods have utilized the principles of inert gas washout: the single breath washout (SBW) method and multiple breath washout (MBW) method.

2.6.4.1 Single breath washout (SBW)

Single breath washout testing, also known as single-breath oxygen testing, is a method of measuring lung function using the technique of gas washout. The principle of SBW is to assess the efficiency of ventilation distribution through gas mixing within the time frame of a single breath (Fowler, 1949). It aims to evaluate uniformity of gas distribution in the lung.

The measurement process

This method is characterized by an inspiratory vital capacity (VC) maneuver, starting from residual volume (RV) and inspiration of pure 100% O_2, in the case of using

endogenous gas (e.g. N_2), until reaching total lung capacity (TLC). This is followed by expiration in which the washout phase of the gas starts to reach RV once again. In case of using exogenous inert gas, such as SF_6, then a wash-in phase is required during inhalation from RV to TLC.

The outcome measures

Breath washout testing aims to assess the uniformity of gas distribution in the lungs. The washout is represented by an expirogram which represents expired gas against expired volume (Figure 8). The expirogram shows the washout process in four phases. In phase I, the inert gas concentration recorded is 0% throughout, reflects gas washout from the conductive airways (i.e. the anatomic dead space). This is followed by phase II, which shows a rise in the curve directed upward, reflecting the gas washout from the dead space and bronchi, where there is mixing of gas but no gas exchange. Phase III represents washout of gas from the alveoli depicted as a slightly sloping plateau; this plateau occurs due to the almost constant inert gas concentration in alveoli. If inspired oxygen or air inhaled during washout is distributed evenly to all alveoli so that all alveoli have equal inert gas concentrations, then phase III of the tracing is almost horizontal (alveolar plateau). However, if inspired oxygen is distributed unevenly (usually due to lung disease), then the end-inspiratory inert gas concentrations are not equal throughout the lung. Clinically, the phase III presents the most informative outcome measure by calculating the slope of phase III. Finally, phase IV represents the closing volume and shows a sharp increase in concentration, reflecting discriminatory small airway obstruction, causing air to be expelled beyond that point (Gold and Koth, 2016).

The slope of phase III is measured over the mid portion of the expirogram [25%-75% of TV of expiration] and it is determined as the line of best fit (Bush and Sly, 2019). The derived indices are SIII which reflects the combined CDI (convection-dependent inhomogeneity) and DCDI (diffusion-convection-interaction-dependent inhomogeneity) contributions and airway closure and trapped gas volume assessment.

The clinical usefulness of the measures

The SBW technique helps assess the efficiency of evenness of gas distribution in the lungs within a single breath. Clinically, getting the measurement of slope lll is the most useful feature of the SBW testing as it indicates the inhomogeneity of gas distribution in the lungs. SBW cannot provide CDI and DCDI in separate form as an outcome of SIII, though it may help locate the defective part of the lung; this ability to localize abnormality is affected by the molar mass of the inert gas used, in which gases of smaller molar masses (i.e. He) localize at the entrance of the acinar area, while heavier gases (i.e. SF_6) show abnormal results at distal aspects of the acinar entrance (Olfert and Prisk, 2004).

The limitation of the SBW

SBW is impractical in infants and younger children as it requires performing conventional Valsalva manoeuvre, which requires a person to cooperate and follow instructions. Moreover, performing SBW requires maintaining the respiratory flow through inspiration and expiration between 400-500 ml and reproduction of vital capacity, limiting this technique to adults and children above 12 years of age. Moreover, in the respiratory phase, the gas distribution and airway closure varies within the regions of the lungs due to

the influence of gravity (Wilson et al., 1987). The SIII in SBW is affected to a great degree by this difference. In addition, SIII does not have the ability of separating CDI and DCDI contribution. The SIII obtained from VC SBW may not be a representation of the actual peripheral airway contribution (Kjellmer et al., 1959).

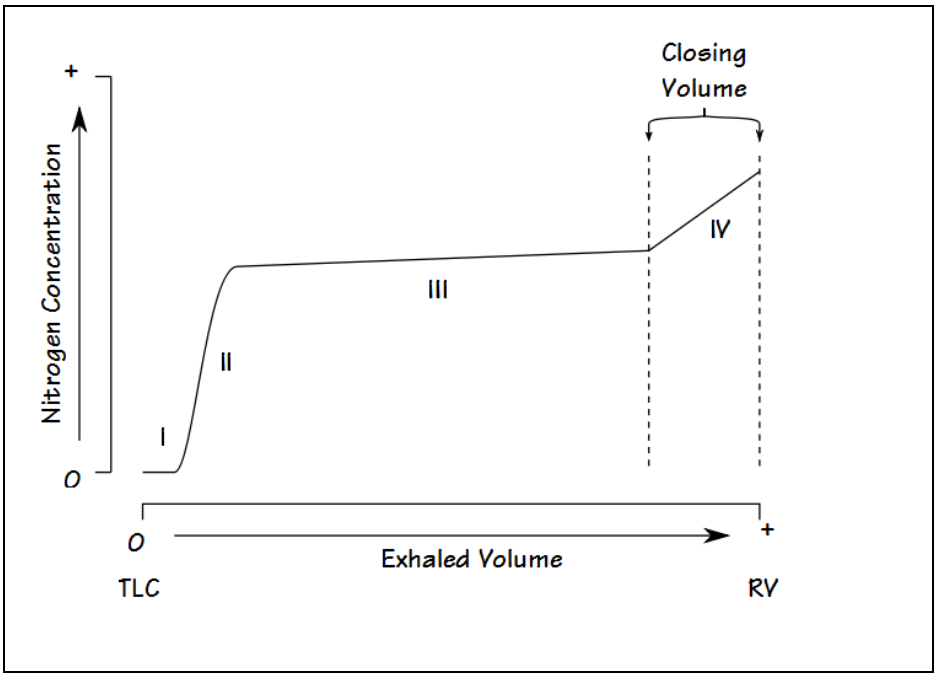


Figure 8 Single breath washout spirogram.
This demonstrated the single breath washout spirogram: Phase I represents the anatomic dead space gas concentration; phase II represents the gas concentration in the dead space and bronchi; phase III (also known as the "alveolar plateau"), represents emptying of pure alveolar gas; phase IV, exhaled gas after the onset of airways closure (PFT BLOG, 2014).

Multiple Breath Washout (MBW)

Multiple Breath Washout (MBW), like SBW, is a lung function test that measures ventilation inhomogeneity (Aurora, 2010). It uses tidal volume for measuring lung function. MBW is an old technique introduced in 1952 by Fowler who described his work on the cardiorespiratory diseases and utilized this method to measure the behavior of gas distribution in the lung (Fowler et al., 1952); following researchers, however, lost interest in the method due to its limitations. The development of the computerized multiple breath washout system and the sensitivity in gas detection were the key features that brought

about the resurgence of the MBW technique in the research community decades after it was first introduced. The MBW technique was developed recently by Gustaffson and Ljungquist (Robinson et al., 2009). The principle of the technique is to use a tracer gas that could be endogenous, such as nitrogen or argon, or exogenous, such as sulfur hexafluoride (SF_6), helium (He), or methane, to assess the efficiency of lungs by assessment of the inert tracer gas clearance (Fowler, 1949).

The peripheral airways are those branches of bronchioles beyond the 8th generation. At this level, airway resistance is not visible. Therefore, the traditional methods that assess airway resistance, such as the spirometer, are unable to detect changes at this level. When peripheral airways are affected by respiratory disease, the air will be distributed unevenly, and inhomogeneity of ventilation occurs (Stewart and Criner, 2013). This is the reason gas dilution methods are preferred in diseases that affect the peripheral airways, as ventilation inhomogeneity becomes a more helpful measurement of pulmonary function than airway resistance.

The measurement process

The MBW process requires the individual to be in a comfortable position using a mouth piece and nose clip attached to the MBW machine. Usually, the individual is seated unless the patient's health status restricts him/her from sitting. MBW technique can be performed either with inhalation of an inert tracer gas such as sulphur hexafluoride (SF_6) or helium, or by using 100% oxygen to wash out endogenous resident nitrogen. The latter technique has been available for several decades and takes slightly less time to perform as it skips the wash-in phase. If an exogenous tracer gas is used, the process is divided into two phases. First, the patient washes in the gas by breathing in the inert tracer gas through

the machine. The subject continues with tidal breathing until he/she reaches a specific gas concentration in the lungs, after which the second phase, the wash out phase, will start. The purpose of this phase is to breathe continuously to clear the lung of the inhaled gas. The main outcome measured by this technique is the LCI (lung clearance index) which indicates the turnovers required to eliminate the exogenous gas out of the lung. Unlike SBW, MBW requires passive cooperation and minimal coordination as it is performed during tidal breathing. Therefore, it is suitable for almost all ages, including infants, and can be done on sedated patients (Aurora, 2006).

The gases used in MBW

MBW assesses ventilation inhomogeneity by washing out an inert tracer gas, which can either be endogenous (naturally resident in the body tissue) or exogenous (not found naturally in body tissue), from the lung. The inert gas must fulfil certain characteristics to be used in breath washout: it should be safe to inhale in the recommended concentration, should not interact with the gas exchange process in the lung tissue, and should not dissolve in the body tissues (Robinson et al., 2013b). Endogenous gases that have been used in MBW testing were nitrogen (N_2) and argon (Ar), while exogenous gases include sulphur hexafluoride (SF_6), Helium (He), and methane (CH_4). The inert gas choice was found to influence the outcome measures of the MBW; this was explained by the difference in behavior of the gas inside the peripheral airways, influenced by molar mass of the gas, gas density, and gas diffusion rate. This affects the location of the diffusion-convection front which occurs at an intermediated zone where convection and diffusion interact in the lung airways. The behaviour of the gas inside the peripheral airways was found to be influenced by gas density (Olfert and Prisk, 2004). The gas diffusion rate is known to be inversely proportional to the molar mass of the gas, therefore affecting the

diffusion-convection front location, which is located less peripherally when using lighter gases compared to heavier ones.

Each gas has its advantages and disadvantages when used in MBW. N_2 is considered endogenous or naturally available as it naturally resides physiologically within the lung. Therefore, no wash-in of inert gas is required. However, to achieve and measure N_2 washout from the lung, 100% O_2 is required. The disadvantage of using 100% O_2 lies in that it may impact the breathing pattern, especially in infants (Singer et al., 2013), which alters MBW values, making the test unreliable. Another disadvantage of N_2 is that it overestimates FRC when used as a tracer gas (Lundin, 1953).

Helium (He) is one of the lowest density gases, especially when compared to SF_6, which is considered a heavy gas due to its high density and molar mass (He versus SF_6 is 4 versus 146 grams/mole). This feature of He increases the rate of leaking during washout testing and is therefore not routinely used. SF_6 is considered a low risk gas and is safe when used within the recommended concentration. SF_6 is non-toxic, non-inflammable, and insoluble in water (Koch, 2003), and is qualified to be used as a tracer gas in MBW. It is free of side effects when used in a concentration of 4% made affordable with an abundant amount of O_2. However, SF_6 has its disadvantages as it is considered a potential greenhouse gas, is considered high cost, and has not been approved for medical use in some countries. Nevertheless, it is commonly used worldwide in medical fields such as ophthalmology and ophthalmic surgery (Koch, 2003).

The outcome measures

Lung clearance index (LCI) is the main parameter that is calculated from MBW curves (depicted in Figure 9) and is used for early detection of peripheral airway changes. It is a measure of ventilation inhomogeneity and is basically a description of how much ventilation is required to completely clear the functional residual capacity (FRC). LCI represents the turnovers of the air volume required to wash out an inhaled inert gas from the lungs until it reaches $1/40^{th}$ (2.5%) of the starting inert gas concentration. The LCI can also indicate the general anatomic location where ventilation inhomogeneity (or alternatively, ventilation heterogeneity) is occurring, specifically the peripheral conducting or acinar airways, which are found at the terminal ends of each bronchiole.

LCI is a quantifiable measure of ventilation inhomogeneity (Robinson et al., 2010). It allows early detection of uneven air distribution and reveals lung defects at early stages, which may direct management before disease progression, and as a result, may delay irreversible lung damage and preserve lung function. Moreover, this technique helps monitor disease progression and is effective in evaluation of treatment strategies. The use of LCI as a method to assess ventilation inhomogeneity is the recent direction in pulmonary care. Nonetheless, this approach may be unable to localize the region of inhomogeneity (Robinson et al., 2013a).

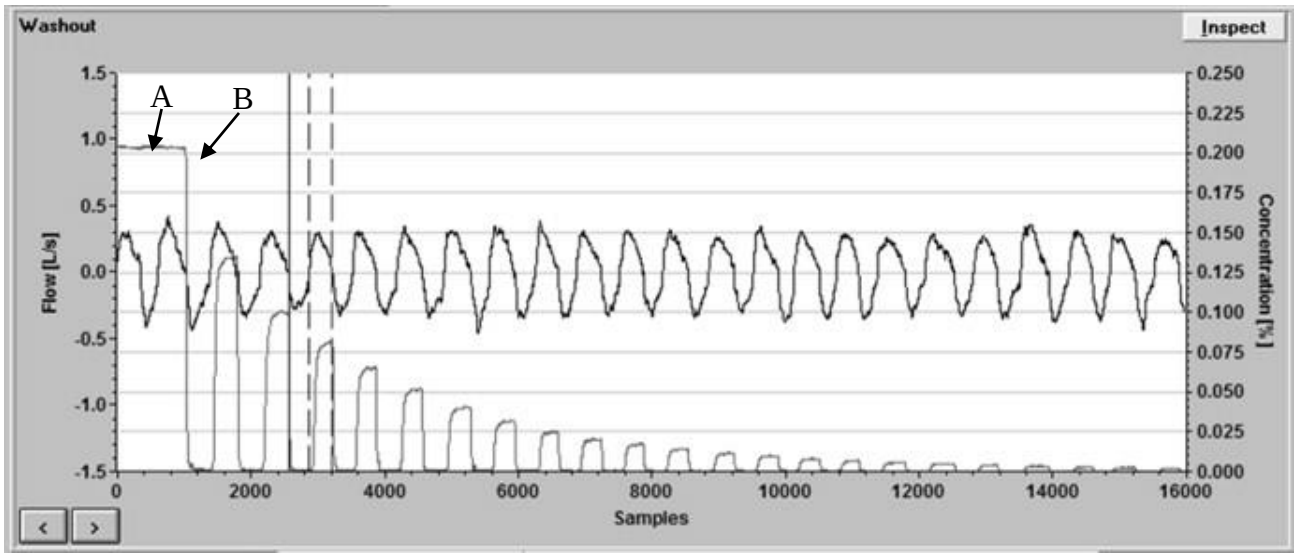


Figure 9 Washout curve.

This illustrated the washout curve where the black trace represents flow (left axis). The grey trace represents inert gas concentration (right axis). A is the end of the wash-in, where gas concentration during inspiration and expiration is identical and equal; B is the start of the washout phase, showing the start of the gradual decrease in SF_6 concentration throughout the washout phase (Aurora et al., 2005b).

LCI is the most commonly used outcome measure in the MBW device. It is an indication of the overall ventilation inhomogeneity of the lung. LCI represents how many turnovers the lung completed to eliminate the inert gas till it reached $1/40^{th}$ of the starting concentration. Mathematically, LCI can be calculated by dividing the cumulative expired tidal volume (CEV) that is required to diminish the inert gas by FRC during washout.

$$LCI = CEV / FRC$$

LCI value will increase when airways are obstructed causing delay in clearing of the inert gas and consequently further turnovers are required to clear the inert gas out of the lung. Therefore, increased LCI value indicates ventilation inhomogeneity and lung function abnormality.

The LCI reported for an individual is the mean of 3 (minimum 2) technically accepted measurements, considering standard deviation (SD) and coefficient of variation (CoV).

FRC is also a calculated measure by MBW. FRC is simply defined as the air volume remaining in the lungs at the end of tidal expiration. FRC is considered an absolute lung volume which cannot be measured by conventional lung function testing methods such as spirometry. It can be measured by plethysmography, helium dilution or MBW. Refer to Figure 4 in section 2.7.1.2. earlier in this chapter for an illustration of lung volumes and capacities, including FRC.

The MBW device measures the tidal breathing of the subject continuously during the test, providing a measurement of the cumulative exhaled volume as well as the expired SF_6. By measuring the initial and end SF_6 concentration, the functional residual capacity (FRC) can be calculated during washout.

$$FRC_{gas} = V_{gas} / C_{initial} - C_{final}$$

Where: V_{gas} is net volume of inert gas expired, $C_{initial}$ is the inert gas concentration at the start of washout and C_{final} is the concentration of inert gas at the end of washout.

The secondary but helpful outcome measures S_{cond} and S_{acin} are more informative than LCI, as they not only indicate presence of inhomogeneity, but they also localise the defect site of ventilation. S_{cond} indicates the inhomogeneity at the conductive airways, which terminates at the small airways at the end of the bronchioles before the alveoli. S_{acin} reflects ventilation heterogeneity at the entrance of the acinar region. These indices reflect the ventilation process in the peripheral and distal parts of the lung.

S_{cond} and S_{acin} are derived from the normalized phase III slope (Figure 10). Each tidal breath when plotted the inert gas concentration against exhaled volume then divided into three phases. Phase III refers to alveolar gas exchange, which represents 65-95% of expiration. These indices reflect the ventilation process in the peripheral and distal parts of the lungs. The gas transport in the acinar area is driven by diffusion, which means that the movement of gas molecules is controlled by the difference in concentration; movement of molecules in diffusion occurs from areas of higher concentration to lower concentration. Gas movements in conductive airways, however, is driven by convection, which is a mechanism controlled by the difference in pressure between two mediums. Essentially when the mechanism of DCDI and CDI is disturbed in conductive and acinar region, heterogeneity results and is expressed in S_{cond} and S_{acin}. Therefore, analysis of phase III slope assists in determining S_{cond} and S_{acin} indices.

In case of lung disease and ventilation inhomogeneity, the slope of phase III gets steeper than that seen in a normal lung. In normal lungs, the slope of phase III is expected to be zero, indicating that ventilation was distributed evenly all over the ventilated lung areas. When there is obstruction in a certain lung area, gas exchange takes longer time, causing an increase in the phase III slope, which indicates inequality of gas exchange and air distribution due to the defected areas. Normalizing the phase III slope is achieved by dividing it by the average of inert gas (SF_6) concentration. The normalized phase III values are plotted against the turnovers required to clear the inert gas out of the lungs, which occurs when the starting concentration ($1/40^{th}$) of the inert gas is reached. S_{cond} and S_{acin} can then be calculated from this graph.

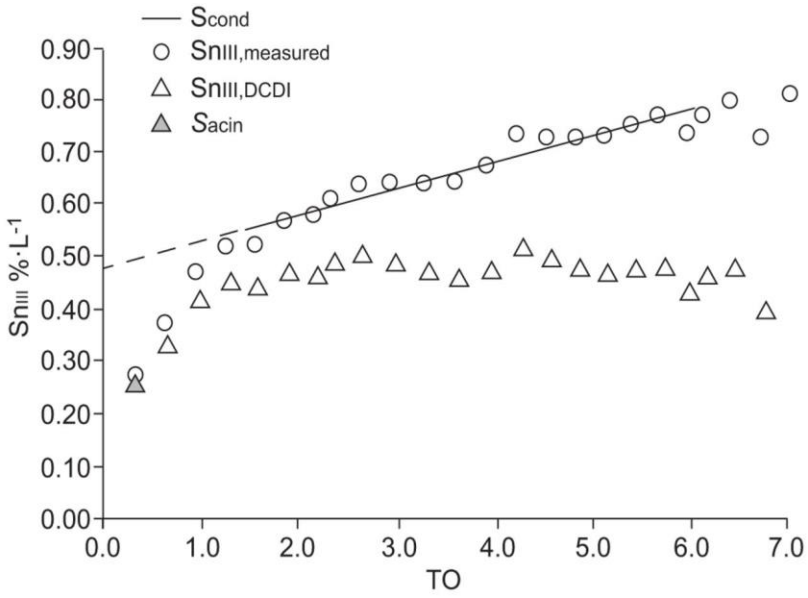


Figure 10 Calculation of S_{cond} and S_{acin} on normalized phase III slope.

This illustrated the calculation of S_{cond} and S_{acin} on normalized phase III slope vs turn overs; x-axis represents the number of turnovers (TO), and y-axis represents the normalized phase III slope (Robinson et al., 2013b).

Ventilation inhomogeneity of gas exchange can be explained by the mechanism that is responsible for the change and increase in the normalized alveolar slope (S_n), which is a diffusion-dependent mechanism. At the first 5 breaths, the increase of Sn is diffusion independent. The reason for the progressive increase in S_n in the MBW process is inhomogeneity occurring between the acinar areas, resulting from uneven emptying of air units. The inhomogeneity occurring in the acinar areas is a result of inequality of airways constructions beside exhalation of air from large gas exchange sites. The notion is that the first 5 breaths reflect the inhomogeneity of ventilation in smaller units which happened by a diffusion dependent technique. Beyond the 5th breath inhomogeneity is attributed to larger units, which caused the increase in S_n. The washout breaths demonstrating the quality of ventilation process representing the better ventilated areas progress to less ventilated areas.

S_{cond} is calculated by the slope of the regression line of normalized phase III slopes versus lung turnover values at 1.5 and 6 values.

$$S_{cond} = \text{deltaTO6-TO1.5}$$

S_{acin} is calculated by subtracting the result of S_{cond} value multiplied by TO of the first breath from SIII of the first expiration.

$$S_{acin} = S_{nIII}\ (\text{1st expiration}) - (S_{cond}\ \text{1st breath} \cdot \text{TO (1st breath)})$$

Normal values of S_{cond} and S_{acin} were found to be roughly around 0.033 and 0.075 respectively (Verbanck et al., 1998). These values were derived from outcome measures done for N_2 washout, which may slightly differ from the actual normal values of S_{cond} and S_{acin} when using SF_6 rather than N_2 as the inert gas in MBW. Still, all these findings were based on N_2 MBW, and there were no standard rules for testing S_{cond} and S_{acin} for diagnostic purposes yet.

Determining normal values of S_{cond} and S_{acin} values has helped identify conditions in which an abnormality in the peripheral airways was present and therefore helped in assessing the abnormal changes occurring in lungs with respiratory disease. The changes of S_{cond} and S_{acin} were obviously identified in asthma and COPD cases; the values were influenced by the degree of severity of the disease. S_{cond} and S_{acin} values were both negatively affected (higher) in cases of asthma, but were impacted differently by the use of bronchodilators. To explain more, S_{cond} in asthma cases was found to show significant ventilation inhomogeneity that may be partially reversed with bronchodilators. However, the abnormal S_{acin} values in asthmatics improved to normal values in response to bronchodilators. A notion that increased S_{acin} was found in COPD patients, though S_{cond}

was subjected to the level of COPD severity referring to the vitality of S_{cond} and S_{acin} as a diagnostic tool for such diseases.

The clinical usefulness of the measures

MBW is a sensitive technique for detecting early changes in small airways and early stages of lung disease. Multiple-breath inert gas washout (MBW) has been found to be an applicable testing method that can be used throughout the different age groups (Aurora, 2006). It is an appropriate tool that assesses gas mixing efficiency and inhomogeneity throughout childhood (Wall, 1985). Unlike other tools, MBW can be performed on unsedated infants (Hjalmarson and Sandberg, 2002) as long as it is done during spontaneous breathing.

MBW is considered a non-invasive testing method. It can be done on patients who are poorly cooperative and does not require a high level of consciousness. It may be the best choice for paediatric lung function testing because it utilizes tidal breathing, and is feasible, reproducible, harmless and sensitive as a diagnostic tool (Fuchs et al., 2012).

The limitation of the MBW

The MBW as a method of measuring lung function still has its disadvantages as is the case with any other method. It is considered a time-consuming technique as each subject is required to perform three trials of the test to take the mean reading of all three results as the final outcome measure. Each measurement trial consumes approximately 5-10 minutes in the case of a child, more time in older subjects, and further time in lungs with abnormalities as they have impaired clearance ability. However, some studies have been

conducted to address and overcome this issue (Hannon et al., 2014; Yammine et al., 2012). Another disadvantage is the use of 100 % oxygen to clear out the nitrogen (when nitrogen is used as the tracer gas), which may be a source of harm especially for neonates; research studies are being conducted to investigate alternative ways to the use of 100% O_2 in nitrogen gas MBW testing (Fuchs et al., 2008). A further limitation involves the high cost of the machine and the fact that it requires special training. Although LCI is sensitive in detecting early airway changes, it has been found to be less informative with more advanced airway disease; in other words, LCI is considered a less valuable indicator of lung function with significant airway obstruction compared with early disease stages (Horsley, 2009).

2.6.5 Comparison between the aforementioned tests of lung function and advantages of MBW

The demand for a more sensitive method that is able to detect the pathological changes at early stages of chronic respiratory disease is increasing. Most of the available common methods have their disadvantages and limitations and are summarized in Table 2. The MBW seems to overcome many of these shortcomings. Finding a single method that has all the desired features is not easy. Early diagnosis and feasibility is regarded as a priority when it comes to choosing a diagnostic modality in respiratory disease.

Spirometry lacks the sensitivity to small airway changes and it is difficult to be used for children aged less than five years old. It also requires patient cooperation which means it is not suitable for unconscious patients as well as children of young age. Normal values in spirometry are different for different ages, genders, stature and fitness level and values

change at different times of day and with time. In contrast to spirometry, MBW is found to be sensitive to early changes in the small airways. MBW can be used for young children and infants either conscious or unconscious as it depends on tidal breathing. LCI obtained from MBW has the advantage of remaining unchanged over time in healthy individuals. Changes occur when the disease has developed in a part of the lung. Therefore, MBW is a better method than spirometry for measuring early lung changes.

CT is an accurate method for detecting structural changes and lung volumes. Nevertheless, it has its disadvantages. It exposes the individual to the danger of radiation which limits its use as a routine procedure. It is a hospital based technique that demands well-trained staff. Additionally, the patient should be in a stable status or sedated to avoid moving during the procedure. In contrast, MBW using SF_6 is a simple procedure that can be performed in or out of the clinic, besides SF_6 is provided as small gas cylinders that can be fixed in the portable Innocor, which makes it more appropriate for routine investigation. MBW is a safe method when compared to CT while it has a similar accuracy in detecting changes in lung function.

Plethysmography is a simple method albeit it may be avoided by a number of people especially those who are intolerant to tight places or are claustrophobic. Some may claim it to be an unpleasant experience and disapprove going through the procedure. Plethysmography must be held in the clinic. On the other hand, MBW with its simplicity, can be performed in and out of the clinic.

Table 2 A comparison between lung function testing methods spirometry, plethysmography, CT and MBW

Method	**Spirometry**	**Plethysmography**	**CT**	**MBW**
Measurement	Lung volume	Lung volume	Lung structure (image)	Lung volume
Advantages	Valid Easy Gold standard for lung function testing Not expensive Portable	Fast Allows for repetitive measurements in quickly assessing the reproducibility of results Results do not vary with the severity of underlying airway obstruction	Non-invasive Sensitive to small airway changes Accurate	Non-invasive Provides portability Sensitive to small airway changes
Limitations	Associates with many potential sources of errors and variability Affected by several factors such as height, equipment, environment and operator Requires patient coaching Requires active participation of the patient Not suitable for all ages (such as preschoolers and infants) FEV1 changes with time (variability during the day and throughout the month) Insensitive to small airway changes	Problems such as bullae or hiatal hernias may be included in lung volume estimates Expensive Some patients cannot tolerate it due to body size (obese) or skeletal abnormalities or claustrophobia May develop shortness of breath	Risk of exposure to radiation Usually requires sedation or anesthesia for infants and young children Requires highly specialized staff Requires patient cooperation and awareness Expensive	Time-consuming Uses 100% O_2 when using N_2 MBW Cost Requires training

2.6.5.1 Devices that measure MBW

The devices used for MBW have been modified and developed over the years. Today, there are four main devices utilized in MBW testing. The gold standard is the mass spectrometer which is custom made for the purpose of MBW testing and can be utilized using any gas; however, it is not commercially available. Three commercial MBW devices are currently available in the market: the Innovision Innocor, the Eco Medics AG Exhalyzer D, and the EasyOne Pro. Table 3 provides a brief comparison of the four MBW devices and a more detailed overview of the Innocor device is presented in the following section, as it is the device we used in our study.

Table 3 MBW devices

	Innovision Innocor	**Eco medics AG Exhalyzer D**	**Easy One Pro**	**Mass spectrometer**
Design	Closed, rebreathing circuit Open circuit washout design	Open circuit wash-in and washout design using bias flow	Open circuit washout design using bias flow	Open circuit washout
Inert gas used	SF_6	N_2 and SF_6	N_2	N_2 and SF_6
portability	Self contained Portable	Semiportable computer required	Self contained portable	Non-portable

This illustrated the comparison between the three commercially available MBW devices (Innovision Innocor, Eco medics AG Exhalyzer D, and Easy One Pro) and the gold standard respiratory mass spectrometer.

2.6.6 The Innocor SF_6-based MBW device

Equipment (Innovision, Odense, Denmark)

The Innocor device (Figure 11) was originally designed to measure cardiac output for cardiac patients. The Innocor principle is based on measuring gas concentration during the expiration phase utilising photoacoustic spectroscopy multi-gas analyzer technology (Agostoni et al., 2005). Adaptation has been made to measure LCI and FRC and a customised interface was used for this purpose. The customised interface consists of a mouth-piece connected to a filter, which is connected to a flowmeter with a gas sampling line.

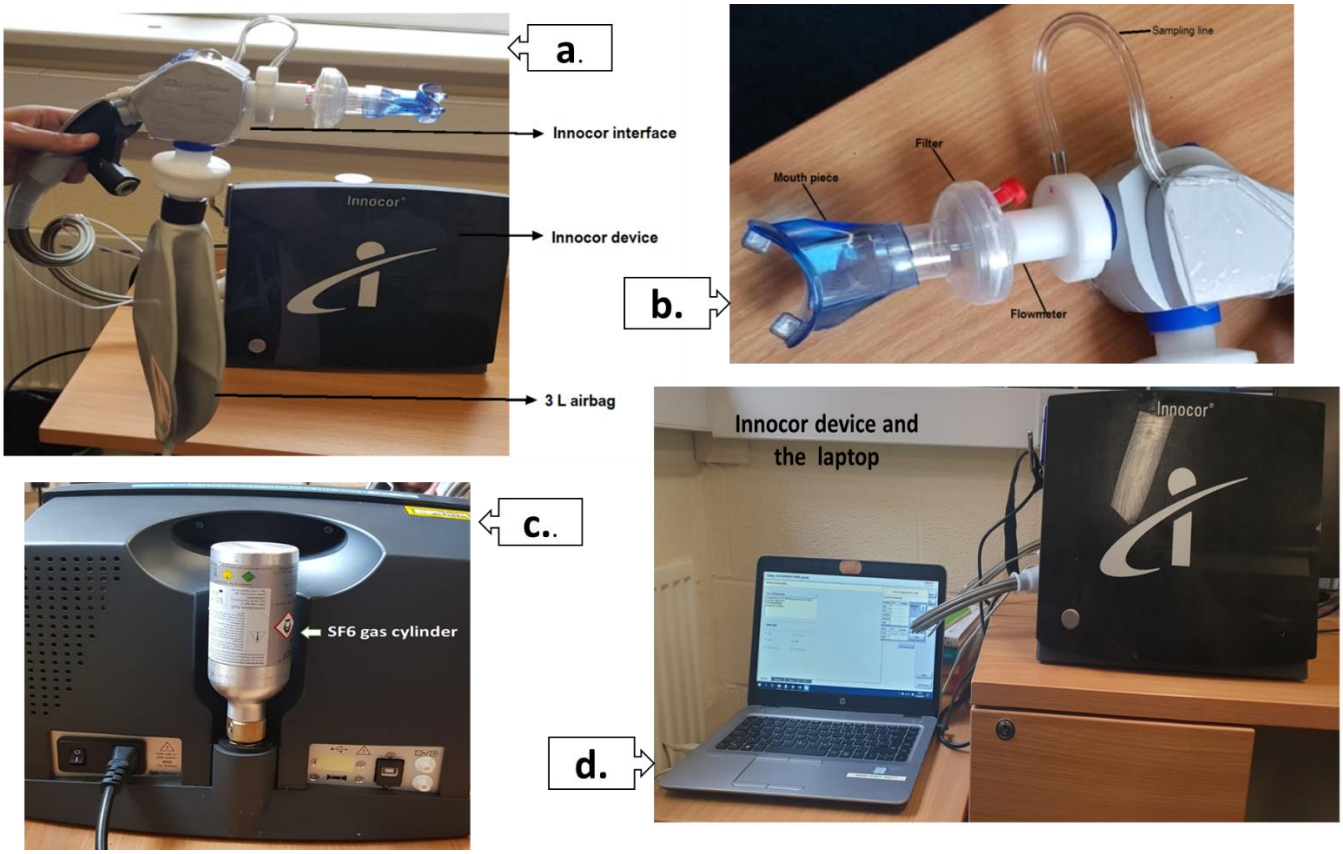


Figure 11 the Innocor device

This illustrated a.) Innocor device with patient interface. b.) MBW interface: flowmeter with gas sampling line, filter and mouth piece c.) SF_6 cylinder fixed in the Innocor. D.) The Innocor device with the laptop.

MBW using the Innocor device derives several outcome measures. The main outcome measure derived was LCI. Several secondary outcome measures are the FRC, S_{cond}, and S_{acin}. The next chapter will review the literature investigating the use and applicability of MBW testing, leading into our research questions.

3 The Literature Review

3.1 The literature search

The search of the articles for this study was done through EBSCO and was conducted through two main databases: Medline and Cinhal. The keywords were classified under four categories: action, structure, measurement, and measurement evaluation.

The CINAHL database (Table 4) yielded 65 articles. The titles and abstracts of the articles were screened to check their relevance to the subject of this study. As a result of the primary screening, 52 articles were found relevant, and 15 were excluded.

Table 4 Search terms and results through CINHAL databasee

	Key words of structure	**Key words of action**	**Key words of method of measurement**	**Key words of evaluation**
Key words	Lung	Breath	MBW	Validity
	Pulmonary	Respire*	FEV_1	Reliability
	Airway	Ventilat*	LCI	Sensitivity
	Alveol*		Spirometry	Reproducibility
				Repeatability
				Error
				SE
Code	S1	S2	S3	S4
Use of OR	113,465	108,118	7,309	252,418
Age filter (0-18 years-old)	15,014	25,523	1,670	53,322
Use of AND	S1+S2+S3+S4 = 65			

Through Medline database (Table 5), the search yielded a result of 39 papers, and after going through the titles and abstracts, the number of relevant articles were found to be 19.

Subsequently, after reading through all the relevant articles and considering any duplication, a total of 24 articles was related to the research subject out of the two

databases.

Selection of the studies for inclusion was based on the following criteria:

1. Studies that measured lung function, as the concern in our study is lung function measurement.
2. A paediatric population, as our study is concerned with paediatric lung function assessment.

Interventional studies were excluded from our search because the focus of this thesis was to analyse psychometric properties of the available methods of lung function measurement.

Table 5 Search results through Medline database

	Key word	Code	Articles number
Key word of structure	Lung	S1	5,877
	Pulmonary	S2	4,794
	Airway	S3	24,094
	Alveol*	S4	7,733
Use of OR	S1 or S2 or S3 or S4	S5	40,127
Key word of action	Breath	S6	18,205
	Respire*	S7	86,288
	Ventilat*	S8	30,182
Use of OR	S6 or S7 or S8	S9	110,385
Key word of method of measurement	MBW	S10	14
	FEV_1	S11	3,299
	LCI	S12	92
	Spirometry	S13	5,322
Use of OR	S10 or S11 or S12 or S13	S14	7,624
Key word of evaluation	Validity	S15	62,358
	Reliability	S16	67,143
	Sensitivity	S17	102,445
	Reproducibility	S18	42,725
	Repeatability	S19	1,160
	Error	S20	53,581
	SE	S21	6,889
Use of OR	S15 or S16 or S17 or S18 or S19 or S20 or S21	S22	266,741

Use of AND	S5 and S9 and S14 and S22	S23	155
Use of AND with age filter (0-18)	S23 with age filter	S24	39

3.2 Review of literature

3.2.1 Introduction

There has been a growing interest in the research community and literature for the assessment of ventilation inhomogeneity by the MBW technique as it showed a distinctive ability to detect early lung disease in a relatively easy and safe manner. These distinctive features of MBW testing have guided efforts in assessing applicability of this method in the diagnosis and monitoring of lung disease particularly in children, as MBW testing relies on measurements obtained during tidal breathing. Over the years, the MBW method has been improved and modified to make it more usable and easier to apply clinically, with special focus on the paediatric population. Many studies have investigated the clinical applicability and sensitivity of MBW testing in diagnosing and monitoring of different respiratory diseases, such as cystic fibrosis. Comparisons have been made with other methods used in assessing pulmonary function. Different MBW devices and tracer gases have also been compared in the literature. In this literature review, we aim to discuss the aforementioned aspects of MBW testing that lead up to our research questions.

3.2.2 The clinical value of MBW as an assessment of pulmonary function

In medical practice, a need for new methods of assessment of lung function was observed over the years, as the standard methods of pulmonary function testing demonstrate limitations that affect the diagnosis and monitoring of chronic respiratory illness. One disease in particular in which this need was observed is cystic fibrosis, as the current gold standard method for diagnosing and monitoring this chronic illness remains to be the computed tomography (CT) image. Because cystic fibrosis presents in childhood, performing serial or annual CT imaging to monitor disease progression and guide treatment presents as a significant source of radiation exposure to a child; this poses an increased risk of future secondary cancers in this population of children. In the paediatric age group, the risk of radiation-induced malignancy is more significant compared with adults due to the highly-proliferating cells seen in the growing body of a child; highly-proliferating cells are at greater risk of undergoing radiation-induced DNA damage and consequently dysplasia of the cells, which may develop into cancer. The risk increases with an increase in the cumulative dose of radiation, meaning that the more CT scans a child undergoes, the greater the risk. Therefore, the presence of a method that spares a child the radiation of a CT scan whilst still providing equally highly sensitive measures of lung function in cystic fibrosis patients has become a goal to medical researchers. Perhaps, that is why much of the literature investigating the usability of the MBW method is conducted on patients of cystic fibrosis.

MBW testing has been suggested to be an appropriate substitute and safer alternative to computed tomography. In a study conducted by Ellemunter. et al (2010), the accuracy of LCI was compared to CT imaging in detecting patients in early stages of cystic fibrosis

that showed normal FEV_1 readings. Results showed that LCI was able to obtain similar diagnostic accuracy compared to lung CT scans. Furthermore, a serial measurement of LCI demonstrated the ability of early identification of progression in cystic fibrosis while avoiding exposure to CT radiation (Ellemunter et al., 2010). This study proved that LCI had equal diagnostic ability and power in identifying lung abnormality comparable to that of CT imaging. Another study conducted by Aurora et al. supported the sensitivity of LCI to early deterioration of lung function (Aurora, 2010). In this study where results of LCI and CT were compared, both were able to detect early CF lung disease, while both had missed some of the cases. A study performed by Gustafsson et al further supports the aforementioned studies by demonstrating that a normal LCI indicates absence of structural lung damage on high-resolution computed tomography (HRCT) (Gustafsson et al., 2008).

MBW testing has also been suggested to be an appropriate substitute to spirometry for the assessment of ventilation inhomogeneity (VI) in the lung. MBW testing demonstrated higher accuracy and sensitivity to abnormalities in lung function by identifying higher than normal measures of ventilation inhomogeneity. One of the most commonly utilized spirometry measures for detecting abnormality of airway function in children is the FEV_1. Studies have found that MBW testing was able to detect abnormal lung function that was missed with a normal FEV_1 reading obtained by spirometry, which means that MBW was able to detect early stages of lung damage that were undetectable by FEV_1 (Green et al., 2011). Furthermore, another study demonstrated that LCI was the earliest measurement to deteriorate in children with progressive CF during serial follow-up evaluation, followed by decreases in spirometry results ($FEF_{0.5}$, FVC, and FEV_1) (Kraemer et al., 2005).

Beside its significantly lower sensitivity to deterioration of lung function in children, spirometry carries a considerable disadvantage in that it cannot be performed on infants and young children as it requires patient cooperation and is effort-dependent. MBW testing, however, depends on tidal breathing and thus requires minimal patient cooperation when performed on young children and can be carried out during sleep for infants. This means that for very young patients in which lung assessment is required, physicians are limited to blood gas analyses and imaging modalities, such as the CT, creating a greater need for lung function testing methods such as MBW in this age group (Sarma et al., 2012).

One study conducted by Bakker et al. aimed to compare nocturnal oxygen level and cough as tools to measure and detect pulmonary changes in CF children with MBW testing. The study showed that LCI can detect significant lung changes in CF, while nocturnal oxygen and cough could not (Bakker et al., 2012).

Studies were also found in the literature investigating the ability of MBW to detect other chronic lung diseases that could not be detected with spirometry. For instance, a study by (Green et al., 2011) found that MBW was able to detect changes in primary ciliary dyskinesia (PCD), which has similar features to CF in that lung damage primarily occurs in the peripheral airways. Although MBW is shown to be more sensitive than spirometry in detecting PCD, further research is required to confirm this conclusion (Green et al., 2011). LCI was also used to detect the abnormal changes that occur in bronchiectasis patients corresponding to detectable abnormalities on CT scan in a study conducted by (Rowan et al., 2014). LCI was found to be more sensitive to such structural changes compared to FEV_1 obtained by spirometry (Rowan et al., 2014).

As the MBW technique is characterized by sensitivity to ventilation inhomogeneity, LCI was demonstrated to be one of the most reliable parameters to quantify ventilation inhomogeneity (VI) (Yammine et al., 2014). A study conducted by Singer et al. (2013) demonstrates the sensitivity and reliability of LCI as a lung function testing method and supports its use as a routine paediatric care procedure as it is considered practical and feasible particularly for this age group (Singer et al., 2013). MBW outcome measures also have the advantage of being reproducible; a study conducted by Aurora et al (2006) concluded that the MBW method yields accurate readings despite repetition between different laboratories (Aurora, 2006).

Furthermore, LCI was found to have the distinctive characteristic of being age-independent especially in childhood, which makes it suitable for research purposes (Yammine et al., 2014). Spirometry, in contrast, varies with age, body size, and gender and therefore makes comparisons between values of different individuals or values gathered on follow-up of disease for the same individual more complex and difficult to interpret (Wang et al., 2009; Wang et al., 2017). Most of the studies that investigated the effect of age and body size on LCI found that LCI was not affected by these factors (Aurora et al., 2005a; Horsley et al., 2008a). One study, however, by Lum et al made a contradicting conclusion; he found that LCI was dependent on age and height, decreasing in a nonlinear pattern as height increases (Lum et al., 2013). However, he stated that these changes were particularly marked in the first five years of life. The literature has explained the reason for this observed pattern in young childhood in that LCI is dependent on structural and functional lung growth in this age group (Habib and Lutchen, 1991; Haidopoulou et al., 2012; Schmalisch et al., 2006). Moreover, LCI was found to be

greatly affected by differences in lung volumes, especially airway dead spaces, in the early years of life. The increased dead space in MBW testing in infants may also be due to the use of a face mask instead of the mouth piece used in older children (Schmalisch et al., 2015).

Another study, which was conducted by Verbanck et al found that LCI, S_{acin} and S_{cond} consistently increased with age; however, the study population age range was 25-65 years (Verbanck et al., 2012). Therefore, this increase in MBW outcome measures with age may occur only in adults beyond a certain age. Further studies are needed to investigate the possible effect of age on LCI in the adult population. In general, however, LCI was found to be an age-independent measure of lung function, particularly among children beyond the first few years of life.

3.2.3 MBW devices

Use of the MBW technique for assessment of lung function was introduced in the literature over 60 years ago. In the past decade or so, however, this method has regained attention and popularity in the medical research community, and a growing interest in developing this technique for use in medical practice has been seen. The respiratory mass spectrometer is the current gold standard technique for performing MBW; however, the lack of having this device commercially limits its use in the clinical setting. This has increased the demand for commercial MBW devices to help increase the clinical applicability of this method. Three commercial devices, as described in the previous chapter, were developed to meet the requirements of clinical use of MBW testing, namely the Innovision Innocor device, the Eco Medics AG Exhalyzer D (a side-stream ultrasonic

flow system prototype), and the EasyOne Pro. These devices vary in several aspects, such as their degree of measurement validation, suitability for different age groups, affordability, regional regulatory agency approvals, portability, the type of tracer gas utilized, and transparency of outcome calculation. For more information about the differences in the devices, refer to Table 3 in the chapter 2.

None of the commercial MBW devices have been designed to suit all ages. However, work is ongoing to create a commercial device that fits all age groups. Currently, the Innocor and EasyOne Pro are the commercially available MBW devices that can be used in school-aged children and beyond. One study has even shown successful applicability of the EasyOne Pro on children in the preschool age group on individuals as young as three years of age (Fuchs et al., 2011). The commercial Eco Medics AG Exhalyzer D was initially designed for infants and very young children, up to five years of age (Fuchs et al., 2009); however, studies have demonstrated successful use in older children as well. The mass spectrometer has been used for all age groups, but as mentioned before, is not commercially available (Fuchs et al., 2006).

3.2.4 Variations in MBW outcome measures between different devices and gases

Studies have shown that the outcome measures that are produced by different devices vary and are thus not comparable to one another and cannot be used interchangeably in assessment of lung function. Mean values of various MBW outcome measures in normal individuals varied across the four MBW devices. For example, the mean LCI for healthy individuals assessed in a study using the mass spectrometer and SF_6 as a tracer gas was found to be 6.19 (Jensen et al., 2013), and in another study using Innocor and the same

tracer gas (SF_6), it was found to be 6.3 (±0.5) (Horsley et al., 2008a). The EasyOne Pro which uses N_2 as a tracer gas was used in a study by (Fuchs et al., 2009) and found a mean LCI in normal individuals of 6.2(±0.4); however, in another study by (Jensen et al., 2013), it was found to be 6.81 in a healthy population using an N_2-based mass spectrometer. These differences between devices is demonstrated in Tables 6 and 7 and illustrated in Figures 12 and 13. As a result of these variations among devices, it was agreed upon collectively by the research community that normative and disease values and ranges must be individualized to each device and that measurements obtained for individuals could not be compared with each other if different devices were used.

The literature has also shown variation in MBW outcome measures with the use of different gases (Table 6Table 7). In a study by (Kent et al., 2014), outcome measures using N_2 gas were found to be higher than measures obtained using SF_6. In another study by (Jensen et al., 2013), a significant difference was found in LCI values measured with a MBW device using two different gases: N_2 and SF_6; mean LCI using N_2 in healthy children was found to be 6.81(±0.05), while mean LCI measured using SF_6 gas in the same individuals was 6.19(±0.05). This variation increased even further in the diseased state; the mean LCI in children with CF was found to be 11.29(±0.05) using N_2 and 10.05(±0.05) using SF_6. In conclusion, and as demonstrated by in the comparison we constructed in Tables 6 and 7 and graph in Figure 12, a variation was observed between LCI measures obtained using N_2 and measures using SF_6 that was greater in the diseased population compared to that observed between the healthy populations.

These differences in the outcome measures obtained using N_2 vs SF_6 was thought to be explained by the differences in the behaviour of these gases in the peripheral airways,

which is influenced by the molar mass or density of the gas and gas diffusion rate (Olfert and Prisk, 2004). SF_6 is a heavy gas and may, therefore, behave differently in the peripheral airways compared with the lighter N_2 gas. However, in studies that compared LCI obtained with SF_6 to LCI obtained using He (which, like N_2, is considered a light gas), an increased variation in LCI measures in the diseased group was not seen with He compared to SF_6 (Jensen et al., 2013). This means that the molar mass alone could not explain the increasing variation in LCI seen with N_2 in diseased subjects.

Further differences in physical properties between N_2 and SF_6 were therefore investigated. The diffusion rate of a gas is known to be inversely proportional to the molar mass of the gas, thus affecting the diffusion-convection location of the gases in the lung, in which lighter gases are located less peripherally in the lung than heavier ones. This places lighter gases in slowly-ventilated lung units compared to the rapidly-ventilated more peripheral lung units where heavier gases, such as SF_6, are found. The endogenous nature of N_2 causes N_2 to diffuse from lung tissues into these slowly-ventilated units contributing to the volume of trapped gas in the lung. This is represented by the higher FRC values obtained with N_2, which has been demonstrated by (Jensen et al., 2013) who compared FRC in healthy subjects and subjects with CF using N_2 and SF_6. Mean FRC in the control group was calculated to be 1.60(±0.06) when using SF_6 and 1.92(±0.07) when using N_2; mean FRC in the CF group was found to be 1.41(±0.06) using SF_6 and 1.89(±0.05) using N_2. N_2, which continuously diffuses from the lung tissue into the slowly-ventilated lung units, acts as a trapped gas and therefore takes longer to be washed out of the lung compared to the heavier SF_6; this phenomenon is amplified in a diseased lung as the volume of trapped gas in the lung further increases pathologically. This was demonstrated by a study that observed that healthy subjects required an additional five breaths to complete washout using an N_2 compared to SF_6, whereas

patients with CF required 18 additional breaths to complete washout of N_2 compared to SF_6. The number of breaths required to complete washout using N_2 was found to increase in direct proportion to the volume of trapped gas measured in the lung (Jensen et al., 2013).

The choice of tracer gas used in research studies as well as in the clinical setting in MBW testing should be made in view of the properties, advantages, and disadvantages of each gas. An overview of the different gases used in MBW can be found in chapter 2 section 2.5.6.12.). Most studies in the literature utilized N_2 and SF_6 as tracer gases in MBW. The fact that N_2 is an endogenous gas presents itself as an advantage as no exogenous gas source needs to be washed into the lung. However as explained above, variation in values is greater with N_2 compared to SF_6, and is more profound in the diseased population. This variation may make it more difficult to set clear normative values for N_2-based MBW testing and thus decrease the method's sensitivity to diagnose lung disease. It may also make monitoring of established lung disease for signs of progression unreliable as variation increases profoundly in the diseased lung. Another disadvantage of using N_2 as a tracer gas occurs due to the need for 100% O_2 to washout the endogenous nitrogen for calculation of the MBW outcome measures. Singer et al (2013) found that 100% O_2 has an impact on the breathing pattern, especially in infants (Singer et al., 2013), which affects the MBW outcome measures. This change in breathing pattern results in a decrease in the tidal volume of full-term infants (Yammine et al., 2014) and a significant decrease in tidal volume in those who are preterm (8%) (Jost et al., 2016). In an attempt to decrease exposure to 100% O_2 so that breathing is not affected, (Singer et al., 2013) conducted a study using N_2 and a 40% O_2 washout protocol in infants and compared it to the traditional 100% O_2 washout protocol. The study demonstrated that, unlike the old protocol utilizing 100% O_2, the new protocol did not change the breathing pattern of the

infants and did not cause hyperoxia (Singer et al., 2013). Further studies are needed to investigate the validity of this new suggested protocol.

A further disadvantage of the N_2-based MBW system is that the required 100% O_2 gas is highly flammable, while SF_6 is not, making SF_6 overall safer for use; however, SF_6 is considered a greenhouse gas, making it more dangerous for the environment (Koch, 2003).

Table 6 A comparison between the mean LCI (SD) values in healthy individuals

Name of the study	Healthy population	Age range	Mean age (SD)	MBW device	Inert gas	LCI mean (SD)
Aurora et al., 2004b	33	6-16	11.3 (3.1)	MS	SF_6	6.45 (0.49)
Poncin et al., 2017	50	8.5-13.9	11.2	Exhalyzer	N_2	7.05 (0.55)
Poncin et al., 2017	50	8.5-13.9	11.2	Easy Pro	N_2	6.78 (0.63)
Poncin et al., 2017	51	23.1-28.3	25	Exhalyzer	N_2	6.92 (0.35)
(Poncin et al., 2017)	51	23.1-28.3	25	Easy Pro	N_2	6.42 (0.54)
(Aurora et al., 2005a)	30	-	4.3 (0.8)	MS	SF_6	6.89 (0.44)
(Horsley et al., 2008b)	17	21-47	31.3 (6.0)	Innocor	SF_6	6.7 (0.6)
(Horsley et al., 2008b)	29	5.3-16.2	11.1	Innocor	SF_6	6.2 (0.5)
Horsley et al., 2008)	12	6-16	11.0	Innocor	SF_6	6.3 (0.5)
Horsley et al., 2008)	48	19-58	33.0	Innocor	SF_6	6.7 (0.4)

Table 6This illustrated the mean (SD) of LCI value in healthy individuals of different age groups using different MBW devices and inert gases from previous studies in the literature.

Table 7 A comparison between the mean LCI (SD) values in CF individuals

Name of the study	CF population	Age range	Mean age (SD)	MBW device	Inert gas	LCI mean (SD)
Aurora et al., 2004b	22	6-16	11.5 (3.2)	MS	SF_6	11.53 (2.86)
Poncin et al., 2017	47	(9.5-16.1)	13.4	Exhalyzer	N_2	9.76 (2.26)
Poncin et al., 2017	47	(9.5-16.1)	13.4	Easy Pro	N_2	8.32 (1.64)
Poncin et al., 2017	34	21.0-32.9	24.9	Exhalyzer	N_2	13.73 (3.63)
Poncin et al., 2017	34	21.0-32.9	24.9	Easy Pro	N_2	10.01 (2.46)
(Aurora et al., 2005a)	30		4.3 (0.77)	MS	SF_6	9.61 (2.19)
(Horsley et al., 2008b)	22	17-47	28.9 (10.1)	Innocor	SF_6	12.8 (3.3)
(Horsley et al., 2008b)	18	7.8-16.7	12.5 (3.5)	Innocor	SF_6	7.3 (2.3)
Horsley et al., 2008)	33	17-49	30.0	Innocor	SF_6	13.1 (3.8)

This illustrated the mean (SD) of LCI value in CF patients of different age groups using different MBW devices and inert gases from previous studies in the literature.

3.2.5 Variability within LCI measures in healthy and diseased populations

LCI values may vary among healthy children and the literature has presented the values of LCI in both healthy and unhealthy populations using different MBW devices and gases. However, within normal individuals, a variability within the LCI values was seen when using the same device and gas. The possible reasons for this variability has not been studied previously. This may raise the question of whether there are any factors that may explain this LCI variance within the normal population.

The values of LCI and other MBW outcome measures for healthy individuals were altered when the MBW device or the inert gas used changed (Robinson et al., 2013a). Therefore, comparing the LCI and any other MBW outcome measures should be done within the same system and same tracer gas utilized.

The values (mean and standard deviation) of LCI for both healthy and diseased individuals were collected from previous studies (Aurora et al., 2005a; Aurora et al., 2004; Fuchs et al., 2011; Fuchs et al., 2009; Gustafsson et al., 2003; Horsley et al., 2008a; Hülskamp et al., 2009). Both healthy and unhealthy (CF, asthma, extremely preterm children) results are plotted in graph in Figure 12 below. The distribution of LCI values throughout the ages in healthy subjects showed that age had no effect on LCI values in healthy individuals. However, the distribution of LCI values for subjects with a known pathology showed a significant increase with age remarkably beyond the age of 15 (Table 7).

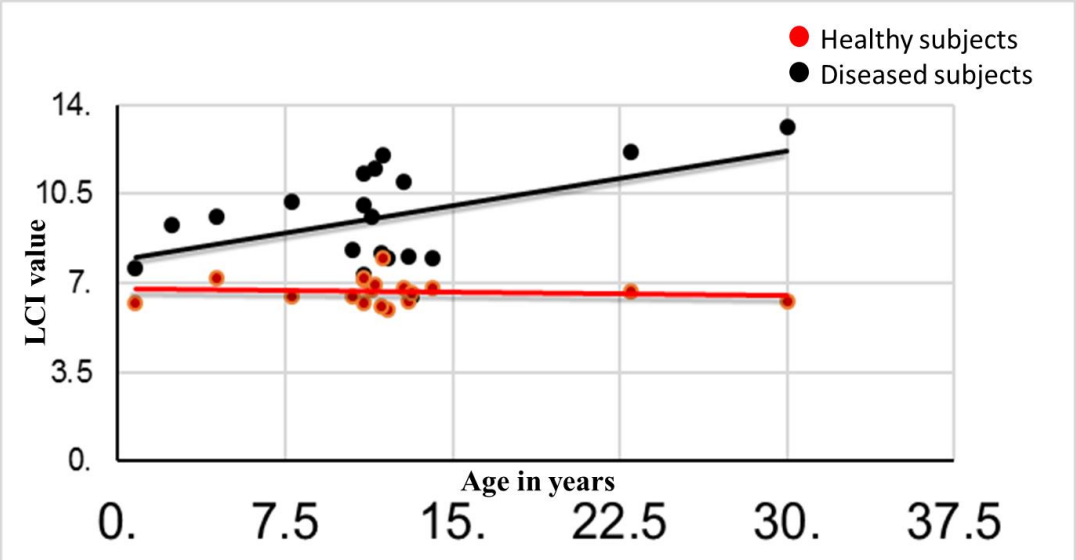


Figure 12 LCI values through age in healthy and diseased subjects

This illustrated the relation between LCI mean values and age in diseased and healthy subjects. The x-axis represents the age in years and the y-axis represents the LCI value. The black and red dots represent the values of LCI for diseased and healthy subjects, while the black and red lines illustrate the linear mean of LCI in diseased and healthy subjects, respectively. The graph shows the difference in LCI values throughout age in both diseased and healthy subjects. LCI appeared to obviously increase in diseased individuals, while healthy individuals appeared maintain LCI values within the same range (Aurora et al., 2004b), (Aurora et al., 2005a), (Poncin et al., 2017), (Poncin et al., 2017), (Horsley et al., 2008b), (Horsley et al., 2008).

We compared the means of LCI from previous studies in the literature to check whether there was a difference between the diseased and healthy groups. A t-test was carried out for the mean values of LCI in the healthy control data that was gathered from previous

studies. The paired sample test showed a p-value <0.001, which indicated a significant difference between the value of LCI means of healthy subjects and the diseased group. The graph in Figure 12 demonstrates that some LCI values of the groups with the disease were in the normal range of healthy values, which again raises the question regarding the presence of associated factors that may influence LCI values within the normal range.

The variation of LCI values for the diseased subjects was found to be greater than the variation of LCI values for the healthy individuals, as can be seen in the graph in Figure 12. This wide variation for LCI values in the diseased subjects may be explained by the differences in disease stage and severity within the group. Although the variation of LCI values in the healthy group was less compared to the diseased group, there was still some variation within it. The reasons for this variance among the LCI values of healthy subjects is not yet clear. It may be due to variable exposure to risk factors that may affect lung function. This is a question to be investigated.

Figure 13 and Figure 14 demonstrated below allowed more detailed comparison between variability of mean LCI values between healthy and diseased (CF) children and adults, respectively. Comparison between variability across different devices and gases can also be observed. For numerical values, reference can be made to the previous tables in this chapter (Table 6 and Table 7).

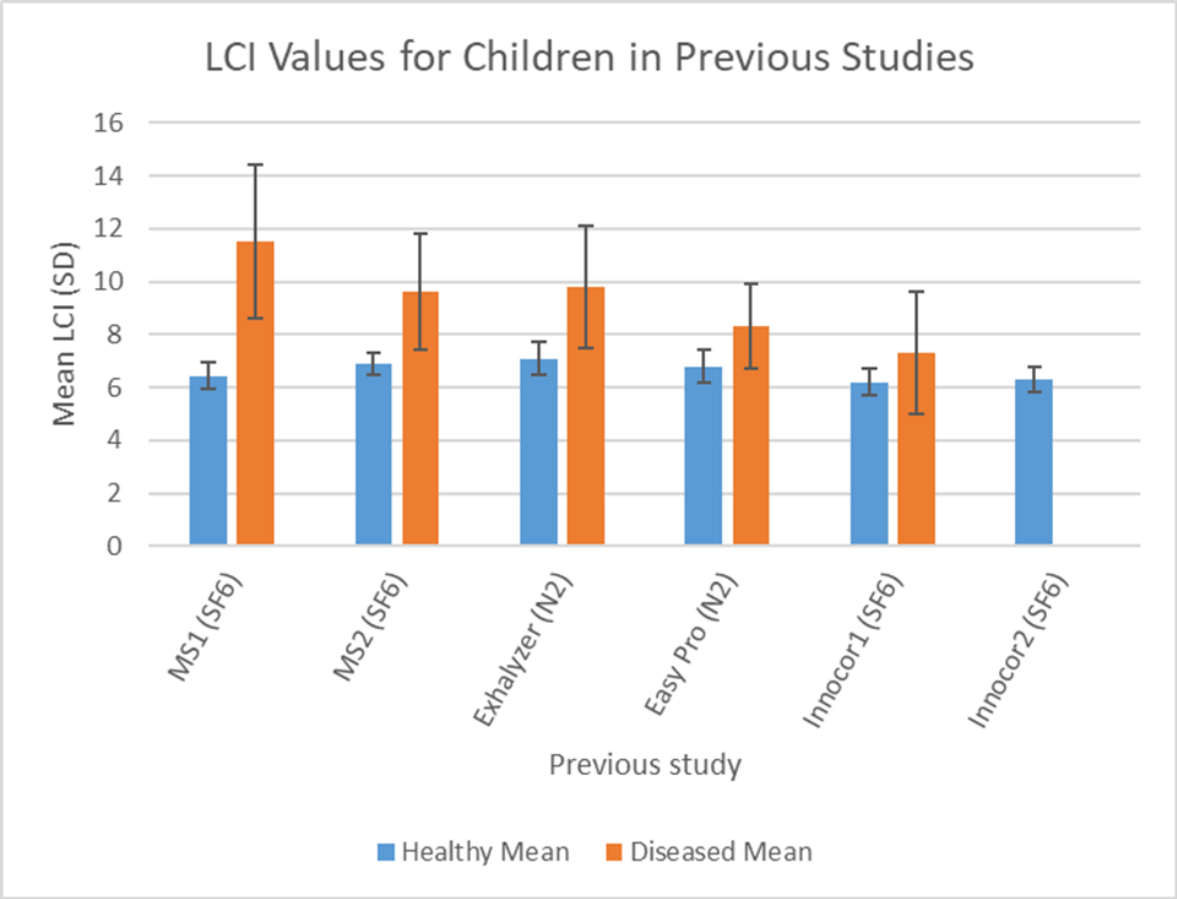


Figure 13 The mean LCI (SD) values for healthy and diseased children population in previous studies

This illustrated the mean LCI (SD) values for healthy and diseased children population in previous studies. MS1 SF_6 (Aurora et al., 2004b), MS2 SF_6 (Aurora et al., 2005a), Exhalyzer N_2 (Poncin et al., 2017) , Easy Pro N_2 (Poncin et al., 2017) , Innocor1 SF_6 (Horsley et al., 2008b), Innocor2 SF_6 (Horsley et al., 2008).

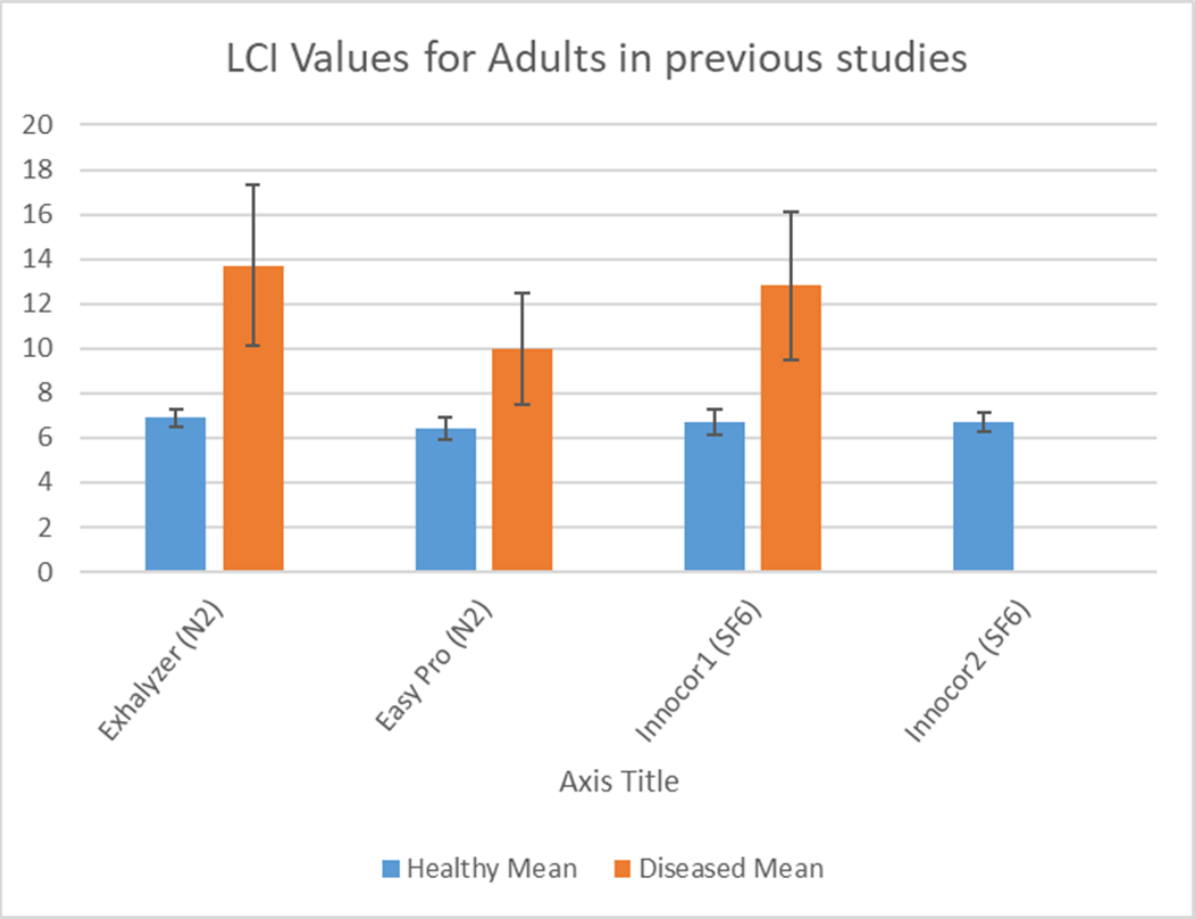


Figure 14 The mean LCI (SD) values for healthy and diseased adults population in previous studies.

This illustrated the mean LCI (SD) values for healthy and diseased adults population in previous studies. Exhalyzer N_2 (Poncin et al., 2017), Easy Pro N_2 (Poncin et al., 2017), Innocor1 SF_6 (Horsley et al., 2008b), Innocor2 SF_6 (Horsley et al., 2008).

3.2.6 Clinical usability and practicality of MBW

The literature has demonstrated the clinical usability and practicality of the MBW method, especially with the continuing evolution of the commercial devices available in the market today. Portability has facilitated research greatly and is obvious by the increasing research being conducted in further investigating this method and its clinical applicability.

MBW testing using SF_6 is found to be a sensitive measure of lung disease, but it has the disadvantage of being a time-consuming procedure (5-10 minutes per MBW run, for a total of 3 runs), when compared to other lung function tests such as spirometry, which takes 2-5 minutes to complete; this may be seen as a limitation to its use clinically; however, the MBW has the advantage of being significantly more sensitive in detecting lung abnormalities than spirometry, as was previously described in the chapter, which may make the longer MBW test duration acceptable in view of its greater sensitivity. In comparison to CT imaging on the other hand, MBW testing was found to take equal or perhaps less time to complete, as CT imaging takes on average a total of 30-60 minutes (Lex Jr, 2008).

MBW is repeated 3 times per session and the average reading is taken into consideration. This repetition contributes to the increased time of testing. To overcome this issue, a research paper was presented by Yammine et al. (2012), who studied the shortened MBW technique for 68 school-aged children; he conducted the MBW test in two ways: by performing two complete runs instead of three, and by applying the three runs but with less concentration of the gas ($1/20^{th}$ instead of $1/40^{th}$). The study found that MBW can be shortened and still obtain sensitive results by following the two mentioned methods (Yammine et al., 2012). A study done by Hannon et al. (2014) found that LCI $1/20^{th}$ is repeatable and sensitive with the same diagnostic performance of the LCI $1/40^{th}$ with the advantage of consuming less time (Hannon, Bradley et al. 2014). Yammine et al (2012) found that decreasing the cut-off concentration to $1/20^{th}$ did not compromise the diagnostic ability of the MBW test in patients with mild CF; further studies may be needed, however, to assess the reliability of the use of this as a cut-off point in calculating LCI for more severe lung disease (Yammine et al., 2012). Therefore, modifying the washout technique by decreasing the cut-off point of the washout phase, achieved by

reducing inert gas concentration to 1/20th, for healthy lungs and lungs with mild lung disease, as well as by performing two complete runs instead of three could be considered a convenient method to shorten the time of MBW testing sessions.

Providing a commercial type of MBW was necessary to increase the practicality of using this method as well as widening the scope of its use in clinical practice. The gold standard MBW method, which is the mass spectrometry, may limit the ability of MBW to be used as a screening tool for testing lung function due to its large equipment size, the fact that it is a hospital-based device, and the high cost of the machine. One such study demonstrated that the sidestream ultrasonic flow sensor (USFS) prototype system, also known as the Eco medics AG Exhalyzer D, MBW outcome measures and tidal tracer gas concentrations are comparable to those obtained using a mass spectrometer (Fuchs et al., 2008). This demonstrates the ability of commercial MBW devices, such as USFS and Innocor, to produce similar results to the hospital-based mass spectrometry machine. Commercial devices may be the key to further usage of MBW to detect early lung disease among children.

3.2.7 ARS/ETS recommendations

MBW has been available for many years, and as researchers investigate this method for potential use in clinical practice. Therefore, a need for a unified protocol to be followed emerged to allow for comparisons of results from one study to the other. As MBW depends on measuring the volumes and concentrations of gases during breathing, an advanced sensor system which can detect and measure the different gases that enter the respiratory system during the test was required. Further analytical processing of the

measurements obtained was also required to calculate measurements such as LCI. These procedures in analysing the data required organization and integration into one set of rules to unite the method of measurement and analysis of the data. As a result, in early 2013 the European Respiratory Society and the American Thoracic Society provided a document (ERS/ATS consensus statement (Robinson et al., 2013a), which included evidence-based recommendations related to the testing, measuring and analysing of the MBW process and the criteria that needed to be followed when conducting the testing procedure. Taking into consideration this statement and following its outlines would result in measurements that follow consistent and uniform principles and protocols that are more valid and easy to compare. The goal of this document was to give a clear description of the SBW and MBW methods both in a physiological and practical aspect, determine the tools required to perform the test as software and hardware, provide the quality control system and validation, outline the outcome measures that are intended, and demonstrate the calculation method that is followed to extract the outcome measures that indicate ventilation inhomogeneity. It also outlines the criteria for accepting the measured data for different systems (SBW and MBW) and different ages (children and adults) (Robinson et al., 2013a).

3.2.8 The current situation with MBW in the clinical setting

MBW testing in children has been shown to be feasible, reproducible, and accurate in making diagnoses. It was shown to be more accurate compared to the other available lung function tests in detecting small airway diseases like CF (Aurora et al., 2011). Aurora et al (2011) conducted a study to assess whether LCI using MBW can predict progression of lung disease in preschool children with CF. This study concluded that abnormal LCI was

found highly predictable for subsequent lung function deterioration in children with CF (positive predictive value of 94%), whereas most children with initially normal LCI results remained normal as time passed (negative predictive value of 64%). LCI was compared to FEV_1as a predictor of progression in the study; FEV_1 positive predictive value in the study group was calculated to be 100%, but negative predictive value was only 25% (Aurora et al., 2011).

MBW testing compared to the other available lung function tests may be the best method in detecting abnormality of the small airways in children, which is considered to represent the early stages of chronic lung disease. MBW has another advantage that makes it suitable for the children population, which is based on the fact that only tidal breathing and no forceful breathing manoeuvres or major cooperation from the child are required. This presents MBW testing as a potential screening tool for chronic lung disease in children.

The MBW method has not yet been used as a routine test in the clinical setting worldwide. Some health centres in European countries, however, have utilized the MBW technique as a follow up procedure performed annually in CF patients, in which an increase in 1 unit of LCI could not be considered negligible, as such an increase indicated disease progression (Singer et al., 2013). As a consequence, the treatment plan for such individuals was re-considered. However, MBW testing has not been applied in clinical practice in other parts of the world such as the United States and Canada (Subbarao et al., 2015).

The MBW method shows promising research results and based on what is published in the literature and the ongoing research being done on this method of lung function assessment, we may very likely see the approval for use of the MBW devices in clinical practice in the near future.

In conclusion, small airway obstruction may not be revealed by means of routine lung function tests that depend on measuring airway resistance, such as spirometry. Therefore, the use of a testing tool that is capable of detecting the obstruction in small airways is required, especially when the obstruction may be the onset of further serious illness, as is the case in CF, asthma and COPD. MBW testing has been confirmed to be a competent method for detecting small airway changes. The use of this method may be the future of early detection of lung diseases that start in the small airways. Consequently, obtaining normative data is essential for providing a reference for detecting lung function abnormalities.

4 Methodology

4.1 Research objectives

The main objectives of this research were:

- Identify normative values of LCI when using SF_6 based portable MBW machine
- Identify normative values of S_{cond} and S_{acin} when using SF_6 based portable MBW machine
- Model the relationship between relevant demographic and environmental factors and LCI, S_{cond}, S_{acin}, and FRC measurements
- Quantify reliability and clinical usability of LCI obtained from SF_6 based portable MBW machine.

4.2 Method

4.2.1 Ethical approval

In preparation for this study, ethical approval was obtained from the ethics committee of Keele University (Appendix 1). The information sheet for the parents (Appendix 2) and for the child (Appendix 3), the consent form for the parents (Appendix 4) and for the child (Appendix 5), the invitation letter (Appendix 6), and the questionnaire (Appendix 7) are presented in the respective appendices.

4.2.2 Study design

There are two approaches one can take to get normative values: longitudinal or cross sectional studies. A longitudinal study acquires repeated measures as a child grows. Such a design will give a clear indication of age-related normative values and data that explores change over time. However, such a design is time consuming. Furthermore, such studies have increased risk of data loss due to attrition. A cross section study design is conducted across a range of ages with a single measure taken on each child. This will give us valid data for the different age ranges for a selected sample without loss of data. However, the time course of change cannot be mapped as accurately as longitudinal studies. As this was a PhD programme study with limited time for recruitment, a cross sectional observational study with a single assessment was undertaken.

4.2.3 The demographic and environmental variables measured by this study

As discussed in section 2.4, it is known that various factors including environmental factors such as exposure to tobacco smoke, early life respiratory infection, premature delivery, and socioeconomic status can reduce lung function. In addition, we wanted to investigate the association between demographic variables such as age, gender and BMI and MBW outcome measures. Accordingly, we needed to have data on these factors for the included children. These factors were considered as the independent variables in this study.

Demographic and environmental data of the children was collected through a questionnaire (Appendix 7) sent out to the parents. In order to maximize the accuracy of the data, the questions were prepared in a simple and clear language.

The questionnaire provided the following:

- Weight = measured in kilogram in the school by an electronic scale
- Height = measured in meter in the school by a height measuring tool
- Date of birth = collected by the researcher on the testing day
- Gestational age / Full-term or premature (derived from gestational age) = collected in the questionnaire from the parents/guardian
- Health status or presence of any medical conditions = answered by the parents/guardian in the questionnaire
- Free school meals representing social deprivation = answered by the parents/guardian in the questionnaire
- Distance from the main road (derived from post code) representing pollution = post code collected in the questionnaire and distance calculated in kilometer using Google Maps
- Smoking or exposure to second hand smoking = answered by the parents/guardian in the questionnaire
- Duration of exposure to smoking = answered by the parents/guardian in the questionnaire
- Early childhood respiratory infection or hospitalization (until the age of 2) = answered by the parents/guardian in the questionnaire

- Level of activity = answered by the parents/guardian in the questionnaire

The invitation letter and accompanied documents were printed and sent to the parents/guardians, via the School, in a sealed envelope given to the child. Parents were asked to read the documents and return the documents signed if they were happy to consent for the child to take part in the experiment. The researcher picked up the signed documents from the School.

4.2.4 Sample size

In the absence of any data related to standard error of measurement a power calculation was not carried out. the sample size for this study was based on estimate that were informed by the following considerations

(a) In order to get descriptive statistics or parametric statistics there is a need for 10 to 20 subjects (Harris and Taylor, 2003).

(b) For modelling purposes and when looking for statistical relationships between age as a variable of demographic factors, a minimum of 10 subjects per independent variable is required (Field, 2009).

To identify a representative sample size in this study, we have 11 independent variables (age, gender, height, BMI, living distance from main road, poverty (represented by free

meal), smoking, exposure to passive smoking, activity level, hospitalization before age of two, and premature birth). For each variable, 10 subjects need to be recruited; this will lead to a required sample size of at least 110 subjects. In other words, in order to obtain meaningful data that could provide valid descriptive statistics such as mean and confidence interval, we determined the need to have a minimum of ten subjects to represent each age group (5-18 years). The minimal clinically important difference is unknown for this measure. Therefore, we decided to take a pragmatic sample size of 140 subjects. During estimation of sample size, some practical issues required consideration such as missing data, participant refusal to continue the study or losses to follow up. To consider these situations, we decided to add 10% to the total calculated sample size, which resulted in 140 + 14 (10 %) = 154 subjects in total.

4.2.5 Inclusion criteria

Children between the ages of 5 and 18 years old (≥5 and ≤18), capable of understanding and following the instructions. The university insurance permits studies on children 5 years of age or older only; access to preschool children within the organization is not allowed.

Consent to participate, given by both child and parents/guardian. Legally, even though children of 16 years of age or more are able to give consent for themselves without parents/gaurdian, parental/guardian consent was still required for such participants. It is important to note that although we got parental/guardian consent, the decision to take measurement was based on verbal consent from the participant on the day of measurement.

4.2.6 Exclusion criteria

- Children who were receiving antibiotics for a lower respiratory tract infection at the time of testing, as it was found to have a decrease in the value of LCI up to 1.27 unit (Sonneveld et al., 2015).
- Children who were diagnosed with asthma, viral-induced wheeze, protracted bacterial bronchitis, CF, or any other chronic respiratory condition.
- Children who had used anti-asthma medication within 3 months prior to the study.
- All of the above criteria may have affected their small airways and since the study aims to create a base for normative LCI data, therefore these were excluded to avoid abnormal results.

4.2.7 Invitation letter

A written invitation letter was prepared and sent by the researcher to 12 schools in the list (Appendix 8) in Stoke-on-Trent as required by the ethics committee (Appendix 1). The Schools listed in Appendix 8 were chosen pragmatically and was based on contacts that existed within ongoing research studies at the School of Health and Rehabilitation. The list included eight primaries, two secondary and two mixed schools. The invitation letter was directed to the head teacher of the school. The letter covered the aim and the purpose of the study and briefly described the intended technique to be used in the study. The letter was accompanied with the following documents: the information sheet for both the parents/guardians and the child (Appendix 2) and (Appendix 3), a consent form to be

signed in the case of agreement to participate in the study for both the parents/guardians and the child (Appendix 4) and (Appendix 5), and a questionnaire investigating the risk factors (Appendix 7). Once a School responded to the letter the researcher visited the Head Teacher (or the authorize representative) to get appropriate consent to contact the relevant pupils.

4.2.8 Contacting the schools

After obtaining permission from the schools, the researcher contacted the head-teacher and a meeting was arranged to provide further information and clarify any doubts regarding the study. During the meeting, several aspects concerning the study were agreed upon: the commencing date of data collection, the room where the testing was going to take place, identifying a named chaperone from within the school who would also help the researcher with data collection, and the arrangement for the participation of the students. By the end of the meeting, I made sure that all inquiries and doubts about the testing procedure and process were cleared for the head-teacher satisfaction.

4.2.9 Consent form

A written consent was prepared, one to be signed by the participant (Appendix 5) and another by the participants/guardian (Appendix 4) before measurements were taken.

4.2.10 Parents/guardians Information sheet

The parents/guardians information sheets (Appendix 2) contained brief information about the aim of the study, the target population, the testing procedure, and the rights of the participants during the study. Contact details of the researcher and research supervisors were included at the end of the information sheet for further information and details.

4.2.11 Child Information sheet

A two-sided leaflet (Appendix 3) was prepared in a simple way to explain the research the young children were participating in and the reason behind their participation. Both information sheets were adopted from ongoing research conducted in Manchester hospital for LCI research, with permission.

4.2.12 Extracting the study measures

For the Innocor outcome measures, there are two methods for calculating the indices of the MBW, the first being a built-in system within the device which is designed to calculate FRC and LCI by the end of each washout test. The second method of calculating the outcome measures of the MBW is an offline washout software that is a specialized custom software designed with algorithms that calculate the required indices. This offline washout analysis package was used, prepared in Igor Pro v6 (Wavemetrics Inc., Lake Oswego, OR, USA). This is based on the same washout analysis package already

deployed in several other clinical studies and clinical trials (Alton et al., 2015; Bell et al., 2018; Davies et al., 2013).

The built-in system of the device provides FRC and LCI, which are considered the most commonly used indices of ventilation inhomogeneity. The customized offline washout software provides further and more precise measurements of washout such as tidal volume, flow gas delay, wash-in and washout start; it also extracts more informative indices for washout, such as S_{cond} and S_{acin}, and amends the faulty parts of the process, such as SnIII slope and leaks.

The outcome measures were extracted and analysed by two persons, myself and an expert in Innocor MBW, independently. Both researchers followed the recommendation of the ERS/ATS consensus statement criteria for measurement acceptability. The measurements which did not comply with the published quality criteria were excluded from the study (Robinson et al., 2013b).

Extracting the raw data from the Innocor device using the special custom built software Igor pro[1] (version 6.37) that is designed to extract outcome measures was represented as an expirogram that showed the flow, O_2, CO_2 and SF_6 concentrations during testing time (Figure 15). The customised offline software program enabled extracting further outcome measures other than LCI and FRC. This software runs on Windows[2]. The LCI file that

[1] Manchester MBW analysis_091114_V4-
[2] Windows 10 education©2018Microsoft corporation

contained the washout raw data was stored in a format that required a special program to convert it into a readable file using Innofileconverter[3].

The main windows included:

- Control panel: the main way of entering the data into MMBWA.
- Main washout window: displayed the washouts tracing the flow, O_2, CO_2 and the SF_6 graphs.
- Flow gas delay: displayed the flow gas delay for the first ten breaths of the washout.
- Shortcut bar: enabled importing the raw data, delete it, set washing and washout start, and save the wash out file.
- Folder table: showed test results contained within the selected folder.
- Expirogram: displayed the main washout graph and enabled the adjustment of phase lll slope Figure 15
- Phase lll slope analysis.

These windows permit the researcher to analyse and adjust the washout of each testing trial individually to determine the LCI and FRC values. The entire extracted outcomes of each test displayed in an Excel file included the values of the outcome measures for each test and for each subject.

[3] Innofileconverterv2 1.2.1.1

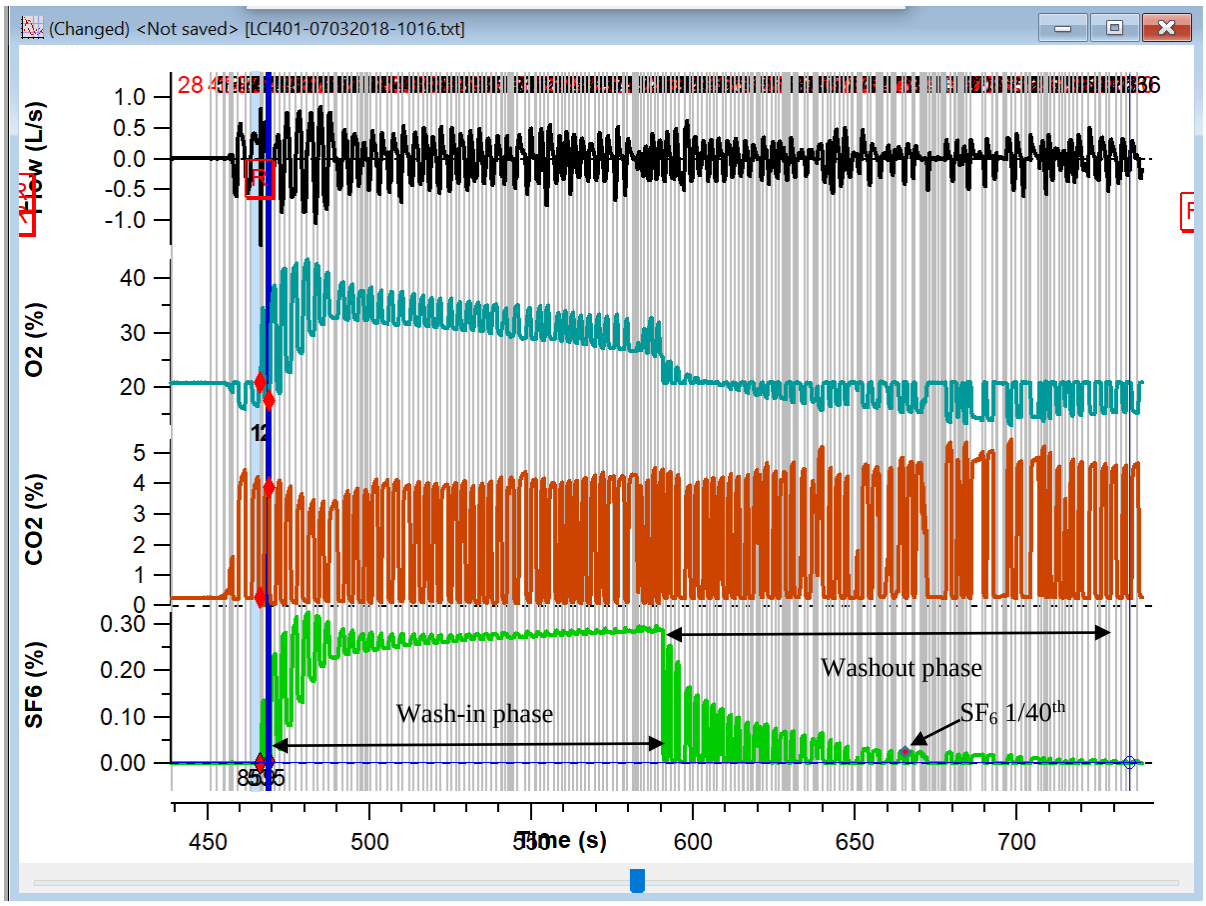


Figure 15 Expirogram of MBW (my data)

This illustrated MBW wash-in and washout measures in volumes vs time in seconds: illustrate flow of gas, O_2%, CO_2 % and SF_6 concentration throughout complete wash-in and washout process

Each expirogram was checked manually by inspecting wash-in and wash-out phases to assure the quality of the test and comply with the recommendation of consensus statement (Robinson et al., 2013a). The testing process had to meet the following criteria to be accepted:

- The participant had to be seated in an upright position with the head in the midline.

- The nose clip had to be tightened and the mouth piece sealed in order to prevent escape of the air in or out of the interface.

- The participant had to complete three acceptable MBW runs, where acceptability is determined by checking the following:

- Wash-in phase should have stable tidal volume and equal inert gas concentration within the breath cycle.
- The washout phase must have a regular breathing pattern and avoid large breaths and coughs.
- Termination of the test is accepted when at least three consecutive breaths were taken beyond reaching $1/40^{th}$ of inert gas concentration at the end of washout, and six turnovers must be completed if intended to calculate SnIII.
- Leaking should not occur; leakage is indicated by a sudden drop in the inert gas concentration during wash-in.
- The participant was allowed an adequate time break between the runs of the test to assure that inert gas concentration has returned to the original value.
- The test underwent further investigation when FRC difference within the three runs exceeded 10%, and was rejected if >25%.

The researcher examined the expirogram for each phase of the testing to assure the quality of testing and remove the outliers which did not follow the criteria. The graph of the phase III slope was checked for each breath to get more accurate S_{cond} and S_{acin} values. Flow gas delay was checked for each test to be identified and corrected when required based on ERS/ATS consensus statement (Robinson et al., 2013a).

4.2.13 Training to undertake the measurements

Using MBW to measure lung function for the children required a training period. The training aimed to qualify the researcher with the testing procedure in order to achieve a total understanding of the measurement process, get the researcher familiar with the testing equipment, and allow the researcher to manage the entire testing procedure independently.

The training plan included two parts, observational and practical. I started attending the cystic fibrosis pediatric clinic in Royal Stoke University Hospital on a weekly basis where MBW was conducted for CF patients in order to get familiar with the technique. I have attended approximately 100 sessions through the observation period for recurrent CF patients. The MBW testing was conducted by a research nurse and eventually a physician who were trained by an expert in MBW. During the observation period, I was not allowed to conduct the test due to governance restrictions. The practical part of the training was conducted by measuring the LCI for colleagues under supervision of the research nurse in the hospital in four separate sessions.

The training for measuring LCI was completed before commencing the data collection and the equipment was available for the study by providing a loan letter from the hospital confirming that this equipment is available for proceeding with the research (Appendix 9).

During collection of the data, I was independently performing the testing process; when there was a problem with the technique or the device, I obtained the advice of the expert to overcome the problem. After completing the data collection, I underwent three training sessions to acquire the process of extracting and analyzing the data. The training sessions were conducted by an expert in Innocor MBW in Manchester Hospital.

4.2.14 Preparation of the Innocor kit

On the day of the testing, the kit should switch on and left to warm up for at least 20 minutes before the arrival of the first participant. All of the required testing parts were prepared in a separate package for each participant for easiness and time efficiency. Each package included a clean washed mouth-piece, a new nose clip, a clean washed air bag, and a new filter.

The connections of the machine parts were checked to be in the right places to ensure no chance of leaks. Lines of the machine were checked throughout their length to ensure they were not tangled or twisted. The gas cylinder was checked to be fixed tightly before commencing the test.

Next, calibration was carried out before testing started to reinforce the test quality and was repeated every four hours during the testing process. In case of replacing or cleaning the flowmeter, calibration had to be repeated to avoid change of pressure interpretation. The calibration procedure started with recording ambient temperature, air pressure and humidity.

Afterwards, oxygen was adjusted and the flowmeter was calibrated using a 3-litre syringe at room temperature and a bacterial filter. The calibration process of the flowmeter was achieved by smoothly filling and emptying the syringe in three different speeds to mimic different respiratory rates. The results were displayed in a table showing the recent gain of the air to be compared to the previous gain. A correct linearization occurs when the difference between the old and new is ±2%.

The last step was the flow-gas delay calibration which required connecting a filter to the RVU. Intense forced inhalation was performed after total air breathed was expired slowly. This manoeuvre aims to create a sharp transition in flow and therefore drop in C O_2 concentration. CO_2 analysis is used to align gas flow and signal, which assists in analysis of raw data. This manoeuver is repeated eleven times. At the end of this process the average is displayed and compared with the previous one. If the difference exceeded 10 ms, then the process must be repeated.

4.2.15 Preparation of the environment

The researcher set-up the weighing scales and the height measure ready for use. A comfortable chair was used to assure the comfort of the participant during the testing procedure. The breathing kit was held by an adjustable stand to modify the different heights of the participants. The use of a tablet device (or a screen) to show an animation

film was aimed to help distract the participant and maintain the tidal breathing mode during the testing process. A small fan was placed in front of the participant to increase the air flow during the wash-out phase and prevent rebreathing of SF_6. A glass of water and tissues were available to be provided to the participants when needed.

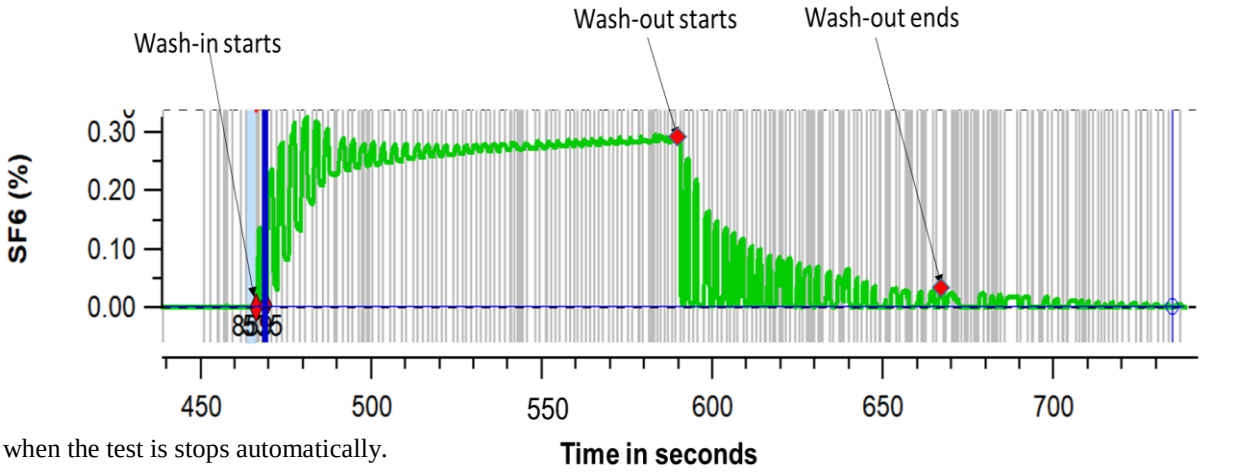
4.2.16 Preparation of the participant

The tests were held in the school with the company of a school teacher/assistant who helped in calling and organizing the attendance of the participants. On the arrival of the participant, I introduced myself and made sure that the participant was familiar with the study and testing procedure. The participant was informed about the testing procedure and what was expected from him/her. Height and weight were measured and recorded in a new file in the Innocor. The participant was instructed about how to perform the measurement including using the mouth piece and the placement of a nose clip. After the participant was seated and it was assured that he/she had no further inquiries, the participant was ready to start the test and the mouth piece and nose clip were adjusted onto the patient. The participant was asked to get familiar with the position and be sure that he was comfortable before starting the test.

4.2.17 Performing the MBW test

The testing procedure was divided into two phases, wash-in and washout. Wash-in was performed from a sealed bag filled with a mixture of room air and test gas (94% O_2, 1% SF_6 and 5% N_2O) up to a volume of 3L. As the wash-in commenced, the participant was asked to take 5 deep breaths at the beginning to speed up the wash-in and then continued

with normal tidal breathing until the inspired and expired concentration of the SF_6 became equal and the concentration reached 4%. Throughout the wash-in phase, I monitored the O_2 and $FiCO_2$ to keep them >18% and <3%, respectively. Simultaneously, I kept a close observation on the participant and the monitor to check for any leaks during the test; leaks can be indicated by sudden step changes in volume. After the wash-in phase was completed, I switched to the washout phase of the gas where supply of the gas is disconnected. The participant was reminded to keep a tight seal around the mouth piece and to breathe normally. A fan was used during the washout phase to prevent rebreathing the expired SF_6 as the room air was used to washout the inert gas. The washout continued until the SF_6 concentration returned to $1/40^{th}$ of the starting concentration, and the procedure was completed. The washout was stopped automatically by the machine when the SF_6 returned to its starting concentration. The measuring of the breathing gases continued for 5 breaths after the washout stopped. This was done to guarantee that the lung was cleared of the inspired SF_6 gas (Figure 16).



when the test is stops automatically.

While the air bag for the following test was being prepared, I made sure that the participant was comfortable and satisfied with the testing procedure and willing to proceed to the next trial. As soon as the bag was prepared, the testing started. The same steps were repeated for the second and third trials. Once the three trials were completed

and no further test was required, the participant was informed that this was the end of the test. After each participant, and before starting the following test, the testing kit was cleaned with antiseptic wipes to control infection, and all the used equipment (mouth piece, filter, nose clip and air bag) were removed and replaced with clean and washed ones.

The test was repeated three times unless one of the trials were unsuccessfully completed because of leak or device fault. In such cases, a fourth trial was performed with the approval of the participant. Each trial consumed between 5-10 minutes.

4.2.18 Method of statistical analysis

Raw data for each participant was presented as three readings of each variable. The intended outcome measures were divided into primary outcomes, which were LCI and FRC, and secondary outcomes, which were S_{cond} and S_{acin}. In order to extract data there was a need for three (minimum of two) valid readings of each outcome measures. The three (or two) valid outcome measures were entered in SPSS[4] in addition to the data extracted from the questionnaire. A simple descriptive data analysis was required to calculate the mean and standard deviation for the three readings to be used in modelling analysis. We also calculated the coefficient of variation (CoV), which is an indication of dispersion around the mean. It is also used to measure variability of measurements (Harrell Jr. et al., 1984).

[4] IBM SPSS statistics 24

Prior to statistical analysis, the data went through pre-processing procedure.

1- Removal of the outlier values by checking the entire readings in order to eliminate the readings that did not correspond to the recommended criteria of the ERS/ATS consensus statement (Robinson et al., 2013a). Outliers were identified by using box plot for each outcome measure and subsequently removed from the data.

2- Checking of serial dependency for the remaining of the data by plotting a graph of each outcome measure values to illustrate the values of first, second and third measurement. If the readings were independent and there was no serial dependency, then we used the mean of the three readings (or the mean of two) to represent dataset as for guidance given by the European guidance (Field, 2009; Harrell Jr. et al., 1984).

3- Testing for the normality was done using Shapiro-Wilk test, which is a simple descriptive data analysis test used to check normality and is used on data obtained from a small sample size. The normality test allowed identifying whether the variables are normally distributed in our sample and therefore helped in deciding to use parametric (when the data is normally distributed) or non-parametric tests (the data is not normally distributed) for statistical/correlation analysis (Harris, 2003).

4- Reliability of the outcome measures: as extracting the data based on applying certain rules and following the recommendation of the ERS/ATS consensus statement, then a simple mistake in extracting the data may negatively influence the results and consequently the entire project. Therefore, making sure that the

process of data extraction is correct and the results were precise, we decided to reassure the process by checking the reliability of the outcome measure data by comparing the analysis of the data that was extracted by the researcher to the analysis of the same data to be extracted and analysed by an expert in the MBW testing. To do this comparison the Bland and Altman test was used to check the difference and agreement between the two data results introduced by a plot graph. In this graph a display of the mean and SD in the scatter plot graph of the difference between the two values (Giavarina, 2015). Bland and Altman test helped to check the reliability of the data and freely interchange between them. The Bland and Altman graph illustrated the X-axis to represent the average of the two result measurements and the Y-axis represented the difference between the two results. The Bland and Altman plot analysis is a simple way to evaluate a bias between the mean differences, within which 95% of the differences of the second method, compared to the first one fall (Bland and Altman, 1999).

5- SPSS was used to enter the data; each participant was represented in a single file that included the entire outcome measures of the MBW values and the outcome measures of the questionnaire.
6- Assess the influence of demographic variables and environmental factors on the outcome measures

The environmental factors were identified as the following: exposure to tobacco smoke, early life respiratory infection, premature delivery, socioeconomic status and living distance from main road, while demographic variables identified as age, gender and BMI.

To carry out the statistical analysis to investigate the relationship between MBW outcome measures and demographic and environmental factors, I used two data sets:

1- Dataset 1: the Keele dataset which I have collected in the schools in Stoke-on-Trent for children aged 5-18-years old and included the measurement of MBW and the questionnaire (Appendix 7). This dataset was used to look at the effect of demographic and environmental factors on MBW outcome measures.

   Dataset 1 was used to answer the following questions:

   - Whether there was any significant difference between the means of the variables based on demographic and environmental factors. Testing the difference between outcome measures based on demographical and environmental factors was investigated using ANOVA and t-test for means.

   - Whether demographic and environmental factors had a significant effect on the variables in the study population. Modelling this relationship was achieved using linear regression.

2- Dataset 2: a combination of Keele dataset + the secondary anonymous dataset that have been collected in Manchester at the same period (as a part of LCI research conducted in Manchester) which goes beyond the age range that we were looking at. Since reporting age related norms and modelling age relationship with MBW outcome measures requires a larger age range, I am using the merged dataset to set a reference for normal LCI values for the intended age group and explore the effect of age on the MBW outcome measures.

   Dataset 2 was used to answer the following questions:

- What are the normative data of MBW outcome measures (LCI, S_{cond} and S_{acin}) throughout the age range.

- Whether there was any significant difference between the means of MBW outcome measures based on age-groups by using ANOVA.

- Whether there was correlation between the age factor and the MBW outcome measures (LCI, S_{cond} and S_{acin}) using Pearson's correlation.

- Modelling the relationship between age and MBW outcome measures and whether LCI is varied with age using linear regression.

4.3 Time scale of the PhD

- The PhD commenced 26th January 2015.
- The progression report submitted in January 2017.
- The observational visits to the Royal Stoke University Hospital started in September 2015 and ended in December 2017; the visits occurred on a weekly basis and depending on the attendance of the cystic fibrosis patients in the outpatient clinic.
- The training sessions on how to use the Innocor device commenced with the expert during the observation period; these sessions were arranged according to the expert's schedule.
- The ethics form was submitted to the University ethics committee in August 2017 and the approval was obtained in January 2018.
- Contacting the schools started in August 2017 and arrangements with the schools were made after the ethical approval was obtained.
- All the children were recruited and assessed between March and August 2018.

- The training sessions on how to extract and analyze the raw data from the Innocor device were arranged with the expert according to his schedule following the end of recruitment.
- The data extraction and analysis commenced in August 2018.
- The submission of the thesis was on 25th January 2019.

4.4 Thesis work

Done by myself

- Conducting a thorough literature review on my research topic
- Filling the ethics form, which was revised by my supervisor, until ethical approval was gained
- Ordering the equipment that was required for the testing procedure [mouth pieces, filters, CO_2 scrubbers, 3-litre airbags, nose clips, SF_6 gas cylinders, disinfectant tablets (for washing the reusable equipment)]
- Writing the invitation emails and communicating with the schools
- Meeting with the head teachers and agreeing on the starting date and the requirements for the testing procedure
- Preparing the required testing equipment for each participant
- Calibrating the MBW machine prior to testing [recalibration was required after 4 hours of testing]
- Conducting the entire testing procedure and collecting the data for all 64 subjects
- Extracting the data from the kit to the offline program
- Entering the data into an excel sheet
- Statistically analysing the data from the excel sheet using SPSS

- Writing up my thesis paper, under the guidance of my advisors

Done with the help of others

- Order the equipment travel case and a new RVU unit [with help of Dr Gilchrist]
- Apply for the loan of the MBW equipment from the hospital by Dr Gilchrist
- Training for using the MBW machine with help of research nurse in the UHNM hospital and Dr Alex Horsley
- Preparation of the testing environment with the help of the teacher assistant
- Organize the participants time schedule for testing
- Training on extracting and analysing the data with Dr Horsley

5 Results

5.1 Dataset 1

5.1.1 The demographics of the Keele dataset population

Sixty four (64) children were recruited in this study with no known respiratory disease or infection at the time of testing, The data from two subjects were not used due to equipment technical factors (n=1) or clinical reasons (n=1) and have not been analysed further. LCI was successfully measured in 62 participants (93%). The participants aged between 9-15 years-old, there were 27 males and 37 females. The mean age was 11.8 (standard deviation (SD)=1.99) years. The weight ranged between 24.2-80.0 kg. The mean weight was 46.11 (SD=13.45) Kg. The height range was 123.5-182.8 cm, and the mean height was 153.78 (SD=14.16) cm. The body mass index (BMI) range was 13.69-33.2 Kg.m^2, the mean BMI was 19.14 (SD= 3.68) Kg.m^2. (Table 8)

Table 8 Summary of the demographics of the study population of dataset 1

Age at testing: mean (SD)	11.7 (1.99)
Age range	(9-15)
Gender (M/F)	35/27
Height: mean (SD)	153.7(13.4)
Weight: mean (SD)	46.1 (14.1)
BMI: mean (SD)	19.14 (3.7)

5.1.2 Preprocessing data

5.1.2.1 Removing the outlier values

All readings collected throughout the study were checked and the readings that did not correspond to the ERS/ATS consensus statement criteria (Robinson et al., 2013a) were

removed from the data. After removing the data that did not fit the criteria, we ended up having 23 out of 62 participants with two MBW test readings, instead of three. Of note, this had no effect on our results, as two valid test readings for each participant is considered reliable in assessment of MBW outcome measures in children (Robinson et al., 2013a; Yammine et al., 2012). It is important to clarify here that the preprocessing of the data and removing of outliers was applied by myself to Dataset 1 but not to Dataset 2, as the data added from Manchester was used after being processed and checked by the research team in Manchester.

5.1.2.2 Checking serial dependency

The data was checked to identify if the three repeated measures had any pattern of serial dependency. This was carried out graphically and is shown in Figure 17. There was no evidence of any serial dependency in the data indicating that the readings are likely to be independent of each other. This indicates that MBW testing provides an accurate measurement of the actual gas exchange occurring in the lungs of each participant across all three serial measurements.

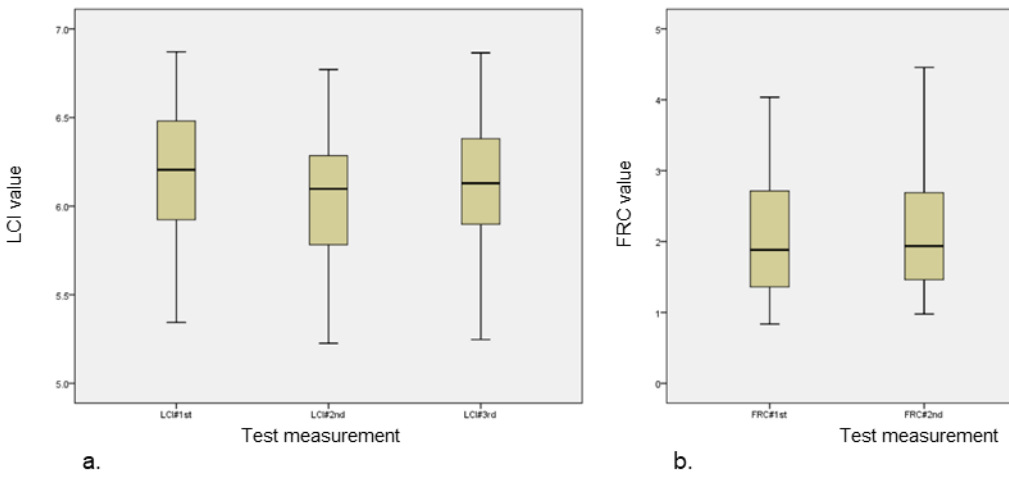


Figure 17 Serial dependency of MBW outcome measures

This illustrated the serial dependency for the MBW outcome measures graphically: the graphs in this figure illustrate the values of each outcome measure (Figure 1.a. LCI, 1.b. FC, 1.c. S_{cond}, and 1.d. S_{acin}) for the three measurement tests and if there was dependency between them. The x-axis identifies the first, second and third measurement tests, and the y-axis shows the values of the outcome measure represented by each graph. The figure shows that the three repeated measures were independent of each other, indicating that there was no pattern of serial dependency.

5.1.2.3 Testing for normality of the variables

All the variables in our study have been subjected to testing of normality to check if they were normally distributed. This was done using Shapiro Walk test. The evidence suggested that data for the variables LCI, S_{acin}, and S_{cond} (p=0.468, p= 0.205, p=0.231) were normally distributed The results for FRC data, however, was not normally distributed (p=0.002). Further exploration of the non-normally distributed variable FRC suggested that the presence of an outlier was responsible for the skew and this was removed to increase homogeneity in the data. This made the FRC variable normally distributed (p=0.200). Normality of all the variables guided our choice of using parametric statistical tests in the SPSS analysis of our results, presenting them as mean and standard deviation.

5.1.2.4 The reliability testing using Bland Altman plot for the difference in the MBW outcome measures

The Bland and Altman plots in Figure 18 demonstrated the reliability of the extracted LCI, FRC, S_{cond}, and S_{acin} values by measuring agreement between the values extracted by myself and another assessor. The LCI mean error (SD) between the two assessors was 0.04 (0.24) and the mean absolute error (SD) was 8.77 (0.15); the variability for the LCI

measurements (the limits of agreement) were between -0.52 and 0.44 (Figure 18a). The FRC mean error (SD) between the two assessors was 0.03 (0.14) and the mean absolute error (SD) was 1.52 (0.03); the limits of agreement were between -0.25 and 0.3 (Figure 18b). The S_{cond} mean error (SD) was 0.0005 (0.010) and the mean absolute error (SD) was 0.01 (0.0001); the limits of agreement were between 0.02- (-0.02) (Figure 18 c). The S_{acin} mean error (SD) was 0.006 (0.05) and the mean absolute error (SD) was 0.36 (0.006); the limits of agreement were between -0.097 and 0.09 (Figure 18 d).

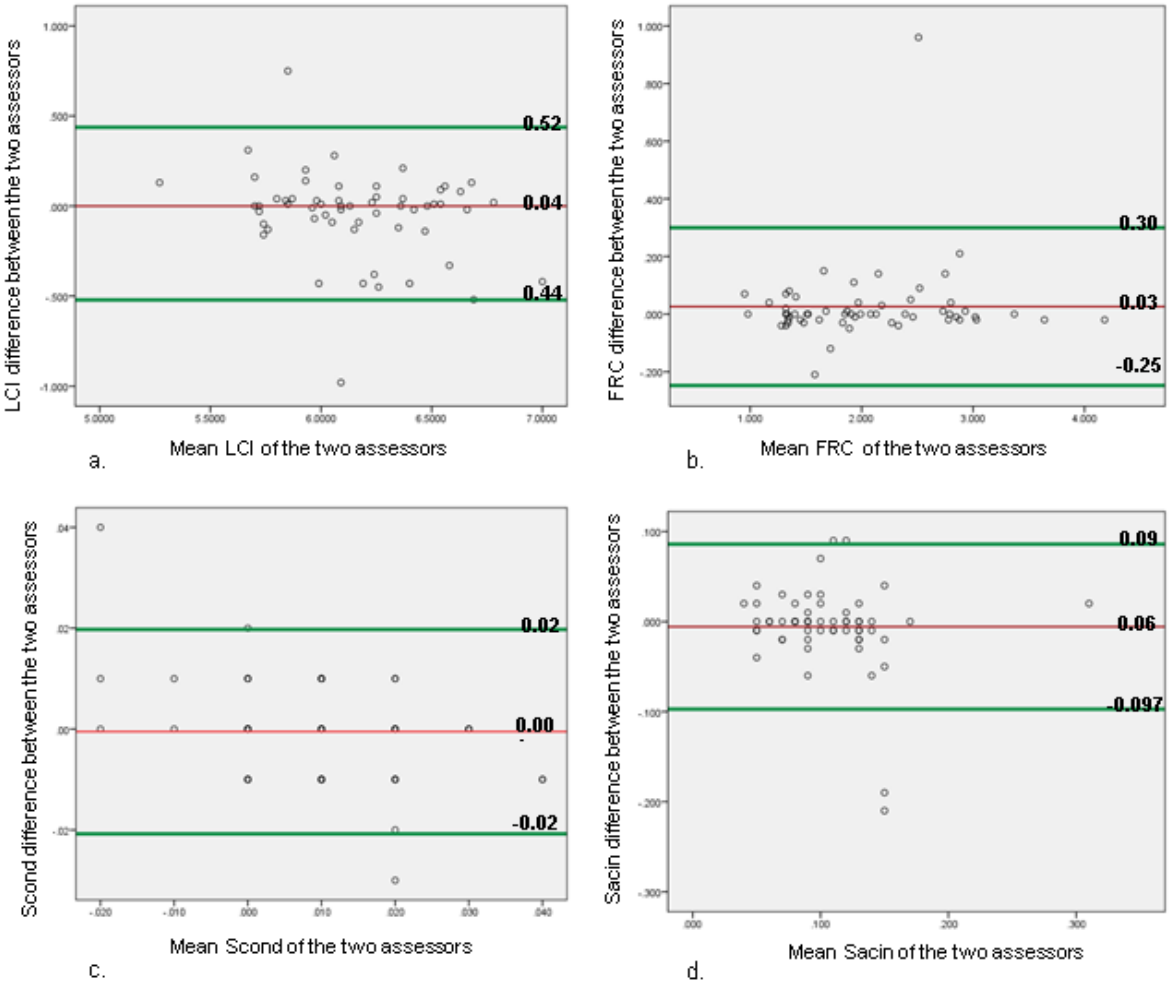


Figure 18 Bland and Altman plots

This Bland Altman plots representing the agreement of the extracted data between the two assessors (Figure 2.a. LCI, 2.b. FRC, 2.c. S_{cond}, and 2.d. S_{acin}): the x-axis illustrated the average of the measurements extracted by the two assessors for each participant, and the y-axis illustrates the difference between the two measurements the two assessors. The mean difference of the differences between the two extracted measurements for each participant was denoted by the red line. The two green lines demonstrated the limits of agreement for the measurements extracted by the two assessors, and they were calculated as mean ±1.96*SD. 2.a. demonstrated the LCI mean difference = (-0.04) and the limits of agreement = (-0.52- 0.44). 2.b demonstrated the FRC mean difference = (0.03) and the limits of agreement = (0.30 - -0.25). 2.c. demonstrated the S_{cond} mean difference = (0.001) and limits of agreement = (0.02- -0.02). 2.d. demonstrated the S_{acin} mean difference = (0.06) and the limits of agreement = (0.085 - -0.097).

5.1.3 The descriptive analysis of the MBW outcome measures

The mean (SD) value for the LCI for our study population was 6.18 (0.34), the minimum value was 5.27 and the maximum was 7.00. The mean (SD) for the FRC was 2.04 (0.72), while the minimum value was 0.95 and the maximum was 4.18. S_{cond} mean (SD) was 0.10 (0.03), the minimum was 0.03 and the maximum was 0.17. S_{acin} mean (SD) was 0.01 (0.02), the minimum was -0.02 and the maximum was 0.09 (Table 9).

Table 9 Summary of the normal values of the MBW outcome measures for dataset

Dataset 1	Mean (SD)	Minimum	Maximum
LCI	6.18 (0.34)	5.27	7.00
FRC	2.04 (0.72)	0.95	4.18
S_{cond}	0.01 (0.02)	-0.02	0.09
S_{acin}	0.10 (0.03)	0.03	0.17

This demonstrated the normal values of the MBW outcome measures for dataset 1 by showing the mean (SD), minimum, and maximum values for LCI, FRC, S_{cond} and S_{acin} respectively

5.1.4 The influence of demographic variables and environmental factors on the MBW outcome measures (LCI, FRC, S_{cond}, and S_{acin})

The environmental factors were identified as follows: exposure to tobacco smoke, early life respiratory infection, premature delivery, socioeconomic status, activity level and living distance from main road (Chapter 2; section 2.4). The demographic variables were identified as age, gender and BMI. Results will be presented as Dataset 1 (Keele dataset) and Dataset 2 (combined Keele and Manchester dataset), which were described in the methodology.

5.1.4.1 Factors excluded from the data analysis

Some factors we assessed in the questionnaire were excluded from the data modelling due to a scarcity of the data relevant to these factors in our study population. None of our participants were exposed to first-hand smoking, only one participant was exposed to second-hand smoking, only one participant was prematurely born (<34 weeks term), and only two participants had positive history of hospitalization for early life respiratory infection. Therefore, statistical analysis could not be performed for assessment of the effect of these environmental factors (exposure to tobacco smoke, early life respiratory infection, and premature delivery) on our MBW outcome measures. Statistical analysis was performed on the remaining environmental and demographic factors: socioeconomic status (free school meals), activity level and pollution (living distance from main road), age, gender, and BMI.

5.1.4.2 Difference between LCI means based on demographic and environmental factors

5.1.4.2.1 Free school meals

The demographical data for the participants who were taking free school meals were (n = 6), three males and three females. Their mean age (SD) was 12 years-old (2.3). Their mean weight (SD) was 51.9 (23.1), mean height (SD) was 157.06 (15.6) and mean BMI (SD) was 21.2 (6.1). The participants who did not have free school meals were (56), 32 males and 24 females. Their mean age (SD) was 10.5 years-old (2.5). Their mean weight

(SD) was 38.4 (10.9), mean height (SD) was 142.01 (12.2) and mean BMI (SD) was 18.7 (3.4).

An independent sample T-test was performed to find that there was no significant difference (p-value=0.14) between LCI means based on free school meals (Table 10).

Table 10 Difference between LCI means based on free school meals

	Free school meals						
	Mean (SD)				Mean difference	Confidence interval	
Variable	Yes (N=6)	No (N=56)	t-value	p-value	(SE)	Lower	Upper
LCI	6.38(0.23)	6.16(0.34)	1.50	0.14	0.02(0.15)	-0.07	0.51

5.1.4.2.2 Gender

The demographical data for the participants were 35 males and 27 females. The mean age for male participants (SD) was 13.3 (1.6) years and 10.59 (1.3) years for the females. The mean weight (SD) for males and females was 54.2 (12.2) kilograms and 39.9 (11.9) kilograms, respectively. The mean height (SD) was 164.7 (10.8) centimeters for males and 145.3 (10.8) centimeters for female subjects. The BMI mean (SD) was 19.8 (3.5) kg/m2 and 18.6 (4.1) kg/m2 for males and females, respectively.

An independent sample T-test was performed and showed there was no significant difference (p-value=0.76) between LCI means based on gender (Table 11).

Table 11 Difference between LCI means based on gender.

	Gender						
	Mean (SD)				Mean difference	Confidence interval	
Variable	Male (N=35)	Female (N=27)	t-value	p-value	(SE)	Lower	Upper
LCI	(6.17)(0.34)	(6.20)(0.35)	0.30	0.76	0.08(0.07)	0.20	0.09

5.1.4.2.3 Activity level

Activity level was categorized into five levels (0-4) as seen in question seven of the questionnaire (Appendix 7):

- 0 for None (no physical activity or exercise)
- 1 for Little bit
- 2 for Medium
- 3 for Often
- 4 for Very often.

The demographical data for the participants based on activity level groups are shown in the Table 12. Of note, there were no participants in the 0 level group.

Table 12 Summary of demographic data based on activity level groups.

Activity level	Number of participants	Mean age (SD)	Male	Female	Mean weight (SD)	Mean height (SD)	Mean BMI (SD)
1	4	12.25 (2.06)	4	0	58.2 (10.4)	158.5 (10.3)	23.2 (4.1)
2	32	10.75 (1.25)	17	15	40.37 (12.6)	146.65 (10.23)	18.4 (3.7)
3	14	10.0 (0.8)	9	5	38.1 (65)	141.6 (5.1)	19.03 (3.2)
4	12	10.75 (2.9)	5	7	37.65(13.05)	142.25 (13.2)	18.1 (3.2)

The results of the ANOVA show that there was no significant difference in LCI values based on activity level groups. (F=2.70, p-value=0.054) (Table 13).

Table 13 LCI difference based on activity level

LCI Vs Activity	Very often (SE)	Often (SE)	Medium (SE)
Little bit	0.12 (0.19)	0.32 (0.19)	0.36 (0.18)
Medium	-0.25(0.11)	-0.04 (0.10)	
Often	-0.21(0.13)		

This represented the differences between the means of the LCI values based on activity level of the participant, e.g. the difference between the mean of the LCI value for the participants who exercised very often and those who exercised little bit = 0.12 (0.19). A significant difference (p-value <0.05) in LCI values between any of the two activity level groups is indicated by a (*). N.B. We did not compare the group with itself as it is not feasible.

5.1.4.3 Differences between FRC values based on demographic and environmental factors

5.1.4.3.1 Free school meals

An independent sample t-test was performed to find that there was no significant difference (p-value=0.84) between FRC means based on free school meals (Table 14).

Table 14 Difference between FRC means based on free school meals.

	Free school meals						
	Mean (SD)				Mean difference	Confidence interval	
Variable	Yes (N=6)	No (N=56)	t-value	p-value	(SE)	Lower	Upper
FRC	2.09(0.63)	2.02(0.73)	0.20	0.84	0.07(0.31)	-0.56	0.69

5.1.4.3.2 Gender

An independent sample t-test was performed to find that there was a significant difference (p-value=0.01) between FRC means based on gender (Table 15).

Table 15 Difference between FRC means based on gender

*significant at p-value<0.05; **significant at p-value<0.01

	Gender						
	Mean (SD)				Mean difference	Confidence interval	
Variable	Male (N=35)	Female (N=27)	t-value	p-value	(SE)	Lower	Upper
FRC	2.23(0.78)	1.78(0.55)	2.54	**0.01***	0.17(0.14)	0.10	0.80

5.1.4.3.3 Activity level

One-way ANOVA was carried out to investigate the difference in FRC mean values based on the activity level of the child. The results of the ANOVA showed that there was no significant difference in FRC values based on levels of activity level groups. (F=1.26, p=0.30) (Table 16).

Table 16 FRC difference based on activity level

FRC Vs Activity	Very often(SE)	Often(SE)	Medium(SE)
Little bit	0.43(0.41)	0.30(0.40)	0.02(0.38)
Medium	0.42(0.24)	0.28(0.23)	
Often	0.13(0.28)		

This represented the differences between the means of the FRC values based on activity level of the participant, e.g. the difference between the mean of the FRC value for the participants who exercised very often and those who exercised little bit = 0.43(0.41) L. A significant difference (p-value <0.05) in FRC values between any of the two activity level groups is indicated by a (*). N.B. We did not compare the group with itself as it is not feasible.

5.1.4.4 Differences between S_{cond} values based on demographic and environmental factors

5.1.4.4.1 Free school meals

An independent sample t-test was performed to find that there was no significant difference (p-value=0.70) between S_{cond} means based on free school meals (Table 17).

Table 17 Difference between S_{cond} mean values based on free school meals.

	Free school meals						
	Mean (SD)				Mean difference	Confidence interval	
Variable	Yes (N=6)	No (N=56)	t-value	p-value	(SE)	Lower	Upper
S_{cond}	0.01(0.01)	0.01(0.02)	-0.39	0.70	-0.002(0.01)	-0.02	0.01

5.1.4.4.2 Gender

An independent sample t-test was performed to find that there was a significant difference (p-value=0.049) between S_{cond} means based on gender (Table 18).

Table 18 Difference between S_{cond} mean values based on gender.

*significant at p-value<0.05; **significant at p-value<0.01

	Gender						
	Mean (SD)				Mean difference	Confidence interval	
Variable	Male (N=35)	Female (N=27)	t-value	p-value	(SE)	Lower	Upper
S_{cond}	0.01(0.01)	0.02(0.02)	-2.01	**0.049***	0.15 (0.14)	-0.02	-0.00004

5.1.4.4.3 Activity level

One-way ANOVA was done to investigate the difference in S_{cond} mean values based on the activity level of the child. The results of the ANOVA showed that there was no significant difference in S_{cond} values based on levels of activity level groups (F=0.397, p-value=0.76). (Table 19)

Table 19 S_{cond} difference based on activity level

S_{cond} Vs Activity	**Very often(SE)**	**Often(SE)**	**Medium(SE)**
Little bit	-0.0001(0.01)	-0.0004(0.01)	0.004(0.01)
Medium	-0.005(0.01)	-0.005(0.01)	
Often	0.0002(0.01)		

5.1.4.5 Differences between S_{acin} values based on demographic and environmental factors

5.1.4.5.1 Free school meals

An independent sample t-test was performed to find that there was no significant difference (p-value=0.33) between S_{acin} means based on free school meals (Table 20).

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Table 20 Difference between S_{acin} mean values based on free school meals.

	Free school meals						
	Mean (SD)				Mean difference	Confidence interval	
Variable	Yes (N=6)	No (N=56)	t-value	p-value	(SE)	Lower	Upper
S_{acin}	0.09(0.03)	0.11(0.04)	-0.987	0.33	-0.02 (0.02)	-0.05	0.02

5.1.4.5.2 Gender

An independent sample t-test was performed to find that there was no significant difference (p-value=0.22) between S_{acin} means based on gender (Table 21).

Table 21 Difference between S_{acin} mean values based on gender.

	Gender						
	Mean (SD)				Mean difference	Confidence interval	
Variable	Male (N=35)	Female (N=27)	t-value	p-value	(SE)	Lower	Upper
S_{acin}	0.10(0.03)	0.11(0.05)	-1.25	0.22	-0.03(0.03)	-0.04	0.01

5.1.4.5.3 Activity level

One-way ANOVA was carried out to investigate the difference in S_{acin} mean values based on the activity level of the child. The results of the ANOVA showed that there was no significant difference in S_{acin} values based on levels of activity level groups (F=1.75, p=0.17) (Table 22).

Table 22 S_{acin} difference based on activity level.

S_{acin} Vs Activity	**Very often(SE)**	**Often(SE)**	**Medium(SE)**
Little bit	-0.04(0.02)	-0.01(0.02)	-0.03(0.02)
Medium	-0.01(0.01)	0.02(0.01)	
Often	-0.03(0.02)		

5.1.4.6 Modelling the relationship between MBW outcome measures (LCI, FRC, Scond, Sacin) and the demographic and environmental variables using linear regression analysis

5.1.4.6.1 The influence of the demographic and environmental factors on LCI

To find which factor had an effect on LCI, a regression analysis was done for each factor individually except the height and BMI which were investigated together due to the interrelationship of the two variables.

The following variables were found to have no significant effect on LCI: gender (F-test= 0.93, p=0.762), BMI and height (1.928, p=0,155), distance from the main road (F=0.318, p=0.575), free school meals (2.259, p=0.138), and activity level (f= 0.958, p=0.332) (Table 23).

Table 23 Regression result of demographic and environmental factors on LCI.

Factor	F-test	p-value
Gender	0.93	0.76
BMI & Height	1.93	0.16
Distance from main road	0.32	0.58
Free school meals	2.26	0.14
Activity level	0.96	0.33

5.1.4.6.2 The influence of the demographic and environmental factors on FRC

A regression analysis was done to investigate the influence of the demographic and environmental factors on FRC. The results of the regression model of FRC vs the factors were shown in Table 23. Gender (F=6.46, p=0.01, FRC value= 2.679-0.448*Gender), BMI (F=19.48, p=0.00, FRC value = -2.461+(0.033*height) -(0.034*BMI) and living distance from the main road (F=31.64, p=0.00, FRC value=2.967-0.235* living distance from the main road) had a significant effect on FRC values (Table 24). Free school meals (F=0.04, p=0.84) and activity level (F=3.55, p=0.06) had no significant effect on FRC (Table 25).

Table 24 Regression result of demographic and environmental factors on FRC

*significant at p-value<0.05; **significant at p-value<0.01

Factor	F-test	p-value
Gender	6.461	**0.014 ***
BMI & Height	19.480	**0.000 ****
Distance from main road	31.640	**0.000 ****
Free school meals	0.044	0.835
Activity level	3.554	0.064

Table 25 Results of significant factors affecting FRC

*significant at p-value<0.05; **significant at p-value<0.01

Factor	B	T-test	P-value	95.0% Confidence Interval for B	
				Lower Bound	Upper Bound
Constant	2.68	10.00	**0.00****	2.14	3.21
Gender	-0.45	-2.54	**0.01***	-0.80	-0.10
Constant	-2.46	-3.05	**0.003****	-4.07	-0.85
Height	0.03	6.24	**0.00****	0.02	0.04
BMI	-0.03	-1.64	0.11	-0.08	0.01
Constant	2.97	15.94	**0.00****	2.59	3.34
Distance from main road	-0.24	-5.63	**0.00****	-0.32	-0.15

5.1.4.6.3 The influence of the demographic and environmental factors on S_{cond}:

A regression analysis was carried out to investigate the influence of the demographic and environmental factors on S_{cond}. The results of the regression model of S_{cond} vs the factors are shown in Table 26. Gender (F=5.25, p=0.03, S_{cond} value= -0.001+0.008*Gender) had a significant effect on S_{cond} values. BMI and height (F=3.09, p=0.053), distance from main road (F=1.58, p=0.22), free school meals (F=0.15, p=0.70), and activity level (F=0.52, p=0.47) had no significant effect on S_{cond}. (Table 27).

Table 26 Regression result of demographic and environmental factors on S_{cond}

*significant at p-value<0.05; **significant at p-value<0.01

Factor	F-test	p-value
Gender	4.041	**0.049 ***
BMI & Height	3.087	0.053
Distance from main road	1.576	0.215
Free school meals	0.153	0.697
Activity level	0.521	0.473

Table 27 Results of significant factors affecting S_{cond},

*significant at p-value<0.05; **significant at p-value<0.01.

Factor	B	T-test	P-value	95.0% Confidence Interval for B	
				Lower Bound	Upper Bound
Constant	-0.001	-0.23	0.82	-0.01	0.01
Gender	0.008	2.01	**0.049***	0.00	0.02

5.1.4.6.4 The influence of the demographic and environmental factors on S_{acin}

A regression analysis was carried out to find investigate the influence of the demographic and environmental factors on S_{acin}. The results of the regression model of S_{acin} vs the factors were shown in (Table 28), and it indicated that the demographic and environmental factors had no significant effect on S_{acin} values: gender (F=1.55, p=0.22), BMI and height (F=1.00, p=0.38), distance from main road (F=1.06, p=0.31), free school meals (F=0.97, p=0.33), and activity level (F=0.36, p=0.55).

Table 28 Regression result of demographic and environmental factors on S_{acin}

Factor	F-test	p-value
Gender	1.56	0.22
BMI & Height	0.998	0.38
Distance from main road	1.06	0.31
Free school meals	0.97	0.33
Activity level	0.36	0.55

5.2 Dataset 2

5.2.1.1 The demographics of the combined dataset population

The combined dataset (Keele + Manchester) was comprised of a total of 162 subjects with no known respiratory disease or infection at the time of testing, 100 from Manchester and 62 from Keele, all of which had acceptable based on ERS/ATS Consensus Statement criteria LCI measurement. (Robinson et al., 2013a). The age range of the subjects in the combined database was five to 59 years of age. Seventy-five (75) were males and (87) were females. The mean age (SD) was 17.3(10.7) years. The weight ranged between 14 to105 kg. Mean weight (SD) was 51.3 (19.2) kg. The height range was 100.0-190.7 cm, and the mean height (SD) was 154.88 (18.6) cm. The body mass index (BMI) range was 10.2-45.6 kg/m2, and the mean BMI (SD) was 20.6 (4.8) kg/m2 (Table 29).

Table 29 summary demographics of the study population of the dataset 2

Age at testing: mean (SD)	17.3 (10.7)
Age range	(5-59)
Gender (M/F)	75/87
Height: mean (SD)	154.88(18.6)
Weight: mean (SD)	51.3 (19.2)
BMI: mean (SD)	20.6 (4.8)

5.2.2 The descriptive analysis of the MBW outcome measures for dataset 2

The mean (SD) value for the LCI for our study population was 6.14 (0.47), the minimum value was 5.07 and the maximum was 7.83. The mean (SD) for the FRC was 2.11 (0.89), while the minimum value was 0.65 and the maximum was 4.76. S_{cond} mean (SD) was 0.01 (0.01), the minimum was 0.01 and the maximum was 0.31. S_{acin} mean (SD) was 0.11 (0.05), the minimum was -0.02 and the maximum was 0.09 (Table 30).

Table 30 Summary of the normal values of the MBW outcome measures for dataset 2

Dataset 2	**Mean (SD)**	**Minimum**	**Maximum**
LCI	6.14 (0.47)	5.1	7.8
FRC	2.11 (0.89)	0.65	4.76
S_{cond}	0.01 (0.01)	0.01	0.31
S_{acin}	0.11 (0.05)	-0.02	0.09

This summarized the normal values of the MBW outcome measures of dataset 2 represented in the mean (SD), minimum, and maximum values for LCI, FRC, S_{cond} and S_{acin} respectively.

To further analyse the relationship between age and MBW outcome measures, we calculated the means, standard deviations, as well as the minimum and maximum values for LCI, FRC, S_{cond}, and S_{acin} for the paediatric age group in Dataset 2 separately and for the adult age group separately (Table 31).

Table 31 The summary of the normal values of the MBW outcome measures for children versus adults in dataset 2.

Dataset 2	**Children**				**Adults**			
Number	111				51			
Age range	5-18				19-59			
MBW outcome	LCI	FRC	S_{cond}	S_{acin}	LCI	FRC	S_{cond}	S_{acin}
Mean (SD)	6.2 (0.4)	1.8 (0.8)	0.01 (0.01)	0.11 (0.05)	6.1 (0.6)	2.8 (0.8)	0.01 (0.01)	0.11 (0.05)
Minimum	5.07	0.7	-0.02	0.01	5.2	1.2	0.00	0.04
Maximum	7.0	4.2	0.09	0.31	7.8	4.8	0.08	0.25

This summarised the normal values of the outcome measures for the children versus the adults represented as the mean (SD), minimum, and maximum values for LCI, FRC, S_{cond} and S_{acin} respectively.

Participants were categorized based on their age into 18 age groups. The youngest age group were 5-6 year-olds and the oldest age group were 49-59 year-olds. The pediatric age group ranges were categorized in increments of one year, while the adult age group ranges were categorized in increments of ten years. This difference in age range was set because the focus of our research was to set normative MBW values for the pediatric population rather than the adult population. Age group categories and the demographical data for the participants based on age groups are shown in

Table 32.

Table 32 Summary of demographic data based on age groups

Age group	Age range	Male	Female	Mean weight(SD)	Mean height(SD)	Mean BMI(SD)
1	5-6	2	3	19.60(2.61)	108.12(5.60)	16.73(2.70)
2	6-7	3	4	21.29(3.86)	117.61(3.09)	15.42(2.61)
3	7-8	1	2	25.00(1.41)	125.70(2.25)	15.99(0.07)
4	8-9	4	1	26.40(2.07)	131.16(3.99)	15.49(1.46)
5	09-10	4	7	34.91(7.76)	139.23(7.08)	17.81(2.95)
6	10-11	6	10	40.73(12.18)	144.31(4.55)	19.42(5.36)
7	11-12	8	12	40.00(10.31)	149.36(11.86)	17.60(2.58)
8	12-13	8	4	52.73(11.93)	157.86(6.61)	20.98(4.30)
9	13-14	8	1	51.00(9.64)	163.63(8.99)	18.91(2.33)
10	14-15	3		58.67(10.07)	165.77(2.93)	21.38(3.95)
11	15-16	12	2	59.64(9.38)	171.35(7.59)	20.29(2.58)
12	16-17		4	67.25(11.53)	165.00(6.27)	24.76(4.47)
13	17-18	-	-	-	-	-
14	18-19	2		173.25(12.37)	64.00(11.31)	21.18(0.64)
15	19-29	8	16	169.66(9.83)	67.00(9.11)	23.25(2.69)
16	29-39	3	16	167.04(8.52)	71.05(15.31)	25.51(5.88)
17	39-49	2	4	171.00(7.07)	76.17(8.89)	26.07(3.13)
18	49-59	1	1	162.95(11.38)	71.00(5.66)	27.09(5.90)

5.2.3 The influence of age on the MBW outcome measures (LCI, FRC, S_{cond}, and S_{acin})

One-way ANOVA was performed to assess:

- Difference between LCI means based on age groups: No significant difference in LCI values based on age group was found (F=1.32, p=0.20).

- Difference between FRC means based on age groups: A significant difference in FRC values based on age groups was found (F=10.83, p=0.000**).
- Difference between S_{cond} means based on age groups: There was a significant difference in S_{cond} values based on levels of age groups (F=1.08, p=0.38). Difference between S_{acin} means based on age groups: A significant difference in S_{acin} values based on levels of age groups was found (F=1.06, p=0.40).

Of note, the significant differences in the means of S_{cond} and S_{acin} can be seen only when the (5-6) age group is assessed.

Pearson correlation was carried out to assess the correlation between age and MBW outcome measures. No significant correlation was found except with LCI (r = 0.07, p = 0.40) FRC (r =0.55, p = 0.00), S_{cond} (r = 0.04, p = 0.63) and S_{acin} (r = 0.06, p = 0.43) (Table 33).

Table 33 The correlation between the age and the outcome measures of dataset 2

*significant at p-value<0.05; **significant at p-value<0.01

Dataset 2 outcome measures	Pearson correlation	p-value
LCI	0.07	0.40
FRC	**0.55****	0.00
S_{cond}	0.04	0.63
S_{acin}	0.06	0.43

This Illustrated the correlation between the age and the outcome measures of dataset 2 the results of correlation showed that there was no significant correlation between age and LCI, Scond and Sacin but FRC was significantly correlated with age.

5.2.3.1 Modelling the relationship between the MBW measures and the demographic variables

A regression analysis was carried out (Figure 19). Results of the regression model of LCI vs age showed that age has no significant effect on LCI values (F-test= 0.72, p=0.40). (Table 34)

Table 34 Regression result of age on MBW outcome measures: LCI, S_{cond} and S_{acin}

Age effect on	F-test	p-value	R^2
LCI	0.72	0.40	0.004
S_{cond}	0.24	0.63	0.001
S_{acin}	0.62	0.43	0.004

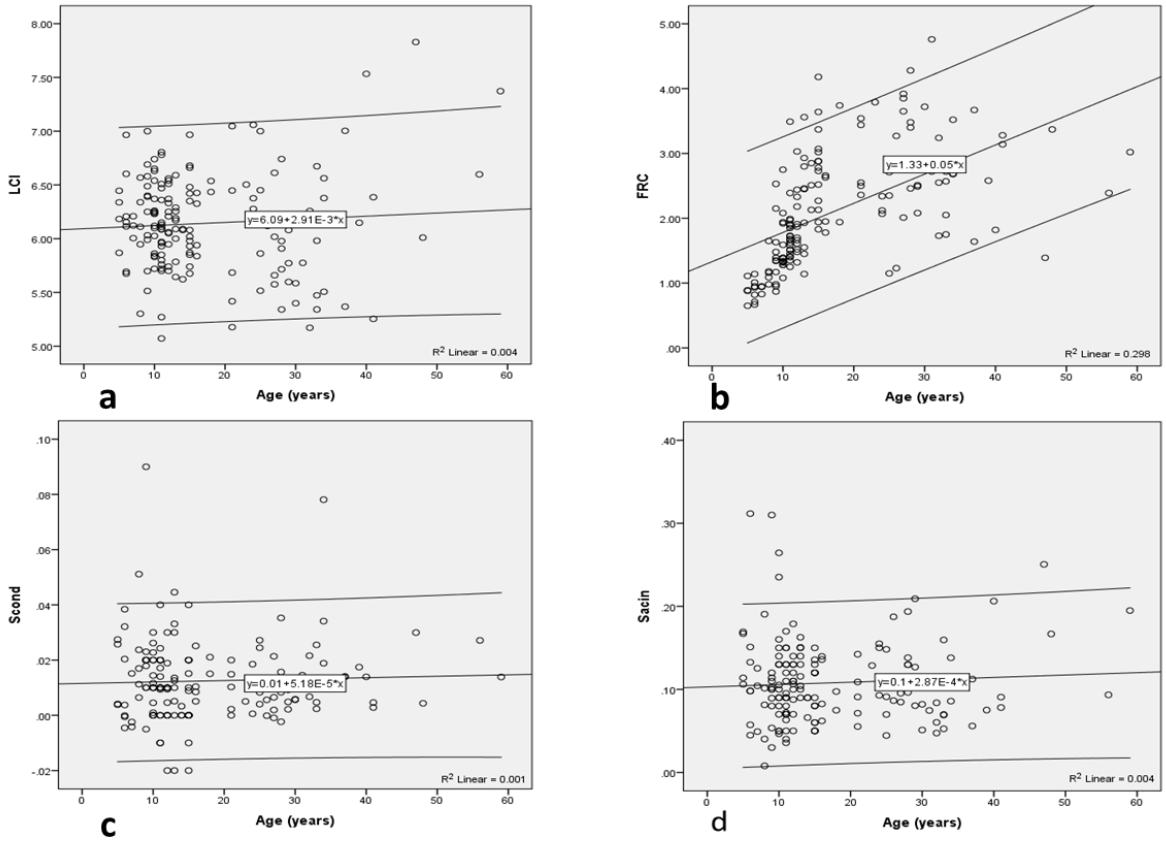

Figure 19 Linear regression for the MBW outcome measures versus age

This represented the linear regression graphs for the MBW outcome measures vs age assessors from dataset 2 (Figure 19.a. LCI, Figure 19.b. FRC, Figure 19.c. S_{cond}, and Figure 19.d. S_{acin}): the x-axis represented the age in years, and the y-axis represented MBW outcome value. The linear regression line was denoted by the middle line with the regression equation was displayed on the line. The upper and lower black lines demonstrated the confidence interval.

5.2.4 Total time of testing session

The total time of testing session was measured and converted into minutes. The mean time for the three reading was 17 minutes and 36 seconds (SD=2.5 minutes). The time was measured and provided by the Innocor device. The maximum time consumed in the three repetitions was 26 minutes and the minimum was 13 minutes and 20 seconds. The two repetitions mean time consumed was 12 minutes (SD=3.3 minutes). The maximum time was 19 minutes and 20 seconds and the minimum was nine minutes and 20 seconds.

6 Discussion

In this study, there were several research questions we aimed to answer. Our main objective was to determine normative values for LCI, the main MBW outcome measure, as well as S_{cond} and S_{acin}. Furthermore, we wanted to identify the effect of a variety of demographic and environmental factors, such as age, gender, exposure to tobacco smoke, and prematurity on MBW outcome measures and model any relationships. We also aimed to assess the practicality and reliability of performing MBW testing using an SF_6-based portable MBW machine in a community.

6.1 Normative LCI, S_{cond}, and S_{acin} values in children

One of our main objectives in this study was to propose normative values for LCI, S_{cond}, and S_{acin} in children when using the SF_6-based portable MBW device (Innocor). I demonstrated that normative values were 6.18 (SD = 0.3, 95% CI = 6.10 to 6.25) The normative paediatric values we present in this research are comparable to values calculated for healthy individuals within a similar age range in previous studies conducted using different MBW devices and gases (Table 35) (Aurora et al., 2004; Fuchs et al., 2009; Gustafsson et al., 2003; Horsley et al., 2008a; Jensen et al., 2013). As discussed in the literature review, the differences in normal values of LCI for the different gases and machines occurs due to the difference in behaviour of the tracer gases, difference in washout of the gases by the body, and differences in the equipment and systems used for each gas (Table 6) (Subbarao et al., 2015).

The normal range for LCI calculated in our study is very similar to that for previous studies involving SF_6 as the tracer gas; however, the mean and upper limit of normal are both lower in our study compared to those described in research studies done on the paediatric population; this comparison is demonstrated in Table 35 (Gustafsson et al., 2003; Horsley et al., 2008a; Jensen et al., 2013; Lum et al., 2013).

Aurora et al (2004) presented a mean LCI value for the normal subjects in their study that was noticeably higher than the normal LCI value we calculated in our study as well as those suggested by the other studies in the literature that used SF_6; this difference may be due to the smaller sample size (N=33) or due to selection bias as the normal subjects in this study were represented by a control group, rather than a random selection from the community (Aurora et al., 2004). It is known that a control group in a case-control study is subject to greater selection bias than other study designs, as it is usually not truly representative of the population being studied due to the nature of recruitment of control subjects in these studies. In fact, most of the studies in the literature (Gustafsson et al., 2003; Horsley et al., 2008a; Jensen et al., 2013) suggesting normal LCI values are case-control studies, subjecting them to more selection bias than our study. Fuchs et al (2009), however, utilized a cross-sectional study approach similar to ours, aiming to set normative values for LCI using the EasyOne Pro N_2-based device; interestingly, the normal mean LCI values calculated was very similar to our results using the Innocor SF_6-based device, although other studies utilizing N_2 as a tracer gas showed higher normal LCI values compared to SF_6 (Fuchs et al., 2009). This may suggest that the EasyOne Pro N_2-based system in particular may yield similar values as SF_6-based MBW systems. Further research, however, is needed to establish such a conclusion.

Table 35 A comparison between the LCI value for healthy individuals in several studies using different MBW devices and different inert gases with our study result from dataset 1.

	Gustafsson et.al (2003)	**Aurora et.al(2004)**	**Jensen et.al(2013)**	**Jensen et.al(2013)**	**Horsley et.al (2008)**	**Fuchs et.al (2009)**	**Our result**
Equipment	MS	MS	MS	MS	Innocor	Easy One Pro	Innocor
Gas	SF_6	SF_6	SF_6	N_2	SF_6	N_2	SF_6
Sample size	28	33	62	62	12	44	62
Age range	4.5-18.7	5.9-16.8	3-18	3-18	6-16	5.3-20.3	9-15
LCI (SD)	6.3 (0.4)	6.5 (0.5)	6.2 (-)	6.8 (-)	6.3 (0.5)	6.2 (0.4)	6.18 (0.3)
Upper limit of LCI	7.1	7.5	-	-	7.3	7	7

6.2 Reliability

To ensure the reliability of the measurements extracted by myself from the Innocor machine, we compared my measurements with those extracted by an expert assessor, which showed no significant difference. Most importantly, this indicates that the measurements that were taken by myself and used in the statistical analysis are reliable, and secondly, that an individual trained to use the Innocor device is capable of extracting measurements that are as reliable as those obtained by an expert, which adds value to the Innocor device especially in assessing its feasibility of use in the community, rather than clinical, setting as a screening tool. Moreover, the limits of agreement provided by the Bland and Altman test may help in providing an acceptable range of error for values extracted from the Innocor device by a trained assessor. Looking at the resulted limits of agreement for the LCI values between the two assessors, we found that the error range was 0.96. This range is representing a ratio of 15% out of the full scale of LCI mean value that was calculated out of our data (6.2) for healthy individuals. Therefore, ± 7.5 of the

mean LCI value is much smaller than the difference between the mean LCI value in healthy and that is seen in people with pathology (referred to Table 7, CF = 8.7).

6.3 The influence of demographic variable and environmental factors on the MBW outcome measures

One of our aims in this study was to assess the relationship between demographic and environmental factors on the MBW outcome measures. the factors I chose were determined by previous research which identified the early life factors associated with future impairment of lung function. (Kouzouna et al., 2016). Most of these factors were assessed through the questionnaire that was given to each participant. However, some were measured by myself on the day of testing before the start of the MBW testing session for each participant; these were height, weight, and the calculation of the BMI. Unfortunately, three of the assessed environmental factors, (tobacco smoke, prematurity, and early life hospitalization for respiratory illness), could not be statistically analysed due to the poor rates of completion of the relevant question. A possible reason for this is the catchment of the school, in which the schools we studied may have been of a well-off socioeconomic status or a well-educated population; such areas are expected to have lower rates of smoking compared to poorer, less-educated communities (Gong et al., 1995; Jenkins et al., 1997; Memon et al., 2000; Pierce, 1989). Moreover, in the UK, there is a noticeable drop in smoking rates likely due to national efforts that aim to decrease the prevalence of smoking by setting laws that restrict tobacco use and launching international awareness campaigns. Moreover, the age of our study population in Dataset 1 did not exceed 16 years of age, making access to tobacco smoke more difficult. (Bauld et al., 2012; McNeill et al., 2015). This drawback in our study may have occurred due to our limited sample size of only 62 participants as well as the limited geographical area

(only two schools) from which our participants were recruited. In the future, this may be overcome by studying a larger sample population and conducting a study over a larger geographical area. Also, it is possible that limiting smoke exposure to tobacco smoke only, in an era where many methods of smoking have become popularized such as electronic cigarettes, illegal smoking, and hookah pipes, may have contributed to an underrepresentation of smoking exposure in our study population.

LCI was not found to be significantly affected by any of the demographic and environmental factors investigated in our statistical analysis, which were age, gender, BMI and height, activity level, social deprivation (represented as free school meals), and pollution level (represented as distance from main road). This is consistent with most of what is found in the literature. We did find, however, some variation in the literature regarding the effect of age and body size (height and BMI) on this outcome measure. Our study was consistent with the findings that the LCI was independent of body size and age (Aurora et al., 2005a; Horsley et al., 2008a). However, this was inconsistent with the findings of Lum et al (2013) who concluded that LCI was body size and age dependent (predicted LCI equation = 5.99 + (73.85 x height^{-1}) and CV=0.08, where height was represented in centimeters) (Lum et al., 2013).

One study by Verbanck et al (Verbanck et al., 2012) found that LCI, S_{acin} and S_{cond} consistently increased with age; however, the study population age range was 25-65 years, which is different from the age range studied in our population, which was 5-59 years. Seventy-four percent (74%) of our study population (120 out of 162 participants), were aged less than 25, and, therefore, the conclusion by Verbanck et al does not necessarily contradict our results, but may be more representative of an older age group

than that studied in our population. Of note, only 6/162 in our study were above 40, representing only 4% of Dataset 2. Three of these six participants were noticed to have LCI values above the upper limit of the confidence interval (Figure 19.a). This may suggest that after the age of 40, LCI results may be affected. Studies done using nitrogen-based mass spectrometry have shown that in people who exceed the age of 40, an increase in LCI seems to occur due to the increased ventilation inhomogeneity that occurs as a feature of aging lungs (Htun et al., 2018; Verbanck et al., 2012; Verbanck et al., 2016). We further noticed that two of these three participants were found to have a higher than normal BMI, which may further suggest that body size may affect LCI values after the age of 40. Further research on adults above 40 years may thus be needed to further investigate the effect of age and body size on MBW outcome measure in this older age group. Overall, however, we found no significant change in LCI with age, BMI, or height.

The fact that LCI was found to be unaffected by all our assessed demographic and environmental factors makes this outcome measure valuable in that a single normative value and range can be set for all genders, body sizes, and age groups, particularly less than 40 years. This makes comparisons across genders, ages, and body sizes easier, whether in clinical practice or for research purposes. Moreover, the stable range of normality across most of the age range studied makes LCI a distinctive lung function measure that can be used in future longitudinal studies in the paediatric population, since it is not affected by changes in lung volumes related to normal growth of lung tissue, as well as changes in activity level. These characteristics are unlike those of spirometry measures which vary normally according to age, gender, and body size (Wang et al., 2009; Wang et al., 2017).

While LCI was found to be unaffected by all assessed factors, S_{cond} and S_{acin} were found to be significantly affected by some factors and not significantly affected by others. BMI and height, activity level, social deprivation, and pollution were found to have no significant effect on both S_{cond} and S_{acin}. Gender, however, was found to have a significant effect (p-value=0.049) on S_{cond}, but no significant effect on S_{acin}. In contrast to our study results, Verbanck et al found a small but significant gender difference for S_{acin} (Verbanck et al., 2012); the tracer gas used by Verbanck et all, however, was nitrogen, which produces different results from SF_6-based MBW testing. Nevertheless, these differences found may direct further research to investigate the relationship between gender and S_{acin} and S_{cond} more thoroughly, although they may be considered secondary MBW outcome measures compared to LCI. A significant difference in both S_{cond} and S_{acin} based on age was found. When we referred to Table 33 and Table 34, we noticed that the significant difference based on age was limited to the S_{cond} and S_{acin} values between the 5-6 year old age group vs all the other age groups. This was also appreciated on the regression graphs modelling the relationship between age and S_{cond} and S_{acin} (Figure 19.c and Figure 19.d) respectively. We traced back these point values and found that they referred to a single participant in the 5-6 year age group provided by the Manchester data in the combined dataset (Dataset 2). We decided, however, to consider this participant's measurements within our analysis since the LCI, our main outcome measure, and FRC were acceptable in accordance with the ERS/ATS consensus statement criteria.

Consistent with our expectations, FRC was significantly affected by age, gender, BMI and height. FRC is known to be affected by these variables because it represents the volume of air present in the lungs at the end of passive expiration. This changes with varying lung size and factors that affect the elastic recoil of the lung. Lung size varies across genders and increases from childhood to adulthood. Elastic recoil decreases with

BMI due to the extra weight opposing the outward recoiling of the chest wall. Aging also affects the ability of the lung to recoil (Aurora et al., 2005b; Jensen et al., 2013). FRC was found to be significantly affected by pollution (represented by distance from main road). However, because FRC was the only outcome measure we found to be affected by this environmental factor, further research is required to investigate the effect of pollution on MBW lung function measures in healthy children. Finally, FRC was not found to be affected by social deprivation or activity level.

6.4 Clinical applicability

This is the first research study to use a genuinely portable MBW system operated by a single researcher (myself) in a community setting.This has demonstrated the possibility of performing MBW testing in a community setting and the feasibility of its large scale use as a screening tool for subclinical respiratory disease in children. It is considered a strength that the assessment of each participant was performed on the same school visit, by the same researcher, in one time-setting, thereby eliminating operator variability in the three sets of outcome measurements.

Use of a compact and robust analyser, combined with an SF_6-gas source that can be stored in small on-board cylinders, means that I was able to package an entire MBW system into a transport case and take it directly into schools. This ease of portability is important for community-based assessment of MBW. Nitrogen-based portable MBW machines, such as EasyOne Pro, on the other hand, require a source of 100% oxygen gas, which is stored in remarkably larger cylinders, making portability of this system in the community-setting more difficult compared to Innocor.

One SF_6-gas cylinder was enough for 9-14 sets of washout; this variability is due to the varying participant sizes, as the amount of gas consumed increases with increasing size of the lungs. Therefore, the younger the child, the less the amount of gas was consumed and the less the number of SF_6-gas cylinders were needed.

The duration of the MBW test during our study was on average 12 min for two test runs and about 17.5 minutes for three runs. The time for gas exchange is less in children than adults due to their smaller lung size. Moreover, healthy lungs wash out the tracer gas faster than diseased lungs. This shortens the time of testing in the healthy paediatric population, especially when two measurements rather than three are applied, making it a feasible screening method in this age group.

In terms of the effect of gas choice on test duration, SF_6-based MBW testing was found to take less time compared with N_2-based MBW systems (Bayfield et al., 2016). This can be explained by two phenomena: (1) the faster washout of SF_6 due to its exogenous nature and gas behaviour compared to the endogenous N_2, and (2) the longer time needed between test runs using N_2-based MBW due to the need for N_2 gas to return to its baseline concentration in the lungs prior to the start of the next washout.

Although MBW testing is more time-consuming compared with spirometry, which takes on average 5-10 minutes, MBW has several advantages over spirometry in clinical application. First of all, it can detect very early changes in lung function, making MBW more sensitive to lung pathology and disease progression (Goksor et al., 2008;

Gustafsson, 2005; Owens et al., 2011); this may make this method appropriate for screening of chronic lung disease. Secondly, unlike spirometry, MBW requires minimal patient cooperation and is not effort dependent, as it assesses lung function while the patient is in passive breathing, which makes MBW attractive to specialists in the field of paediatric pulmonary medicine. In our study, the children that underwent the MBW test cooperated well and found no difficulty or challenge in following the instructions of the test throughout the testing period.

Computed tomography (CT) and high resolution computed tomography (HRCT) are two other widely used methods of lung assessment in medical practice. Although CT images are able to provide information about early lung abnormalities, it is not justified to use CT scanning on young, healthy children as a screening tool as it exposes them to unnecessary radiation that increases their risk of developing future malignancies (Ward et al., 2017). This risk is higher in the growing child compared to the adult because growing cells are more sensitive to radiation-induced cell damage, which is why CT imaging in paediatric medicine is reserved for cases in which an accurate diagnosis is more important to the child's health than the risk of future malignancy posed by the radiation. Moreover, CT and HRCT are more time-consuming than MBW testing; CT imaging may take a total of 30-60 minutes, including the time spent getting the patient ready for the scan, which is much longer than the total of 3 runs of MBW testing conducted in our study. Further disadvantages of CT are that it can only be carried out in a hospital setting, requires the attention of more medical personnel, and requires more patient cooperation or sedation as movement throughout the scan duration must be minimized for acceptable image resolution (Sarma et al., 2012).

Many studies have been conducted over the past decade assessing the sensitivity and repeatability of LCI in disease conditions such as CF, bronchiectasis and COPD. They have all agreed that LCI is considered a repeatable and highly sensitive measure of lung function abnormality compared to spirometry outcome measures as well as computed tomography imaging of the lung parenchyma. (Green et al., 2011; Gustafsson et al., 2008; Rowan et al., 2014; Trinkmann et al., 2016)

6.5 Limitations

As with any research study, our results and findings have to be taken into consideration in light of some limitations. Firstly, our study was subjected to selection/sampling bias, as we only received permission to recruit participants from two out of an intended 14 schools, which also resulted in a limited sample size for us to work with. This may have occurred as a result of our choice of email as a method used in contacting the schools. A direct meeting with the school principal or head teacher to explain the study and deliver the invitation personally may have been more effective in eliciting a response. However, we were restricted to the used of email as a means of sending the invitation in accordance with the recommendations of the ethics committee. Being able to reach a larger number of schools would have made our sample more representative and generalisable to the UK, as a larger geographical area and sample size would have been achieved. Moreover, the small sample size and limited geographical representation may have contributed to the fact that we were unable to test for the effect of tobacco smoke exposure, prematurity, and early life hospitalization on the MBW outcome measures due to the very few and insufficient reports of exposure to these factors. In addition, a larger sample size may have provided further strength to our statistical results, increasing confidence in our normative values for MBW outcome measures. In order to set normative data that can be

generalised to a variety of populations, we suggest that further research be done in different geographical locations, covering all age groups, ethnicities and social classes. Our study may be considered as an initiative in producing widely generalisable LCI, S_{cond}, and S_{acin} normative values for future clinical use.

Secondly, the use of a questionnaire to identify many of the factors we investigated in our study in itself is also considered a source of bias. Questionnaires are known to introduce recall bias to data. It is considered a systematic error caused by differences in the accuracy or completeness of the recollections retrieved by the study participants regarding past events. However, we found that using a questionnaire was the most practical and efficient way to assess the demographic and environmental factors we aimed to analyze in our research study.

Smoking exposure could have been measured using a urinary or salivary sample from the children being studied. This method, however, is considered invasive when working with children in a public sector such as schools, and therefore, consent may not be available. Furthermore, the cotinine test was expensive and could not be justified for a preliminary study. As result, we relied on the questionnaire to assess exposure to tobacco smoke, although reliability of this method may be less favorable.

Assessment of tobacco smoke exposure was limited to cigarette smoking with no assessment of exposure to other types of smoking (e.g. illegal smoking, electronic cigarette smoking, and hookah pipe smoking). The agreement of assessing only for tobacco smoking followed a discussion between the supervisor and myself, in which it was agreed that cigarette smoking was the most frequent legal method of smoking. This agreement was made to emphasize more truthful answers.

Parents/gaurdians may have difficulty recalling the occurrence of early-life respiratory infections in their children's lives, and therefore, to ensure obtaining more accurate answers in assessing this risk factor, we asked about the frequency of hospital admissions during childhood for a respiratory infection, as hospitalization is more likely to be remembered. This also indicates the seriousness of the lung condition and the need for treatment. When people visit the general practitioner (GP) however, medication may not be prescribed, and because of not having the permission from NHS ethics, then exploring the participants' medical history was only possible through the parents'/guardians' knowledge.

Finally, we faced a few technical limitations at the beginning of our testing period, as we were working with a machine that – like any machine – was subject to manufacturing flaws and technical problems. At the initial phase of our testing trials, we faced recurrent device failures that showed up as pauses during the test, which resulted in delay of the testing process. These technical issues required that I travel to meet with the machine expert in order to solve the technical problem I had faced. Unfortunately, this issue resulted in a delay our in testing and decreased the time available to collect more data.

7 Conclusion and future recommendations

This research aimed to identify normative values for the main MBW outcome measures for the paediatric population, investigate and assess any relationships between demographic and environmental factors and MBW outcome measures, and demonstrate clinical applicability and usability of the portable MBW method outside the clinical setting. Based on quantitative and qualitative analyses of the data collected in our research study, it can be concluded that MBW outcome measures, namely LCI, S_{cond}, and S_{acin}, in children are not affected by demographic factors, such as age, gender, height, and BMI, but further investigation is needed to assess the effect of environmental factors, such as exposure to smoke, prematurity, and early life respiratory infections, on these measures. This may be an aim for future research.

The normative values for the paediatric age group that have been suggested in our study have been found to be very similar to normal values reported previously in the literature. Further research is needed, however, to identify normative values for the adult population as well as explore the effect of demographic factors, particularly age, on these measures, as age may possibly affect outcome measures after the age of 40. Further work also needs to be directed in setting normative values of MBW outcome measures for infants and preschoolers, as this age group was excluded from our study population.

Supporting what has been suggested in the previous literature, LCI has been found to be a reliable and reproducible measure of lung function that has shown a consistently narrow range of normal values throughout childhood and early adulthood (from 5-40 years of

age). This makes LCI a possibly very valuable measure in serial assessments of lung function in this age range, particularly in monitoring of chronic lung disease.

In our research, we also successfully demonstrated the usability and practicality of performing MBW testing on children in the community setting using the portable SF_6-based Innocor device and thus the possibility of taking MBW testing outside of the clinical setting. This may direct future efforts in exploring the feasibility of using the MBW method as a screening tool for detecting early chronic respiratory disease in children, as the medical literature has shown that most chronic respiratory diseases, such as asthma, COPD, and CF, can be detected prior to development of symptoms and the occurrence of irreversible lung damage. Establishing an early diagnosis for these potentially debilitating diseases may significantly improve prognosis by guiding earlier intervention when damage is still reversible. We further recommend that future work is directed in identifying the group of children in the population who will benefit from undergoing such a MBW screening test according to a risk score determined by research that aims to identify the risk factors that affect lung function in early life and progress to symptomatic lung disease. In assessing exposure to smoking, further research may reassure reliability of responses by using cotinine testing rather than questionnaires. As this study follows a cross-sectional approach, there is a need for longitudinal studies (cohorts) in the future to better assess the effect of known environmental risk factors for chronic lung disease on MBW outcome measures obtained throughout childhood.

Further recommendations for future work include setting normative values for MBW outcome measures using the other available commercial devices and gases, such as the

EasyOne Pro and Exhalyzer D, as our normative values cannot be applied to machines other than the SF_6-based Innocor device.

Furthermore, in order to establish normative values that can be generalised for use in the clinical setting in the UK, further research should aim to recruit a larger study population from different cities and areas in order to make the sample more representative of the general UK population. Future similar international efforts are further needed to generalise values to a global level.

Finally, to improve the clinical applicability and feasibility of MBW testing in the community setting, studies have demonstrated ways to decrease test duration, which include taking two rather than three measurements and decreasing the final washout concentration measured to $1/20^{th}$ rather than the standard $1/40^{th}$ of the initial tracer gas concentration inhaled. Further studies, however, need to be conducted to investigate the reliability of LCI($1/20^{th}$) in normal individuals compared to LCI($1/40^{th}$), as not enough studies have investigated the reliability of this method.

In the end, MBW testing seems to be emerging in the field of pulmonary medicine as a promising method of lung function assessment, which may very well be a part of the standard clinical care of respiratory disease patients in the near future. Research investigating this method is therefore in high demand.

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9 Appendix

9.1 Appendix 1

11/01/2018

Dear Amnah

Applicant: Amnah Alrumuh
Project title: Determining the clinical usefulness of MBW methods to study lung function in children
ERP Ref: ERP1348

Thank you for submitting your application for review. The proposal was reviewed by the Panel Chair. I am pleased to inform you that your application has been approved by the Ethics Review Panel.

If the fieldwork goes beyond the date stated in your application, or there are any amendments to your study you must submit an 'application to amend study' form to the ERP administrator at research.governance@keele.ac.uk. This form is available via http://www.keele.ac.uk/researchsupport/researchethics/

If you have any queries please do not hesitate to contact me, in writing, via the ERP administrator, at research.governance@keele.ac.uk stating **ERP1348** in the subject line of the e-mail.

Yours sincerely
PP.

Dr Andrew Rutherford
Chair – Ethical Review Panel

9.2 Appendix 2

Keele University

School of Health and Rehabilitation and The Institute for Science & Technology in Medicine

Determining the Clinical Usefulness of MBW Method to Study Lung Function in Children- Information sheet for Parents/Guardians

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How to contact us

If you have any questions about this study please talk to
Amnah AlRumuh

Tel: 07799304194*
a.k.a.alrumuh@keele.ac.uk

*This is a temporary number which I am using for my PhD study.

You are invited to take part in a research study

- This study looking at new ways of measuring the function of the lungs. The details of the supervisors of this project are listed at the end of this form.
- Before you decide if you would like your child to take part, it is important for you to understand why this research is being done and what taking part would involve for your child.
- Please take your time to read the information carefully.
- Your child's participation is entirely voluntary. They do not have to take part if you do not want him/her to.
- Please ask a member of the research team if there is anything that is not clear, or if you would like more information before considering letting your child take part.
- Thank you for reading this information. We hope this research will be of interest to you and your child.

Important things that you need to know

- This study is looking at measuring lung function in children with no respiratory illness.
- This method is not a diagnostic procedure
- The new measurement is called the lung clearance index (LCI). Taking part in this study involves your child having LCI measured (described in this information sheet).
- We are looking for up to 143 healthy children aged 5- 18 years with no lung problems to undergo an LCI assessment on **one** occasion.
- The test itself is anticipated to last 20-30 minutes (in total) and this measurement will be repeated 3 times.
- Is your child **currently receiving antibiotics for a lower respiratory infection;** or **diagnosed with asthma, viral induced wheeze, protracted bacterial bronchitis, CF;** or **any other chronic respiratory condition; and or have used anti-asthma medication in the last 3 months prior to the study.** If yes, then the child is **not suitable** to participate in this study

N.B. Should the child be ill on the day of the test, the researcher may exclude them from the study

Why are you doing this research?

This study is a research looking at the value of a measurement called **Lung Clearance Index (LCI)** in healthy children and the factors that may affect this value.

Lung function tests are designed to tell doctors how well a person's lungs are working. A common test used in clinical practice is called spirometry, which involves "blowing" into a machine until your lungs are empty. The Lung Clearance Index (or "LCI" for short) is a non-invasive test which can be performed during simple relaxed breathing. It has been shown to be better at picking up early changes in the lungs which is important for patients with lung problems. LCI has been measured in a number of research studies and has been shown to be an excellent research tool, but it has not previously been measured in routine clinical monitoring.

LCI has previously been measured in children and adults and children with lung disease such as Cystic Fibrosis, asthma and COPD. It is particularly useful at picking up early changes in lung function. In this study we are aiming to measure LCI in children aged 5-18 years, using a portable system that allows a quick test time. In this study we aim to measure LCI in children with no lung problems.

Why do you want my child to take part?

Your child has been invited to take part in this research because they are not known to have any problems with their lungs.

What happens if I agree?

Your child will need to attend **one** measurement session where three readings will be taken. An experienced researcher will explain the study to you and your child in more detail and give you both the opportunity to ask any questions before asking you to sign a consent form. If your child is old enough we will ask them if they would like to sign an additional assent form.

Study visit

LCI assessments will take place at the School. We anticipate the test taking approximately 20-30 minutes. We anticipate your child's study visit lasting approximately 40 minutes in total. You are welcome to be present at the time of the measurement.

Measurement of LCI

The test is performed during simple relaxed breathing. Your child will need to breathe in and out of a mouthpiece whilst sitting down. A nose clip will be applied to ensure no air leaks through his or her nose.

The first part of the test involves breathing air mixed with a small amount of a tracer gas, called SF_6 (sulphur hexafluoride). This gas is present in very small amounts, is inert (meaning it has no effect on the body), odourless and colourless and is not absorbed (taken up) by the body. It has been used for many decades in assessing lung function in similar tests. Your child will breathe the air and tracer gas in and out for a few minutes until it is evenly mixed with the air in their lungs (this phase of the test is known as the **wash-in**).

The mouthpiece will then be switched to breathing room air, and we will measure how the tracer gas is breathed out of your child's lungs. This second phase is known as the **wash-out**. We will repeat the test three times.

Additional measurements
We will also measure your child's height and weight, and ask them to perform a spirometry test.

What else will I be asked to do?
We are very interested to know your child's experience of the wash-out testing, and we may ask him or her some short questions about it afterwards. If your child is old enough we will also ask him or her to rate their current health (so we can compare how LCI changes when symptoms change). Alternatively, we will ask you to answer this question.

Does my child have to take part?

You do not have to agree to your child taking part if you do not wish to.

What happens if I change my mind?

It is okay if you agree to your child taking part in the study but later change your mind. You do not need to give a reason. If you decide to pull out of the study we will continue to use the data we have already collected on your child, unless you expressly ask us not to.

Are there any benefits from taking part?

There will be no direct benefit for participants in this study.

Are there any risks from taking part?

There are no risks from taking part. The tracer gas (SF_6) has been used for many decades and at much higher concentrations than we are using in lung function testing in infants, children and adults. It is already licensed for use in this way.

What happens if something goes wrong?

If you have a concern about any aspect of the study, please speak to the researcher in the first instance. If you remain unhappy and wish to complain formally contact Dr Gilchrist (Fran), Professor Pandyan (Anand) or Ms Nicola Leighton: n.leighton@keele.ac.uk.

If something did go wrong and your child was harmed during the research due to someone's negligence then you may have grounds for legal action or compensation against the University.

Non-negligent harm arises where an individual has been harmed during research, through no fault of an individual or institution involved, even though all the correct policies and procedures have been followed.

Additional information about the study

Will we receive a payment for taking part?
You and your child will not receive any payment. At the end of the test, we are also offering children a simple present as a token of our appreciation for their time taking part in this research.

Will my child's details be kept confidential?
We follow strict ethical and legal guidance regarding patient confidentiality. Any information we have about your child will be handled in confidence. All data recorded will be coded and your child's name will remain anonymous.

When taking part in research it is occasionally necessary for details to be made available to authorised research staff who are bound by the same duty of confidentiality. This may include people who conduct quality assurance and quality control checks to confirm that the research was done correctly. These people may include research ethics committees, inspectors, monitors and auditors.

Who has reviewed this study?
All research in the University is looked at by an independent group of people called a Research Ethics Committee. The Research Ethics Committee is made up of experts, non-experts and members of the general public. Together they review research applications to ensure your safety, rights, wellbeing and dignity are protected at all times.

This study has been reviewed and approved by Keele University Ethics Committee.

What will happen to the study results?
This work forms part of a research. It is intended that the results of the study will be presented at conferences and published in medical journals so that we can explain to the medical community what our research results have shown. Your child's name will *never* appear in any report or publication arising from this study.

Who is organising and funding the research?
This research is being carried out and supervised by Dr. Fran Gilchrist and Professor Anand Pandyan.

Contacts for further information

For more information about this study, you can speak to a member of the research team to

Dr Fran Gilchrist:
Children's Department
University Hospitals of North Midlands
Royal Stoke University Hospital

Newcastle Road, Stoke-on-Trent, ST4 6QG

Tel: **01782 675289**

Professor Anand Pandyan:

Professor of Rehabilitation Technology & Head of School
School of Health & RehabilitationPhysiotherapy

Keele University, Staffordshire, ST5 5BG

Tel: **01782 734252**

Ms Nicola Leighton

Research Governance Officer

Directorate of Engagement & Partnerships

Academic Legal Services

Keele University Science & Innovation Park | Keele University | ST5 5NH

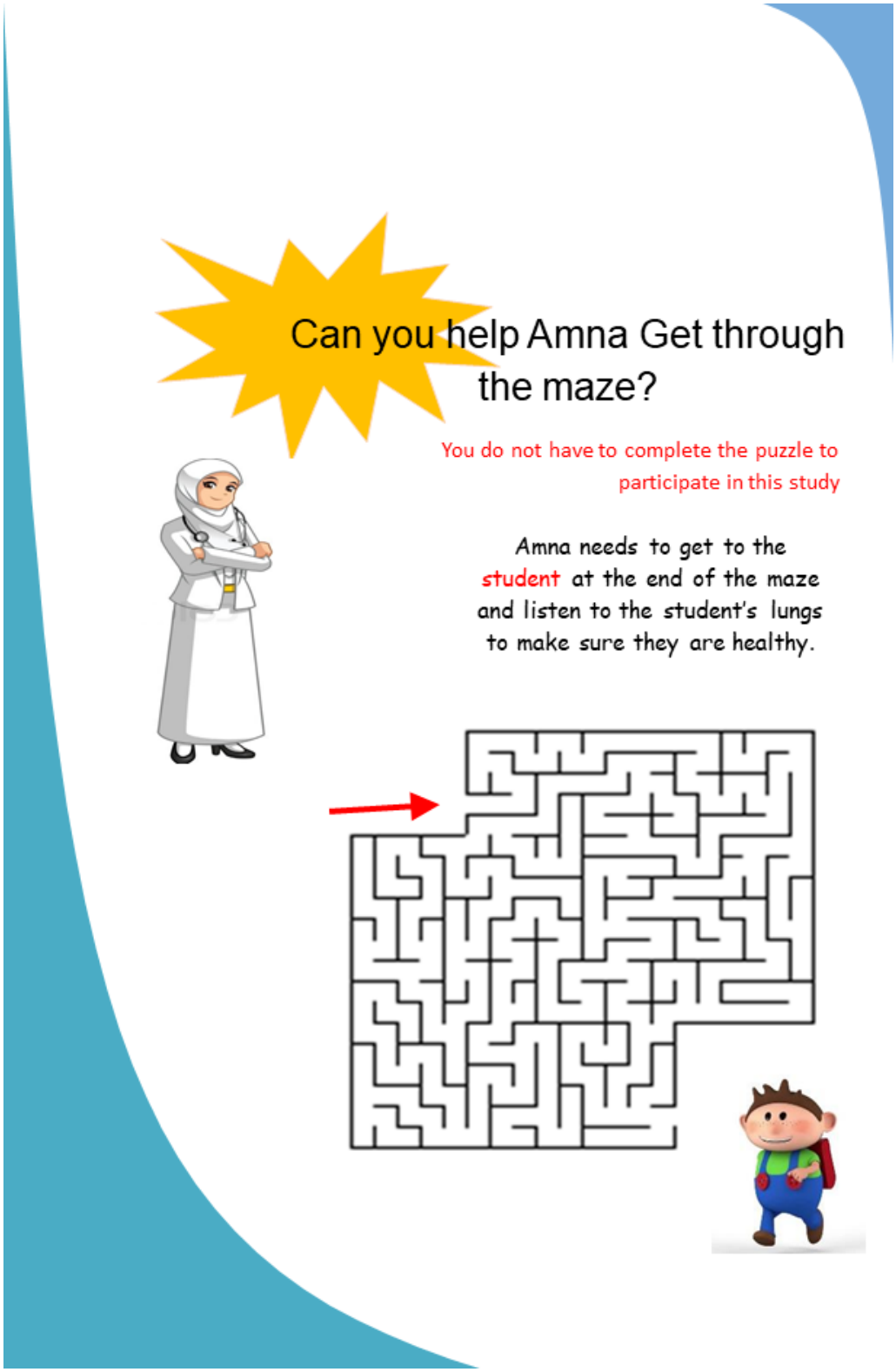
Tel: 01782 733306 | www.keele.ac.uk

Who will be happy to answer any questions or concerns you might have.

Thank you for reading this information sheet.

Adaptive from LCI-Rescue, with THANKS to Dr Alex Horsly

9.3 Appendix 3



School of Health and Rehabilitation and The Institute for Science & Technology in Medicine

LCI-NORM

Information Leaflet for Healthy Children

For children aged 5-18 years

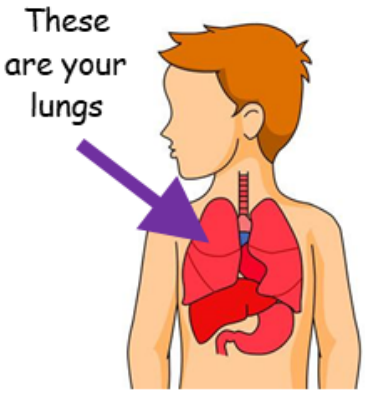
Hello!

You are invited to take part in the **LCI-NORM**
This leaflet will tell you what the study is about.

What is research?

Research involves collecting information to find out more.
It's a bit like homework for grown-ups.

Why are you doing this research?



Can you see the little boy's lungs?

Your lungs help you to breathe.

In this study we are collecting information about how well your lungs work compared to children who sometimes find it hard to breathe

What will I need to do if I help?

If you would like to help we will ask your mum or dad, or a grown up looking after you, if you can take part in a special test.

The test involves breathing into a tube with special gas in it with a RESEARCHER in the room. Your mum, dad or a grown up looking after you (e.g. your teacher) will be with you all the time. You will get to watch the television whilst I do my homework and collect the information I need.

You might get asked whether you thought the test was good or not afterwards as well.

Do I have to help?

No, only if you want to and your mum, dad or grown up looking after you say it's okay too.

Will anything hurt me?

Children with sticky lungs have taken part in the special test before and did not tell us that it hurt them.

Thank you for reading this leaflet

Adaptive from LCI-Rescue, with THANKS to Dr Alex Horsly

9.4 Appendix 4

Keele University

School of Health and Rehabilitation and The Institute for Science & Technology in Medicine

LCI-NORM

School of Health & RehabilitationPhysiotherapy

Keele University, Staffordshire, ST5 5BG

Tel: **01782 734252**

Participant Consent Form – PARENTS (HEALTHY CHILD)

Version 1.0 Dated: 01.09.2017

Participant Initials: ..... Study number: .....

Instructions on completing this form:

i) Use a **black** pen.

ii) Please read the following statements carefully, and **initial the box** if you agree.

Eg.	I confirm that I have read and understand the Participant Information Sheet.	JB
1.	I confirm that I have read and understand the LCI-NORM Participant Information Sheet, version 1.1 dated 01.09.2017. I have had the opportunity to consider the information and ask questions, which have been answered satisfactorily.	
2.	I understand that my child's participation is voluntary. I may, at any time, withdraw my child from the study without giving any reason.	
3.	I give my consent for my child to undergo LCI measurements on a single occasion	
4.	I wish to receive written information on the study findings at the end of the study	

Parent name: ..... Signature: ..... Date: / /

Researcher name: ..... Signature: ..... Date: / /

1 copy for participant; 1 copy for researcher notes;

Parent Consent Form- Healthy Child, Version: 1.0 Dated: 01.09.2017

Adaptive from LCI-Rescue, with THANKS to Dr Alex Horsly

9.5 Appendix 5

School of Health and Rehabilitation and The Institute for Science & Technology in Medicine

LCI-NORM

Assent Form - HEALTHY CHILDREN

Version 1.0 Dated: 01.09.2017

School of Health & RehabilitationPhysiotherapy

Keele University, Staffordshire, ST5 5BG

Tel: **01782 734252**

Child's Initials: ..... Study number: .....

Instructions on completing this form:

i) Use a **black** pen.

ii) Please read the following statements carefully, and **initial the box** if you agree.

Please read the following statements and **tick the happy face** if you are happy to carry on.

1.	Have you read (or had read to you) the information about this study?	☺
2.	Do you understand what this study is about?	☺
3.	Have you asked any questions you want to?	☺
4.	Have you had all of your questions answered in a way you understand?	☺
5.	Do you understand that it's OK to stop taking part at any time?	☺

Child's name: ..... Signature: ..... Date: / /

Researcher name: ..... Signature: ..... Date: / /

1 copy for participant; 1 copy for researcher notes;

Parent Consent Form- Healthy Child, Version: 1.0 Dated: 01.09.2017

Adaptive from LCI-Rescue, with THANKS to Dr Alex Horsly

9.6 Appendix 6

To the Head teacher dd/mm/yyyy

Dear …….,

I am a student reading for a PhD in Keele University with Dr Fran Gilchrist (a local paediatric respiratory consultant in UHNM) and Professor Anand Pandyan (from Keele University). My study aims to measure lung function in children and explore what factors (e.g. living next to the main road, smoking/exposure to cigarette smoke, physical activity etc.) affect the measures of lung function.

I am looking for potential participants for this study. Would it be possible for us to meet in person to discuss if it is possible to use your School as a site to recruit participants for my study? This letter outlines the measurements I propose to undertake in this research study.

I will be using two methods to measure lung function:

The spirometer (the current standard of measurement) and a newer technique called the Multiple Breath Washout test. The newer method is easier on the children, however, the child will need to breathe an inert gas which is proven to be safe. The time to take both measurements is approximately 30 minutes. I am in a position to take all the measurements in your school.

In order to conduct this study, I would require the following support from your school:

- Provision of an area to collect my data
- The help of an assistant who is familiar with the children to help with measurements (a stipend will be available to this person or the school to cover the costs)
- As I do not have contact details for children and the parents/guardians, your support would be needed in sending out study-related information and paperwork via the students to the parents/guardians on my behalf. (N.B. I will bring copies of all relevant documents that I will be using to advertise this study to our meeting).

Just to reassure you I will not start this study until I first have your permission and the permission from the University. The University approval processes will confirm that this study meets all the ethical requirements as currently required by law. Currently, this study is estimated to start in (to be confirmed). I will call you in the next week to arrange for a meeting to discuss the potential of you supporting this study.

Yours sincerely,

Amnah Alrumuh (a PhD student)

a.k.a.alrumuh@keele.ac.uk

Mackay Building

School of Health & Rehabilitation

Keele University, Staffordshire, ST5 5BG

Tel: +44 (0) 1782 734191

9.7 Appendix 7

The LCI-Norm Study

This questionnaire has to be filled by the participant's parents/care taker.

Child' name: Study ID No: Gender Weight

Height DOB Address Post code

-Does your child currently receive antibiotics for a lower respiratory infection? Yes

No

- Is your child diagnosed with asthma, viral induced wheeze, protracted bacterial bronchitis, CF, or any other chronic respiratory condition?

Yes No

- Has your child used anti-asthma medication in the last 3 months prior to the study? Yes

No

***if you answer 'yes' to any of previous questions, unfortunately your child can not take part in this study**

Would you answer these questions?

1- Was your child born full term (>36 weeks)? Yes
No
If No, how many weeks? ……..……..

2- Is your child exposed to cigarette smoke at home? Yes
No
If yes, for how long has he/she been exposed to cigarette smoke?

(Month/year)…….……

3- Does your child smoke? Yes
No

If yes, for how long has your child been smoking? (Month/

year)…….……..

4- Does your child have a free meal at school? Yes
No

5- Do you live near a main road? Yes
No

6- Has your child been hospitalised for respiratory/breathing problem/infections before age of 2?
Yes No
If yes, for how many times? ……..……..

7- Which one of the following describes your child activity out of school hours (e.g. played sports, went running, swimming, bike riding, did aerobics) best for the last 7 days?

Read all five statements. Before deciding on the one answer that describes him/her.

- None 0 hours
- Little bit 1 hour/week
- Medium two and half hour/week
- Often 5 hours/week
- Very often > 5hours/week

9.8 Appendix 8

Name of the School	Address	Head teacher	E mail
Woodhouse Academy **Tel: 01782 973600**	81 Woodhouse Ln, Biddulph, Stoke-on-Trent ST8 7RL	Ms Claire Butler	principal@woodhouseacademy.staffs.sch.uk>
JCB Academy **Tel: 01889 506100**	Mill St, Rocester, Uttoxeter ST14 5JX, United Kingdom	Mr Jim Wade	https://jcbacademy.com
Forsbrook CE Primary School **Tel: 01782 392577**	Cheadle Rd, Stoke-on-Trent, Staffordshire ST11 9PW	Mrs Natasha Richards	office@forsbrook.staffs.sch.uk
Thistley Hough Academy **01782 883 500 (Main Office)**	Thistley Hough, Penkhull Stoke-on-Trent, ST4 5JJ	Holly Hartley	Secondary office@thistleyhough.org.uk
St Margaret Ward Catholic Academy **01782 883000**	Little Chell Lane Tunstall Stoke-on-Trent ST6 6LZ	C.Smith	office@smwca.org.uk
Harpfield Primary Academy **01782 234984**	Palmers Green Hartshill Stoke on Trent	Mr R T Woollacott	Primary enquiries@harpfieldprimaryacademy.org.uk

	ST4 6AP		
Sutherland Primary **01782 594133**	Beaconsfield Dr, Stoke-on-Trent ST3 3DY	Garry Boote	sutherland@sutherlandacademy.co.uk
Hillside Primary **01782 235350**	Stoke-on-Trent ST2 7AS	Mrs N Morris	office@hillsideprimary.org.uk
Forrest Park **01782 234979**	Woodall Street Hanley Stoke on Trent Staffordshire ST1 5ED	Miss S L Irving	admin@forestpark.org.uk
Sneyd Green Primary School **Tel: 01782 234460**	Sneyd Street, Burslem, Stoke-on-Trent, ST6 2NS	Mrs Rosina Lee	sneydgreen@sgfl.org.uk
St Mary's Catholic Academy **01782 234820**	Norton-Le-Moors, Ford Green Rd, Stoke-on-Trent ST6 8EZ	Mr I. Beardmore	stmaryrc@sgfl.org.uk
St John the Evangelist Catholic Academy	The Avenue Kidsgrove	Mrs H Butters	headteacher@stjohnskidsgrove.org.uk

01782 296736	Staffordshire ST7 1AE		
St Wilfrid's Catholic Academy, **Tel: 01782 235676**	Queen's Avenue, Tunstall, Stoke-on-Trent ST6 6EE	MRS MASON	office@stwilfridsnewman.co.uk

9.9 Appendix 9

University Hospitals of North Midlands NHS
NHS Trust

ACADEMIC DEPT of PAEDIATRICS
Women & Children's Diagnostics Centre
Paediatric Respiratory Offices, PAEDS127
Newcastle Road
Stoke on Trent
ST4 6QG
Tel: 01782 675289

Email: francis.gilchrist@uhns.nhs.uk

Department of Paediatrics

21st June 2016

Dear Professor Pandyan

I am writing to confirm the loan of our Innocor Gas Analyser to the School of Health Rehabilitation, Keele University. The duration of the loan is from 1st September 2017 and 30th August 2018. This is to allow the measurement of lung clearance index in healthy children as part of LCI-NORM. The equipment is for use on Wednesdays, Thursdays and Fridays.

Yours sincerely

Dr Francis J Gilchrist
Senior Lecturer and Consultant in Paediatric Respiratory Medicine