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The Relationship Between Added Lung Sounds And Airway Dimensions

by

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ABSTRACT

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The aim of this study was to explore potential relationships between added lung sound characteristics measured by Computer Aided Lung Sound Analysis (CALSA) and airway dimensions measured by High resolution Computed Tomography (HRCT). CALSA has been proposed as a new objective measurement to record and analyse lung sounds. However, there is still a lack of evidence as to whether and how added lung sounds relate to the geometry of airways. HRCT is considered to have the highest sensitivity of imaging measurements and is capable of generating three dimensional pictures of airways from which the dimensions may be measured. Twenty-six participants (9 healthy non-smokers, 9 healthy smokers and 8 patients with COPD) were recruited. Lung sound data were recorded using a digital stethoscope. HRCT scans were conducted using a Siemens Sensation 64 CT scanner and the resulting data were analysed using the Pulmonary Workstation 2 software to give airway dimensions. Lung sounds were characterised in terms of Crackle 2-cycle durations (crackle 2CD), the number of crackles per breathing cycle (NCpB) and lung geometry were characterised in terms of airway diameter, length, branching angle, internal perimeter, wall thickness and percentage of wall area. The analysis showed that there was a significant positive correlation between crackle 2CD and airway wall thickness at generations 3 and 5. Crackle 2CD also significantly correlated with the branching angles at the main bronchus and at generation 3. There was also a significant negative correlation between NCpB and percentage of wall area at generation 2 and airway wall thickness at generation 5. Moreover, NCpB recorded at anterior right region of chest wall was found to predict the percentage of wall area at the right upper bronchus. These initial results suggest NCpB might be useful to predict changes in percentage of wall area caused by the chronic inflammation of the main bronchi, though a larger sample size would be needed to confirm it. This suggests that crackles could potentially be used as a biomarker of COPD.

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DECLARATION OF AUTHORSHIP

I, Surussawadi Mackawan

declare that the thesis entitled

The Relationship Between Added Lung Sounds And Airway Dimensions

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

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Definitions and Abbreviations

CALSA Computer aided lung sound analysis

HRCT High resolution computed tomography

COPD Chronic obstructive pulmonary disease

FEV_1 Forced expiratory volume in one second

FVC Forced vital capacity

IDW Initial deflection width

2CD Two cycle duration

Fmax Frequency of maximum intensity

F25 Frequency at a quarter in power spectrum intensity

F50 Frequency at a half in power spectrum intensity

RMS Total spectral power in root mean square

WA Wall area

WA% The percentage of wall area

IA Internal area

Ai Airway luminal area

Chapter 1 : Introduction

1.1. Introduction

This chapter begins with a brief introduction to the motivation and justification for this research aiming to determine the validity of using Computer Aided Lung Sound Analysis (CALSA) to detect abnormalities in airflow limitation diseases in comparison to the gold standard of imaging using high resolution computed tomography (HRCT). An overview of this thesis will then be presented.

Respiratory disorder has been reported to be a major cause of illness globally (Bousquet et al. 2007). Chronic Obstructive Pulmonary Disease (COPD) is predicted to become the third leading cause of death worldwide by the year 2020 (Murray & Lopez 1997). Additionally, the number of people diagnosed with interstitial lung disease has been increasing, while pulmonary infections such as pneumonia and acute bronchitis continue to be diagnosed commonly (Sovijärvi et al. 2000c). There is therefore a need for improved means of diagnosis.

In order to diagnose respiratory diseases, there are many possible measurements ranking from the basic non-invasive to the complicated invasive methods. Among these, standard auscultation has been the simplest tool used by the clinician (Loudon & Murphy 1984; Sovijärvi et al. 2000c). However, auscultation relies heavily on the skill and training of the individual clinician and therefore offers subjective results with a high degree of inter- and intra-operator variability (Grenier et al. 1998; Murphy 2007). Computerised methods for recording and analysing lung sounds, in contrast, can provide objective information, with little or no inter- and intra-operator variability to aid diagnosis of many cardiopulmonary diseases (Sovijärvi et al. 2000c; Murphy 2007; Murphy 2008; Gurung et al. 2011). It has also been shown that it is possible to monitor the process of lung diseases (Piirilä 1992) and the effectiveness of treatments (Pasterkamp et al. 1997a; Beck et al. 2007).

However, although different lung sounds have been found to be associated with various pathologies, very little is known about their relationship with the underlying geometry of the airways. By using CALSA and comparing the data to

imaging data it may possible to explore this relationship. The aim of this research was to compare data derived from CALSA with data derived from high resolution computed tomography (HRCT). This gold standard imaging technique is able to detect abnormalities of the respiratory system and can show cross sectional data of tissues. From these data, three dimensional models of the bronchial tree can be derived and measurements of the airways can be estimated. If relationships between the data derived from CALSA and those from HRCT could be found, it would support the application of CALSA to aid diagnosis and monitor respiratory diseases. This study also aimed to be able to make useful lung sound recordings in a clinical setting in order to demonstrate the application in a real clinical environment.

1.1.1. CALSA, HRCT and COPD overview

CALSA is a method used to objectively quantify the acoustic properties of lung sounds (Sovijärvi et al. 2000c; Murphy 2008; Gurung et al. 2011). Lung sounds can be recorded very simply using a digital stethoscope connected to a portable computer or a personal digital assistant (PDA) and transferred to a digital format for analysis. At the time of writing, the analysis is not possible in real time but requires storage and analysis offline. Using CALSA it should be possible to detect abnormal sounds in patients with lung disease, which would be useful for both diagnosis and monitoring the effectiveness of medical treatments (Piirilä 1992; Pasterkamp et al. 1997a; Sovijärvi et al. 2000c; Gurung et al. 2011). It has been hypothesised that CALSA might provide an accurate, non invasive, bedside measurement of lung function requiring minimal patient collaboration (Marques 2008).

The addition of CALSA to spirometry has been reported to increase the sensitivity of spirometry as an objective screening test for the presence of respiratory dysfunction (Gavriely et al. 1994). In a study which involved 91 patients with COPD and 12 with restrictive lung disease, the overall sensitivity to detect abnormalities increased from 71% to 87% when they combined the information from both measurements. Abnormal lung sounds were also found in 32 of 388 normal subjects (screened by a pulmonologist). This was initially presumed to reflect a number of false positives. Then these 32 were invited to be re-examined at 12–18 months after the first tests. In 21 of 24 follow-up examinations published they still presented with abnormal lung sounds and 3

of them had developed a diagnosable lung or heart disease. Thus, CALSA could provide an early sign of lung or heart disease and the combination of spirometry and CALSA significantly improved the sensitivity to detect respiratory dysfunction (Gavriely et al. 1994). CALSA may also be able to reflect more closely abnormalities of airway geometries than forced expiratory volume in one second (FEV_1). Schreur et al (1994) found that there was a significant decrease in lung sound intensity in nine symptom-free subjects with mild asthma when compared with 8 healthy subjects, while FEV_1 remained unchanged in both groups Thus, CALSA may be able to provide valuable additional objective data to aid early diagnosis of some pulmonary diseases (Schreur et al. 1994).

HRCT has been suggested to be a gold standard in detecting focal and diffuse lung diseases with the highest sensitivity and specificity among other imaging techniques (Müller 2001). It can also provide indirect information reflecting narrowing of the small airways by determining quantitative changes in lung density between inspiration and expiration (Kalender 2000). The measurement of airway wall dimensions is useful in COPD because it is known that chronic airflow limitation can be caused by a combination of airway and parenchyma changes (Coxson 2008). CT imaging can also show three-dimensional geometry of the human airways down to around generation six (Sauret et al. 2002). This may provide useful data to explain the pathogenesis of COPD in the peripheral airways. The variables measured from the three dimensional data include airway diameter, airway length and airway branching angles any of which may also be changed in COPD patients and may affect the mechanism of airflow that generates breath sounds in the airways (Schreur et al. 1992; Schreur et al. 1994; Malmberg et al. 1995). However, despite the many advantages of HRCT, it is prohibited by accessibility and cost, making mass screening impractical. Additionally, exposing the patient to the dose of radiation given by a HRCT scan may cause tissue damage. This also limits the application of HRCT because of this safety issue.

COPD is defined as an airflow limitation disease which causes significant morbidity and mortality of people worldwide (Rabe et al. 2007). COPD is an expensive disease. The cost of COPD treatment in both Europe and the United States has increased gradually since 2002. In the United States, for example, indirect costs of COPD were $18 billion and direct costs were $14.1 billion in

2002 (National Heart Lung and Blood Institute 2004). In the European Union, the costs of COPD were around €38.6 billion annually. In the United Kingdom, each COPD patient costs an estimated £1,639 annually, equating to a national burden of £982m (Devereux 2006). Additionally, the increase of this cost depends on the severity of diseases. It has been reported from a prospective study in 1,510 COPD patients in Spain that the expense of severe COPD patients was almost double those of mild stage (Miravitlles et al. 2003). Thus, if COPD could be detected at an early or mild stage of disease and appropriate intervention provided it may help to decrease the cost of overall health care. Moreover, Devereux claimed that the average life expectancy of someone with severe COPD in the UK, when compared with a control group, was reduced by 4.1 years, while this number in mild stage reduced by 1.1 years (Devereux 2006). If COPD can be detected at the mild stage of disease and proper intervention provided it could also improve the life expectancy of the patients.

Lung diseases like COPD place a considerable burden on individuals and society, affecting health, occupation and social roles (Rabe et al. 2007). Despite falling smoking rates, with the projected rise in the number of older people, COPD prevalence is increasing (Jones 2006). “Under-diagnosis and under-treatment contribute to the growing burden of human misery and healthcare costs (Jones 2006) p 652). Key interventions such as smoking cessation are most likely to be successful in early disease. It is therefore necessary to devise simple, safe, reliable and valid measures of early pathological changes. It is hypothesised by the researcher that these changes may be detected by lung sound analysis. The relationship between structural airway geometry and lung sound variables in healthy subjects is currently not known. It is also not known if lung sounds change predictably with changes in airway geometry through pathology. It is hypothesised that lung sound analysis could provide a non-invasive measure to detect early abnormalities. Therefore, this research aims to investigate whether quantitative data from lung sound analysis has any relationship with airway geometry measured using HRCT in either healthy people or in people with known pathological changes.

1.2. Thesis overview

This thesis begins with the literature review presented in chapter 2. Normal/abnormal lung sounds and their respective characterisation, the development of CALSA and CALSA as a measurement to detect airways obstruction are addressed. The introduction to HRCT as a standard imaging technique in COPD, the principles of CT scan working followed by the development of HRCT are also included. The parameters measured by HRCT in COPD are discussed. Then, the possible correlation between HRCT and CALSA is evaluated. Chapter 3 presents the equipment and methodology used in the study. Chapter 4 presents the results and findings. Then, a discussion of research findings is presented in chapter 5. Finally, the conclusion of this study and summary of the main areas suggested for further work are discussed in chapter 6.

Chapter 2 : Literature Review

2.1. Introduction

This chapter will present a critical overview of computer aided lung sound analysis (CALSA) and investigated its ability to detect airway obstruction. The basic knowledge in normal and added lung sounds will be presented first, followed by the mechanism of airway closure relating to crackles and airway wall mechanics. The development and role of CALSA in various pulmonary research studies will be included. Then the currently available methods for recording lung sounds and the procedure for their analysis will be discussed. The characteristics of both normal and added lung sounds in COPD will be included. CALSA as a measurement to detect airway obstruction will be addressed. The principle of CT scan working will be introduced to the reader followed by the history and growth of HRCT. The parameter measured by HRCT in COPD will be determined. Then previous research studies involving HRCT in COPD will be reviewed. Finally, the possibility of comparing HRCT data with CALSA data will be evaluated.

2.2. Lung sounds

Lung sounds have a long history since Laënnec invented the standard stethoscope in 1816. However, the difficulty of describing lung sounds using only the clinician's judgement means that there was a lack of formal definition of lung sounds until the year 2000. In this year, the EU BIOMED I programme and the European Respiratory Society (ERS) task force provided funding for the Computerized Respiratory Sound Analysis (CORSA) projects to standardise computer lung sound recording and analysis methods to create standard guidelines in order to compare the results between different institutes and also allow commercial development of respiratory sound analysis equipment to be possible (Sovijärvi et al. 2000c).

This process brought together a group of experts in the field from Belgium, Finland, Britain, Italy, Germany and the Netherlands. They examined around 320 original and review articles in international scientific journals, official guidelines (European Respiratory Guidelines for Lung Function Studies, American Thoracic Society publications) position papers as well as statements

and reports of international societies (Sovijarvi et al. 2000a). The CORSA project has provided a standardised approach to recording and analysing lung sounds which has been followed by several authors since 2000 (Piirila et al. 2000; Elphick et al. 2004; Marques 2008; Parthak et al. 2008; Marques et al. 2009).

The CORSA projects have provided a formal definition of terms used in lung sound analysis, environment and patient preparation and respiratory sound capturing and analysis procedures. The first author on many of the CORSA guidelines is Sovijarvi (Sovijarvi et al. 2000a; Sovijarvi et al. 2000b; Sovijärvi et al. 2000c). Although these are not the only possible definitions or procedures, they have been followed within this thesis because they represent the consensus of experts based on the best available knowledge at the time.

2.3. Definition of lung sounds

CORSA defined lung sounds as sounds which are heard over or within the chest wall including normal lung sounds and added lung sounds (Sovijarvi et al. 2000a). The detail of each type of lung sounds such as their origin and characteristics will be reviewed in the following sections.

2.3.1. Normal lung sounds

Normal lung sounds are defined as sounds arising from breathing which are detected over the chest wall of healthy people (Gavriely et al. 1995; Pasterkamp et al. 1997b; Sovijarvi et al. 2000a). They are also characterised by a quiet, low frequency sound during inspiration and are hardly audible during expiration (Sovijarvi et al. 2000a). The acoustic properties of normal lung sounds presented both low and high frequency sounds with a range between 60 and 1,000 Hz (Pasterkamp et al. 1997b).

Normal lung sounds have been reported to provide information about regional ventilation and pathophysiological changes in the airways (Pasterkamp et al. 1997b) but no objective evidence has been published to support the statement. Therefore, it is still unclear whether the sound generated in the airways and transmitted through the chest wall during breathing can reflect the degree of lung ventilation and changes in the configuration of the airways.

2.3.1.1. Origin of normal lung sounds

There may be many mechanisms involved in generating normal lung sounds, such as velocity and direction of airflow (Kraman & Wang 1990) and differences of air pressure and volume among structures of the chest wall (Sovijarvi et al. 2000b). However, a change of airflow rate and direction along the geometry of the bronchial tree, which creates turbulent flow, has been believed to be the main factor to generate normal lung sounds at different generations of the airways (Dalmay et al. 1995). It is believed that the turbulence intensity depends on the dimensions of the conducting airways, which bifurcate into smaller daughter bronchi, and also the airflow velocity (Dalmay et al. 1995; Sovijarvi et al. 2000b). In larger and medium airways, for example, such as the trachea and proximal bronchi the velocity of airflow is very high. Consequently, this high speed of airflow can generate turbulence and hence lung sounds. In smaller airways, it is believed that the speed of airflow decreases to a value less than the critical point to generate turbulence. Thus, airflow in such areas is laminar and generally does not generate any sound (Dalmay et al. 1995). One study explored the origin of inspiratory and expiratory sounds using an array of 16 microphones and applied an algorithm to localise the origin of inspiratory and expiratory sound signals (Kompis et al. 2001). They found that inspiratory sounds predominantly originate from the peripheral airways, whereas the expiratory sounds come from more central airways. However, their sample size was very small, consisting of only four healthy males and one male child with lung consolidation, so their findings may not be representative of the general population and it would not be possible to demonstrate any statistically significant difference in the distribution of the lung sounds with the disease as they have used subjects of different ages (healthy adults vs. unhealthy child) and only one unhealthy subject. Therefore, there is still a need to verify whether inspiratory and expiratory sounds are generated from the different airway generations.

Sound transmission from mouth to chest wall is influenced by the anatomical variation between individuals (Kraman & Austrheim 1983) and its propagation and attenuation are dependent on the density of the pulmonary parenchyma and the chest wall, which together function as a low pass filter (Pasterkamp et al. 1997b). Additionally, age, gender (Gross et al. 2000), height, and body size

(Pasterkamp et al. 1997b) have been reported to affect the intensity of lung sounds.

From the literature, normal lung sounds result from turbulent flow within the airways. The airflow in the small airways is believed to be laminar and therefore does not contribute to normal lung sounds. However, any lung disease which alters the properties of the airways, such as their diameter, will alter the flow and could therefore have an effect on the generation of normal lung sounds. In theory there is therefore a possible link between normal lung sounds and airway diameter, but this has not been explored. The reason behind this may be that there is no objective measurement to show airway geometry and then relate those measurements to normal lung sounds. Thus, more research is needed in this area to validate the proposed claims mentioned above, particularly that inspiratory and expiratory breath sounds originate from different areas of the lung and that lung diseases which alter the airway geometry may alter the characteristics of the normal breath sounds.

2.3.2. Added lung sounds

Added lung sounds, in contrast, are divided into two categories consisting of discontinuous sounds (crackles) and continuous sounds (wheezes). They are found in many cardiopulmonary disorders (Sovijarvi et al. 2000b) and their characteristics have been reported to provide clinically significant meaning (Murphy 2008).

2.3.2.1. Crackles

Crackles are intermittent, short and explosive sounds which have been reported to be the most frequent of added lung sounds that are indicative of pulmonary diseases (Loudon & Murphy 1984; Piirilä et al. 1991; Piirila & Sovijarvi 1995; Murphy 2008).They have a wide range of frequencies between 100 and 2,000 Hz or higher with duration less than 20 ms. Crackle duration is a continuous variable, but crackles have conventionally been divided by clinicians into two groups i.e. coarse crackles and fine crackles depending on their intensity, frequency and duration.This division into two types (coarse and fine crackles) is believed to reflect different types of lung diseases. For example, fine crackles are found frequently in pulmonary fibrosis while coarse crackles are commonly detected in COPD and congestive heart failure (Piirilä et

al. 1991). Although crackles are mostly associated with lung diseases, they were also found in healthy adults (median age 21.33 years, range 19–33 years) during slow inspiration from residual volume (Workum et al. 1982). There may therefore be some association between crackles and lung volume (Workum et al. 1982).

2.3.2.1.1 Origin of crackles

There have been several theories to explain the mechanism to generate crackles; sudden reopening of airways for inspiration crackles and gradual closure of airways for expiration crackles (Forgacs 1967), pressure equalisation or the change of elastic stress after sudden reopening of abnormally closed airways (Sovijarvi et al. 2000b), sudden airway closure for expiratory crackles and sudden airway reopening for inspiratory crackles (Vyshedskiy et al. 2009) and airway wall stress-relaxation (Pasterkamp et al. 1997b). The current consensus is that crackles are caused by the sudden reopening or closing of abnormally closed airways in pulmonary or cardiology disorders such as inflammation and/or oedema (Kiyokawa et al. 2001).

The characteristics of crackles are therefore likely to depend on the dimension of airways as they close or open. Smaller peripheral airways are believed to generate shorter duration crackles than central airways where crackles have longer duration and are generated in early inspiration (Piirila & Sovijarvi 1995). At one time coarse crackles were believed by Loudon and Murphy (1984) to originate from the bubbling of air through secretions in proximal airways such as bronchi or bronchioles while fine crackles were claimed to be caused by the sudden reopening of small airways such as alveolar ducts and alveoli (Loudon & Murphy 1984). However, both studies demonstrated no objective evidence to show that different types of crackles come from different generations of the airways. Thus, it is questionable whether coarse crackles arise from more central locations than fine crackles. If it is possible to test the theory that different type of crackles come from different airways it would be possible to use crackles to localise the origin of airway diseases. Therefore, further research is needed to verify this hypothesis.

2.3.2.2 Wheezes

Wheezes are defined by CORSA as continuous adventitious sounds with a musical character and frequency more than 100 Hz and duration more than 100 ms (Sovijarvi et al. 2000b). They are usually superimposed on normal breath sounds, having sinusoidal wave forms, and can be described as monophonic or polyphonic depending on the number of frequencies which are detected.

Wheezes are also found in many other conditions such as bronchospasm, narrowing of airway, mucosal oedema, intraluminal tumour, secretions, foreign bodies, external compression by masses, or dynamic airway compression. There are several known disorders, for example, which are associated with wheezes such as infections, tracheobronchitis, laryngomalacia, bronchiectasis, laryngeal tumours, cystic fibrosis, tracheal stenosis, emotional laryngeal stenosis, asthma, COPD, pulmonary oedema, or even forced expiration in healthy people (Meslier et al. 1995).

2.3.2.2.1 Origin of wheezes

The mechanisms for generating wheezes are still unclear. Movement of secretions is one of the possible sources, but the flutter of airway walls may also be prominent (Pasterkamp et al. 1997b). The fluttering model claims that wheezes are probably generated by the fluttering of the airway walls and fluid together, induced by the acceleration of the gas flow through the collapsible tubes in both peripheral and central airways. Due to insufficient flow velocity, one study claimed that wheezes cannot be generated after the 7^{th} generation of the airways (Sovijarvi et al. 2000b). However, there is no objective evidence to support their claim, especially in a clinical study. Thus, the study to explore whether wheezes come from specific levels of airway generation using an objective measurement which can show the picture of airways is necessary to verify the claim.

The airway wall oscillation appears when the airflow velocity reaches a critical point which is called the flutter velocity. In this model, wheezes always occur with flow limitation, but flow limitation can exist without giving rise to wheezes. The fluid dynamic flutter theory mentioned above has been used to describe expiratory wheezes but there still is no theory for inspiratory wheezes (Grotberg & Gavriely 1989).

2.4. The mechanism of airway closure relating to crackles and airway wall mechanics

Crackles have been reported to be the most frequent sound which are found in many pulmonary diseases (Piirila & Sovijarvi 1995). Crackles are believed to be generated by the reopening and closing of closed airways (Kiyokawa et al. 2001). In order to understand this mechanism it is first necessary to consider the basic mechanism of airway closure before it can re open and close again and hence generate crackles.

There is a thin liquid film which lines pulmonary airways from the trachea until the alveoli. This liquid film at alveoli level has been believed to assist the transportation of gases between pulmonary circulation and the inspired air (Heil et al. 2008). The surface tension between the thin film and the air space affects the mechanics of the lung and also influences around 50-60% of the elastic recoil of the lung (Heil et al. 2008). This surface tension can be decreased by pulmonary surfactant which also facilitates the fluid to extend over the airway surface. The liquid film is a thin and quite uniform layer on the airway wall, however it is able to cause blockages of the airway (Heil et al. 2008). The basic mechanism of airway closure depends on the surface tension acting at the interface between the liquid lining and the air in the lumen (Heil et al. 2008). A simple model of an elastic airway (Heil et al. 2008) is shown in Figure 2:1.

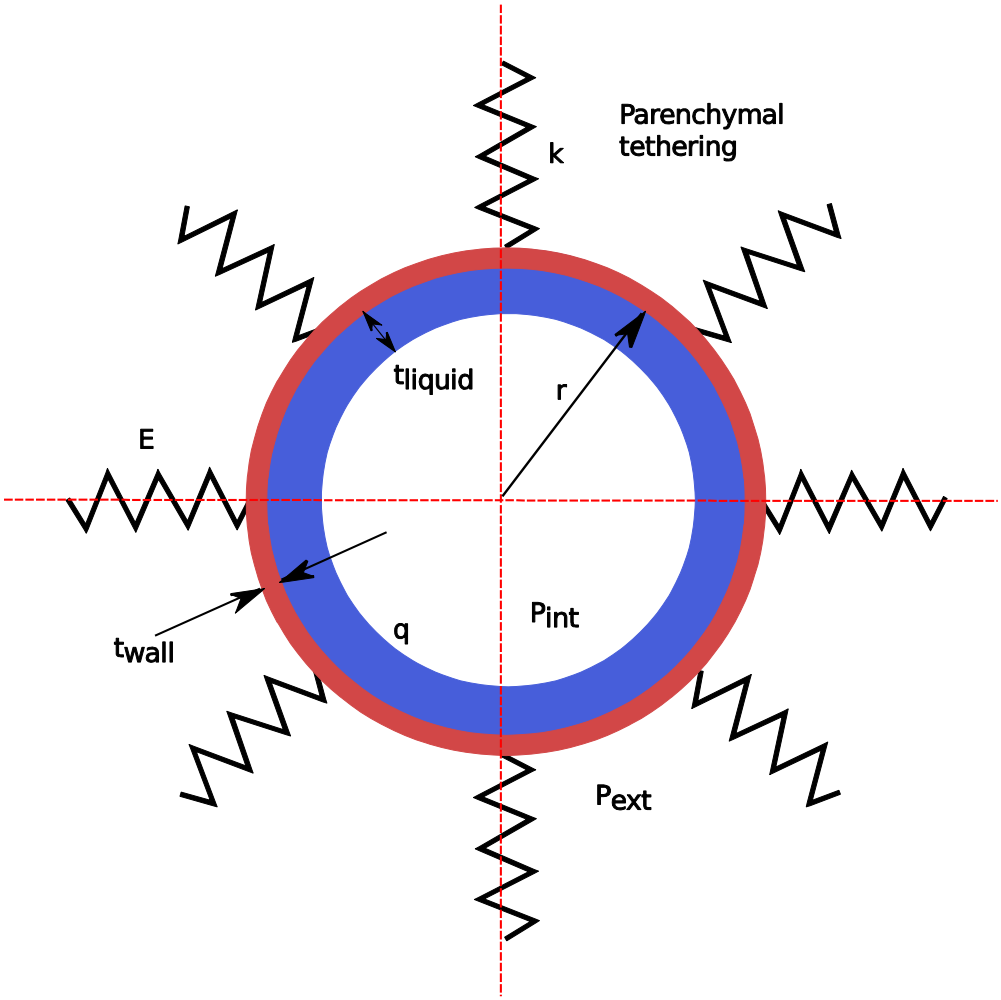


Figure 2:1: A simple model for pressure load on airway wall (Heil et al 2008 with permission)

Where, E= Young's modulus, q= surface tension, r= radius of the airway, Pint= airway pressure in the lumen, Pext= pleural pressure or external pressure, twall= airway wall thickness, tliquid= liquid thickness, k= elastic springs stiffness

The airway is represented here as an idealised, straight circular tube with radius r, lined with a thin static film of uniform thickness. The surface tension acting at the curved interface causes pressure across the interface and the fluid pressure (P*fluid*) is given by the Young–Laplace equation.

$$P_{fluid} = P_{int} - qc \quad \text{Equation (1)}$$

Where P*fluid* = fluid pressure, P*int*= air pressure in the lumen, q= surface tension, c= the mean curvature of the air–liquid interface. Equation 1 demonstrates that spatial variation in the interfacial curvature causes pressure gradients in the fluid. If the uniform liquid lining is disturbed, the induced pressure gradients drive flows that will redistribute the fluid and if the pressure is adequate this may pull the fluid together and cause a fluid bridge in the airway which can lead to airway closure via the film collapsed mechanism (Kamm & Schroter 1989). The process of film collapse by liquid plug formation in rigid axisymmetric vessels is beyond the scope of this thesis and is described in detail by Heil et al (2008) pp 215–8.

The other airway closure mechanism results from the elastic nature of the airway walls, which deform in response to the pressure exerted on them (Heil et al. 2008). The external or pleural pressure can form a compress overload (compressive load) on the airway wall following the equation 2.

$$P_{wall} = P_{ext} - P_{fluid} = P_{ext} - P_{int} + qc \qquad \text{Equation (2)}$$

Where P*wall* = wall pressure, P*ex*t= pleural pressure or external pressure, P*fluid* = fluid pressure, P*int*= air pressure in the lumen, q= surface tension, c= the mean curvature of the air–liquid interface. The compressive load is resisted by the wall's internal stiffness (characterised by its Young's modulus E and the airway wall thickness, twall) and the structural support by the parenchymal tethering (represented by the distributed elastic springs of stiffness k).

Equation 2 shows that the structural collapse of the airway wall can be caused by an increase in pleural pressure, a reduction in the internal pressure, an increase in the compressive load generated by the liquid lining and a decrease of structural support by parenchyma. If the airway wall is compressed, the curvature of the air–liquid interface will increase, resulting in an increase in the compressive load on the wall, which will further increase the compression. This phenomenon is called "compliant collapse" (Kamm & Schroter 1989). Since the curvature of the air–liquid interface is normally high in small airways, these airways are more likely to be susceptible to compliant collapse (Heil et al. 2008). In contrast, in larger airways the airway walls are rigid and the surface

tension is small, so the surface tension induced compression is unlikely to produce wall deformation. Hence, in the larger airways closure may result from the redistribution of the liquid from a thin film into an occluding liquid bridge. However, in disease condition (e.g. emphysema) the loss of support due to parenchymal destruction and the decreased tethering can cause a reduction in the airway wall stiffness leading to collapse even in the larger airways (Heil et al. 2008).

2.5. The methodology to record lung sounds

Lung sound data have been used in clinical practice for more than a century to assist diagnosis of pulmonary diseases. In order to detect lung sounds, the standard stethoscope was first invented by Laënnec to provide information of sounds which related to diseases such as pulmonary tuberculosis (Pasterkamp et al. 1997b). Since then this tool had been widely used among clinicians and became part of routine medical examination (Loudon & Murphy 1984) as a basic clinical diagnostic tool. Despite their popularity, the limitations of standard stethoscopes are that they are subjective, dependent on the user judgement and hence potentially provide unreliable data (Grenier et al. 1998; Murphy 2007). Thus, if lung sounds are believed to provide useful clinical information, there is a need to develop lung sound measurement tools which are more objective, and less dependent on the skill of the user.

With the development of computer technology in the twentieth century, it was possible to improve the method to record and analyse lung sounds in an objective way. Computer aided lung sound analysis (CALSA) is a technique which engages an electronic device to record the patient's lung sounds, then uses the computer to analyse and classify lung sound data based on the specific signal characteristics (Gurung et al. 2011). It can be used to quantify changes in lung sounds, make permanent records of the measurement and produce graphical representations that help with the diagnosis and management of patients with respiratory disease (Sovijärvi et al. 2000c).

Measuring and analysing lung sounds by computer is affected by many factors such as environment of the recording room, type of recording equipment used, type of sensors, breathing procedure and signal processing techniques

(Charbonneau et al. 2000; Rossi et al. 2000; Vannuccini et al. 2000). Therefore, these aspects will be discussed in more detail below.

2.5.1. Environment

Although CALSA is superior to standard auscultation in allowing objective lung sound data to be recorded, stored and analysed (Pasterkamp et al. 1997b; Sovijärvi et al. 2000c; Murphy 2007; Murphy 2008), there are still some limitations to applying CALSA in day to day clinical environments. One of these issues is that the environment where the lung sounds are recorded needs to be controlled. This is because the lung sound signals could be corrupted by background noises which occur during the recording. For example, wheezes are particularly sensitive to background noise (Beck et al. 2007; Guntupalli et al. 2008; Parthak et al. 2008). This corruption of the signals can affect the performance of the algorithm, leading to misinterpretation of lung sounds. Therefore, it is necessary to manage background noise in every lung sound recording session in order to get the highest quality of sound recordings as possible.

Background noise can be measured by recording the zero flow over the chest wall while subjects hold their breath, then applying the same algorithm as lung sound analysis algorithm to measure the actual values of background noise (Piirilä et al. 1991; Piirila et al. 2000). However, this procedure may not be comfortable for the patient and may lead to hypercapnia (high carbon dioxide in their blood) in some cases. Also, the sounds recorded using this technique will still be contaminated by the heart sound. CORSA guidelines recommend the background noise should be less than 60 dB when recording lung sounds in clinical environments (Rossi et al. 2000). However, the measurement of background noise in this way requires special equipment, which may not be feasible in a real clinical setting. Thus, it is essential to apply another technique which is more suitable when recording lung sounds in the real clinical setting. Therefore in this study it was decided to record background noise with the stethoscope head pressed against the surface of the bed. This damps the direct sound to the recording head but allows the pickup through the back surface of the head of any ambient sound as it would be picked up with the stethoscope pressed against the subject's chest wall. The sound recorded in this way was then analysed to make sure that the amplitude of

background noise was at least 10 times less than the amplitude of lung sounds. Although this method may not be completely equivalent to having the stethoscope on the chest wall during a breath hold, it seems to be more comfortable for the patient and can also get rid of heart sound contamination.

There are three environmental lung sound recording conditions which have been reported in the literature (Piirilä et al. 1991; Schreur et al. 1992; Pasterkamp et al. 1993; Schreur et al. 1994; Rossi et al. 2000; Elphick et al. 2004; Murphy et al. 2004). One is the sound proof chamber where the background noise is completely controlled. The other is the quiet room where the background noise is partially controlled. The last one is in a hospital ward where the background noise is uncontrolled. It seems that sound proof rooms are the best situation to achieve the high quality of lung sound recordings, however these environments cannot be feasible for clinical settings (in which this thesis aims to apply lung sound recording). Thus, the quiet room would be the proper, although not perfect, condition to avoid background noise contamination of lung sound signals and therefore was adopted in this thesis.

2.5.2. Type of recording equipment

Although CALSA recording equipment comes in two main types (single channel and multiple channel)(Murphy 2007; Murphy 2008), there are no publications which compare the sensitivity and specificity between these two types in the same environment and groups of subjects. Thus, there is still no strong evidence to show which type of CALSA recording equipment is better than the other. Therefore, it seems that deciding which system to use depends on other factors, such as whether it is available in the research facility, comfortable for a patient, and the aim of recording the lung sounds e.g. localisation of the origin of sounds.

Single channel devices can be used at the bedside with a handheld computer, personal digital assistant or smart phone. The single channel system consists of a digital stethoscope (which can be held by clinicians' hands) which is connected to the computer (Murphy 2007; Murphy 2008; Marques et al. 2009). Lung sounds are collected and transferred to the computer for analysing, processing and reporting. This type of equipment would be suitable to apply in clinical practice because it is similar to the standard stethoscope apart from being connected to the computer. Thus, it would be easier for the user who is

familiar with standard stethoscopes. Also, this system does not require special equipment such as the embedded microphone shirt which is needed in multiple channels system. The requirement for just one microphone and simpler recording technology would make this method less expensive than multiple channels. Despite the many advantages of single channel devices, especially in clinical practice, they cannot provide the information needed to localise the origin of lung sounds.

Multiple channel recording equipment is used to detect sounds simultaneously at many sites of the chest wall. This type of eqiupment needs a garment to fix the microphones to multiple sites of chest wall. This may result in three dimensional data being collected simultaneously, which may provide more information about the location of disease than two dimensional views from chest x-ray (Murphy 2007; Murphy 2008). Multiple channel recording equipment can feasibly locate the origin of sounds which would be useful to relate to the location of lung diseases, which could be helpful for planning treatments or monitoring the process of diseases.

There are however some limitations to apply multiple channels in routine clinical practice. At the time of writing this thesis, there is also no report about the varied size of the garment of multiple channel systems, especially for small babies. This would result in the technique being limited to use in only the subject groups which can fit the specific size of the embedded microphone shirt. Also, the requirement for multiple microphones and more sophisticated recording equipment to handle signals from many microphones (8-16 microphones) simultaneously make this approach relatively expensive compared to single channel systems. In order to accurately locate the origin of the sounds, it would be necessary to precisely control or at least record the locations of the microphones over the body of the patient. This would be difficult to achieve in practice and at the very least would be time-consuming to set up. This would place limitations on the groups of patients that could be monitored using this technique to those that would be more compliant, thus excluding small children for example. Additionally, the presentation of the results to the clinician in a manner which would be easy to interpret would be difficult to achieve, especially when compared to the single channel system with its inherent familiarity owing to its similarity to the standard stethoscope.

Finally, if this technique will be used in clinical practice it would be necessary to improve the aesthetic appearance to suit patient comfort.

As there has been no formal investigation into which technique (single or multi-channel) is better for diagnostic purposes, it is impossible to judge which one would be the best. One of the principle objectives of this thesis was to investigate techniques that could be applied in real clinical practice as easily as possible. Therefore, even though a multi-channel system would be able to offer significantly more information in terms of the origin of the lung sounds, it was decided to use a single channel system that could be readily deployed in a real clinical setting.

2.5.3. Type of sensors

Sensors or microphones to record lung sounds provide the connection between patients and recorder devices (Dalmay et al. 1995). Therefore, their type and quality affect the quality of lung sound data. According to the literature, there are two common types of microphones for lung sound recording and their efficiency has been reported to be different (Pasterkamp et al. 1993; Wodicka et al. 1994; Pasterkamp et al. 1997b). Piezoelectric microphones or contact sensors are more sensitive to movement artefacts because they are designed to record the movement directly; while an electret microphone or air-coupled sensors are more sensitive to background noise (Pasterkamp et al. 1993; Vannuccini et al. 2000). Thus, it seems that if the background noise cannot be completely controlled an electret microphone may not be the best choice because it would pick up ambient noises which would consequently corrupt the lung sound signal being recorded. Therefore, a piezoelectric microphone is more practical in clinical settings where the background noise can be partially controlled. However, piezoelectric microphones are more fragile and expensive (Pasterkamp et al. 1997b; Vannuccini et al. 2000). Thus, they may have shorter average lifetime and also higher maintenance cost. Another limitation of piezoelectric microphones is that their response depends on the pressure against the body surface (Vannuccini et al. 2000). Thus, to guarantee the same response of piezoelectric microphones the pressure which is applied to chest wall of the subject should not be significantly different between subjects.

Only one study has investigated the ability of piezoelectric and electret sensors under the same situation (same subjects and environment) to record

respiratory signals and found no significant differences in their performance (Pasterkamp et al. 1993). This study recorded respiratory sounds in a sound proof room, thus eliminating background noise. Therefore, their conclusion may not apply in a real clinical setting with significant ambient noise, because of the difference in the sensitivity between the two types of microphones to background noise.

Lung sound recording sensors should also present flat response in the frequency range of respiratory sound signals (Vannuccini et al 2000).This means that sensors should respond with the same sensitivity for the whole frequency range of the respiratory sound (100–2,000 Hz) to make sure that all of the frequency components of the respiratory signal will be captured with the same sensitivity.

In this research the ds32a+ Stethoscope, Thinklabs digital stethoscope (Thinklabs, USA) was used, which contains a piezoelectric sensor. This device was deemed to be suitable in the clinical environment because of its relative insensitivity to background noise. The pressure which was applied on subject's chest wall was also taken into account and efforts were made to ensure that this was consistent between all of the lung sound recordings.

2.5.4. Breathing procedure

Breathing patterns used during lung sound recording depend on the characteristics of the specific type of lung sounds which are of interest. For instance, if the intensity of normal lung sounds is of interest, breathing with airflow control should be implemented to standardise between subjects (Schreur et al. 1992; Schreur et al. 1994; Malmberg et al. 1995). This is because the intensity of normal lung sounds directly depends on the volume of airflow which passes to the lungs (Schreur et al. 1992; Dalmay et al. 1995; Fiz et al. 2008). However, breathing with airflow control needs special equipment such as mouth pieces, noise clip and other devices to monitor the airflow. Also patients need to practice to perform the breathing in order to achieve the target airflow which is set by the investigator. This makes it difficult to apply airflow controlled breathing in the clinical setting. Moreover, the method itself is very complicated and uncomfortable for patients. Therefore, this technique can only be applied to patients with good communication and cooperation;

hence it is not appropriate for non-compliant patient groups such as young children and elderly patients.

There is no published information on the effect of using or not using airflow control when recording adventitious lung sounds. Previous research has successfully collected good adventitious lung sound data from a clinical setting, without airflow control (Marques 2008; Marques et al. 2009). As this research was also to be conducted within a clinical setting, it was decided to record without airflow control, to optimise patient comfort.

2.5.5. Signal processing techniques

2.5.5.1. Signal recording sampling rate

Sampling rate or sampling frequency in lung sound recordings is defined as the number of samples per second which are taken from the lung sound signal to be converted into digital form for analysis (Bore 2006). When recording lung sounds, the sampling rate must be selected carefully to ensure that the original lung sound signal is captured as accurately as possible. If the sampling rate is lower than twice the maximum frequency in the original lung sound signal then the recorded lung sound signal can be corrupted and cannot accurately represent the characteristics of the original lung sound signal (Bore 2006). This effect is called aliasing and can be avoided by applying the proper sampling rate and filtering (Bore 2006). According to the maximum frequency of the original lung sound signal is around 1,000-2,000 Hz. The sampling frequency should be around 2,000-4,000 Hz. In this research a sampling rate at 44.1 kHz (standard setting for audio-recording system of MATLAB) was used, which was more than ten times of the maximum expected frequency in the lung sound signals. Thus, aliasing was not apparent in this study.

2.5.5.2. Filtering

Filtering is the process that transforms an input signal into an output signal by removing unwanted signals (Stearns & Hush 1990). There are two types of filters which are applied in lung sound analysis (Vannuccini et al. 2000). A high pass filter is the filter which allows frequencies higher than the cut-off frequency to pass while a low pass filter is the filter which allows frequencies lower than the cut-off frequency to pass (Stearns & Hush 1990). After filtering, the sound signal should contain only the frequency range of interest. For

example if crackles are the sounds of interest, after filtering the lung sound signal should contain frequencies between 100-1,000 Hz (see section 2.3). The high pass filter is applied in lung sound analysis to prevent low frequency sounds such as heart sounds, muscle sounds or sensor motion sounds to distort the lung sound signal, while the low pass filter is applied to prevent aliasing (Vannuccini et al. 2000). This thesis applied high pass filtering with cut off frequency of 95 Hz and low pass filtering with cut off frequency of 2,000 Hz to ensure that the lung sound signal with the frequency band of interest was passed and analysed.

2.5.5.3. Lung sound analysis algorithms

Signal processing means operating on a signal in some ways to extract useful information from the signal (Bore 2006). Signal processing procedures in lung sound analysis depend on the nature of specific lung sounds which are of interest and also the aim of analysis. There are many signal processing methods such as time expanded waveform analysis (TEWA) which uses a slower playback mode and a conventional chart recorder for analysing detailed information of waveforms with a time scale of ≥ 800 mm/s (Murphy Jr et al. 1977; Sovijarvi et al. 2000a). This makes it possible to study waveforms of breath sounds and adventitious sounds by expanding the time axis. The Fourier Transform (FT), another signal processing method, is a mathematical tool that decomposes a time signal into another representation which allows the frequency content of the signal to be analysed. It can detect and analyse hidden periodic signals which time-expanded waveform analysis could not do (Charbonneau et al. 2000). However, FT can provide the spectral content of the signal (frequency and amplitude) but it is not able to give information regarding where in time those spectral components appear. For this reason, FT is not a suitable technique for non-stationary signal such as crackles. FT can be used for non-stationary signals, if we are only interested in what spectral components exist in the signal, but not interested where these occur. Thus, another signal processing technique to overcome this problem is the wavelet transform. This technique can provide the time and frequency information simultaneously, hence giving a time-frequency representation of the signal (Polikar 1996). Therefore, it is better than FT to analyse non-stationary signals.

However, although it can be shown in theory that the wavelet transform is better than TEWA for the analysis of crackles, it has not been applied

extensively in clinical practice while TEWA is more common (Murphy Jr et al. 1977; Murphy Jr et al. 1989; Piirilä et al. 1991; Piirilä 1992; Vannuccini et al. 1998; Murphy et al. 2004), Vannuccini 1998, Murphy 2004, Piirilä et al 1990, Piirilä et al 1992). This inconsistency between the technique which is best in theory and that which is most commonly used, led to the development of the CORSA guidelines to enable standardisation for signal processing techniques. Therefore, the CORSA guidelines for signal processing techniques were applied in this thesis.

CORSA projects have published guidelines which cover lung sound recording and analysis methods such as sensors, filters, environmental control, breathing procedure and signal processing methods. However it is difficult to follow all details of CORSA guidelines when recording lung sounds in clinical environments, especially the measurement of background noise in which they recommended the level less than 60 dB as measured by acoustic metre which compared to the generally accepted threshold of hearing of 20 μPa. This measurement is unlikely to be achieved in clinical settings. Therefore, in the opinion of the author, the CORSA standard about background noise should be revised for recordings in clinical environments.

2.6. Characterisation of lung sounds

After applying signal processing methods to analyse lung sound signals, it is possible to show the characteristics of each specific sound which can provide a meaningful value to assist diagnosis of pulmonary diseases. The formal definitions of terms applied to characterisation and analysis of lung sounds are presented in appendix 2.

Normal lung sounds can be characterised using CALSA by their power spectral slopes and quartile frequencies (Schreur et al. 1992; Malmberg et al. 1995; Charbonneau et al. 2000). Power spectrum is the frequency domain data representing the power distribution of a sound with respect to frequency (Sovijarvi et al. 2000a). The power spectral graph plots the amplitude (dB) of each sine wave component against the frequency and its slope can be calculated from the change of log amplitude versus the change of log frequency (Gavriely et al. 1995). Sometimes, normal lung sounds can be plotted by their power spectrum in the root mean square (RMS) units instead of

log units. Because there is a wide range of frequencies in normal lung sounds, a common way to characterise this frequency spectrum is to separate it into parts. Thus, each part presents the same amount of energy. Quartile frequencies are the fractions of frequency spectrum which divide the power spectrum into quartiles as F25, F50, F75 and Fmax (Malmberg et al. 1995; Sovijärvi et al. 1996). The maximal frequency (Fmax) is the frequency at the maximum intensity or amplitude in power spectrum (Malmberg et al. 1995; Charbonneau et al. 2000) and can also be used to characterise normal lung sounds.

To represent normal lung sounds intensity, average sound power in log power at specific frequencies is used to show the three dimensional plots of power spectra (x axis = airflow, y axis = frequency, z axis =intensity) during inspiration and expiration (Schreur et al. 1992; Fiz et al. 2008). The specific frequencies can be two ranges of frequencies such as 150–300 Hz and 300 to 600 Hz (Fiz et al. 2008) or only one frequency such as 200 Hz (Schreur et al. 1992). This kind of plot gives a visual indication of how the frequency content of the sound varies with airflow.

Although there are many variables for the characterisation of normal lung sounds, only their intensity in log power and some frequency quartiles such as Fmax and F50 have been used in clinical practice to differentiate between healthy subjects and COPD or asthmatic patients (Malmberg et al. 1995; Sovijärvi et al. 1996). Therefore, it would be necessary to carefully choose some characteristics of normal lung sounds when applying these sounds in clinical practice.

Crackles can be described by CALSA in terms of frequency, duration, number and timing in breathing cycle (Piirilä et al 1991, (Piirilä et al. 1991; Piirila & Sovijarvi 1995; Sovijarvi et al. 2000a; Marques 2008). The waveform of crackles commonly begins with a deflection followed by a long and damped sinusoidal wave (Figure 2:2) (Piirila & Sovijarvi 1995). Because of their regular features, crackles can be described by their duration as a) initial deflection width (IDW): the duration between the starting point of crackle and the first deflection; b) two cycle duration (2CD): the duration from the starting point to the end of two complete cycles; c) largest deflection width (LDW): the duration of the deflection of the largest amplitude.

Crackle characteristics are essentially continuous, but have been conventionally used to divide crackles into either ‘coarse’ or ‘fine’ groups, according to specific characteristics (Sovijarvi et al. 2000b). However, the parameters used to define coarse and fine are inconsistent. In the CORSA guidelines coarse crackles are defined as low pitch, high amplitude sounds and long duration with 2CD more than 10 ms, and fine crackles are defined as high pitch, low amplitude sounds and short duration with 2CD less than 10 ms, (Sovijarvi et al. 2000b). However, the American Thoracic Society defines coarse crackles as loud and low pitch sounds with durations of 2CD around 10 ms and IDW around 1.5 ms. Fine crackles, in contrast, are defined as less loud and higher pitch sound with the duration 2CD less than 5 ms and IDW around 0.7 ms (American Thoracic Society 1977). This leads to the different cut off duration to verify crackles which would be useful to differentiate between diseases (Piirilä et al. 1991). Therefore, although there has been some effort to provide standardisation for researchers and clinicians in this field, it seems to be inconclusive about the definition of crackles between the two institutes. This needs to be remembered when comparing studies that have applied different definitions. In this research, the CORSA definition was used because it has been reviewed more extensively in the literature (Sovijarvi et al. 2000a).

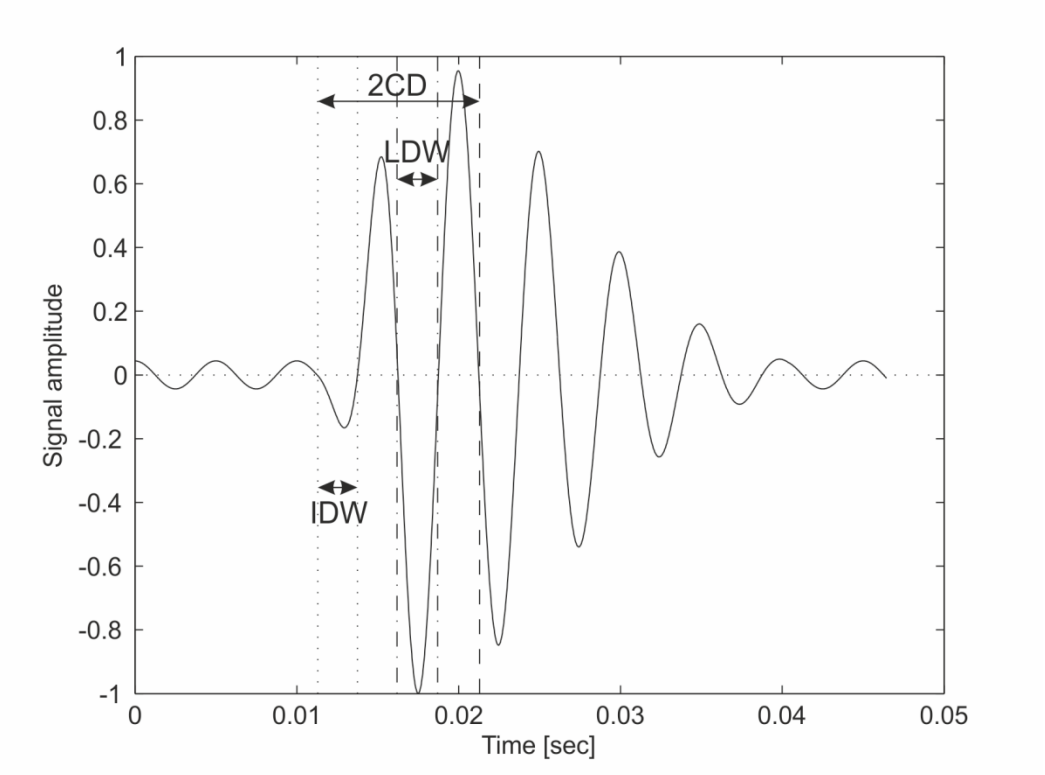


Figure 2:2: Plot of a crackle (time versus amplitude). IDW- initial deflection width; 2CD- two cycle deflection; LDW- largest deflection width (Adapted from (Marques 2008) with permission).

Wheezes are characterised by their pitch location in frequency, duration and timing, histogram of wheezing episodes and mean frequency balance between inspiratory and expiratory wheezes (Charbonneau et al. 2000). They are also characterised using acoustic lung sound analysis by their intensity, number of wheezes per breathing cycle and proportion of the respiratory cycle occupied by wheezing (Sovijarvi et al. 2000b). Wheezes at a higher frequency (higher pitch) are detected at the trachea rather than at the chest wall because these sounds transmit better through airways than through lung tissue, which acts as a low pass filter (Pasterkamp et al. 1997b; Sovijarvi et al. 2000b).

Similar to crackles, the definition of wheezes is different between CORSA and ATS. CORSA defined wheezes as continuous added lung sounds with frequencies equal or greater than 80 Hz and duration more than 100 ms (Sovijärvi et al 2000b) while ATS defined wheezes as continuous added sounds with frequencies greater than 400 Hz and the duration of more than 250 ms (American Thoracic Society 1977). This inconsistency means that it is therefore

difficult to compare the results between researchers who applied different standards. In this research, the CORSA definition was used because it has been reviewed more extensively in the literature (Sovijarvi et al. 2000a).

2.7. Added lung sounds detection algorithms

It has been reported that the occurrence of added lung sounds in the respiratory system normally relates to pulmonary disorders (Sovijarvi et al 2000b). However, added lung sounds have also been reported within healthy subjects (Kataoka & Matsuno 2008). It is believed to be the number, frequency, timing and duration of these sounds which determines the presence of pulmonary disorders. CALSA makes it possible to record and objectively analyse lung sounds and hence has the potential to assist in the diagnosis of many pulmonary conditions. There have been many algorithms developed to detect crackles and wheezes in order to differentiate and aid diagnosis among respiratory diseases (Murphy Jr et al. 1989; Sankur et al. 1996; Hadjileontiadis & Panas 1997; Vannuccini et al. 1998; Taplidou et al. 2003; Murphy et al. 2004; Taplidou & Hadjileontiadis 2007; Jain & Vepa 2008; Lu & Bahoura 2008; Bahoura 2009; Riella et al. 2009; Taplidou & Hadjileontiadis 2010). However, to understand these algorithms it is first necessary to consider the basic principle to identify these sounds in respiratory signals.

2.7.1. Automatic characterisation and quantification of crackles

The basic principle to classify crackles is usually based on their appearance in the time domain. TEWA is a technique which expands the time axis of the recorded signal to make the identification of certain features easier. Usually, the time axis is expanded to 3,000 mm/sec, as recommended by the CORSA guidelines. The waveform of crackles presents a specific appearance with a sudden short deflection followed by a series of deflections with a greater amplitude than background signal (Charbonneau et al. 2000)(see Figure 2). There were some criteria suggested by Murphy Jr et al (1989) to identify crackles in TEWA: 1) the waveforms have to cross the baseline between three and sixteen times; 2) the amplitude of the largest peak has to be greater than double the amplitude of the background sound; 3) the beginning of the event needs to have a sharp deflection in either negative or positive direction and 4) the crossing of the baseline after the initial deflection has to be progressively

wider (Murphy Jr et al. 1989). Another method to identify crackles in the time domain (the plot of signal between amplitude and time) is based on the computation of the first derivative of the respiratory signal with respect to time (Vannucini et al. 1998), which gives a measure of the rate of change, or slope, of the suspected crackle signal. As a crackle signal will contain regions of high rate of change, a threshold based technique can be used to identify parts of the respiratory signal which are likely to correspond to crackles. The authors tested the performance of their algorithm by comparing the results to those obtained by a single expert observer who manually identified crackles in the lung sound signals from 15 patients with cryptogenic fibrosis alveolitis (CFA). One weakness to this approach is the need to choose the threshold value which is used to identify when the first derivative of the signal might correspond to a crackle. Variations in this value could affect the performance of the algorithm significantly and the authors state that there is no automatic way of determining the correct value. They suggest that an initial value can be computed as the 80th percentile of the frequency histogram of the first derivative signal and then manually changing this value up or down until the best results are achieved. In reality, this would be quite difficult for a clinician to do as part of their routine clinical practice.

Wavelet transformation and adaptive nonlinear filters are signal processing methods that can locate and remove data that is recognised as noise. It looks at each data point and decides if that data is noise or valid signal. If the point is noise, it is simply removed and replaced by an estimate based on surrounding data points, and parts of the data that are not considered noise are not modified (Brigo et al. 1998). These techniques are also applied to extract crackles from vesicular sounds (Sankur et al. 1996; Lu & Bahoura 2008).

Due to there being no formal comparison between TEWA and wavelet under the same conditions (patients, type of CALSA, type of sensor, breathing procedure and environment), it was difficult to decide which technique was superior to the other to verify crackles. The decision of which signal processing method to apply to detect crackles in the clinical setting may depend on other factors, such as whether that algorithm can provide sufficient data to aid diagnosis of pulmonary diseases.

2.7.1.1. Automatic crackles detection algorithms

According to the literature (Table 2:1), the sensitivity and specificity of the algorithms to detect crackles have been reported to be between 84 and 93% for sensitivity and 78% and 89% for specificity (Sankur et al. 1996; Hadjileontiadis & Panas 1997; Vannuccini et al. 1998; Murphy et al. 2004; Lu & Bahoura 2008). These studies compared their algorithms using the experts' opinion and previous algorithm as a gold standard. TEWA seems to be the standard method to present the characteristics of crackles. This is because the time parameters of crackles are believed to have clinical meaning in terms of diagnosis of diseases (Piirila & Sovijarvi 1995). For example, coarse crackles with longer are believed to be associated with COPD while fine crackles are found in fibrosing alveolitis (Piirilä et al. 1991, Piirilä et al. 2000, Murphy 2008, Pasterkamp et al. 1997).

There are different approaches e.g. discrete wavelet transform, non-linear filter, wavelet-based filter and wavelet package filter to separate crackles from vesicular sounds before identifying and classifying them into coarse and fine crackles (Sankur et al. 1996; Hadjileontiadis & Pandas 1997, Lu & Bahoura 2008). Wavelet technology is used to detect crackles because 1) crackles are non-stationary sounds and wavelets are a good signal processing technique for this type of signal 2) they are also low amplitude compared to normal lung sounds 3) they can be overlapping with other respiratory signals and hence be distorted by the normal lung sound signal 4) they are also difficult to establish in the time domain parameters (Sankur et al. 1996; Hadjileontiadis & Pandas 1997; Lu & Bahoura 2008). Unfortunately, most studies which applied wavelet to detect crackles reported only the sensitivity and positive predictive value of algorithms but not the specificity (Sankur et al. 1996; Hadjileontiadis & Pandas 1997; Lu & Bahoura 2008) and therefore have not provided any data about the number of false positives when no crackles were present. This could have a significant impact if the technique were to be used to aid clinical diagnosis. Therefore wavelets may not be a suitable technique to apply in real clinical settings compared to TEWA.

The description of lung sound data including the method of recording, the breathing procedure, the environment and the detail of patients which are used to verify crackles are important information to include in the study or articles which develop crackle detection algorithms (Vannuccini et al. 2000;

Charbonneau et al. 2000; Rossi et al. 2000). None of this information has been provided in a series of studies which used a lung sound database (Sankur et al. 1996; Hadjileontiadis & Pandas 1997; Lu & Bahoura 2008) to test the algorithms that were described. Therefore it is difficult to draw any firm conclusions about how the algorithms would perform in a real world clinical environment.

In conclusion, according to the literature it seems that TEWA is the basic principle to define crackles. There have been many algorithms developed to detect crackles in respiratory signals but some of algorithms reported only the sensitivity or detectability but not the specificity. This may not provide the ability of algorithms to define non crackles which is necessary in the diagnosis process to differentiate between normal and abnormal conditions. Thus, the algorithm which can provide both sensitivity and specificity is more feasible to apply in the clinical practice. Additionally, the description of lung sound data (mentioned before) should be included in the process of testing the algorithms. Therefore, the recording and signal processing methods following CORSA (which recommended TEWA) are probably a promising and standard method to increase the efficiency of the automatic detection and hence were applied in this study.

Table 2:1: Summary of articles described crackles detection algorithms

No.	Year/ Authors	Algorithm/ filter	Confirmation	Population	Type of CALSA/ area of recording	Environment/ Method	Parameter to be detected	Sensitivity/ Specificity/ Detectability/ Positive predictive value
1.	1996 Sankur B, Güler EÇ and Kahya YP	The discrete wavelet transform	Experts' opinion	132 crackles 4 Pneumonia 3 Bronchiectasis 2 Asthma 2 Chronic bronchitis 2 Emphysema 1 Interstitial lung disease adult	Two microphones over chest walls Do not indicate area of recording	Mouthpiece with nose clip and airflow control	Crackles	Sensitivity 96.21% Positive predictive value 90.71%
2.	1998 Vannuccini L, Rossib M and Pasqualic G	Time expanded waveform analysis A finite impulse response filter	An expert based on the criteria used to define crackles in Murphy's study (1989)	200 inspiratory crackles 15 Cryptogenic fibrosing alveolitis adult	Single channel lung sound recording with an air-coupled condenser microphone Do not indicate area of recording	Mouthpiece with nose clip and airflow control	Crackles versus no crackles	Sensitivity 84% Specificity 89%
3.	2004 Murphy RL, Vyshedskiy A, Power-Charnitsky VA, Bana DS, Marinelli PM, Wong-Tse A and Paciej R	Time expanded wave form analysis	Physicians and radiologists	100 Pneumonia 100 Healthy adult	Multiple channel recordings Sixteen channels (two over trachea and 14 over chest wall)	Deeper breath than normal with mouth open while lay on 16 microphones embedded in a soft foam pad	Acoustic pneumonia score	Sensitivity 90 % Specificity 78% in practice sample Sensitivity 94% Specificity 88% in other samples Positive predictive values 94% in practice samples and 87% in other samples.

No.	Year/ Authors	Algorithm/ filter	Confirmation	Population	Type of CALSA/ area of recording	Environment/ Method	Parameter to be detected	Sensitivity/ Specificity/ Detectability/ Positive predictive value
4.	1997 Hadjileontiadis LJ and Panas SM	Wavelet-based filtering	Expert's opinion	Three lung sound databases 146 crackles 5 Pulmonary fibrosis 4 Interstitial fibrosis 5 Chronic bronchitis 2 Allergic alveolitis	Do not indicate	Do not indicate	Crackles	Detectability 97.5±5.59%
5.	2008 Lu X and Bahoura M	Wavelet packet filter	Another algorithm	182 crackles from The lung sound database (RALE and ASTRA) and the audio files accompanying with two books	Do not indicate	Do not indicate	Crackles	Sensitivity 92.9% Positive predictive value 94.4%

2.7.2. Automatic characterisation and quantification of wheezes

Wheezes are usually quantified according to their spectral appearance. The processes can be divided into three categories; splitting and windowing from respiratory sounds, extracting the particular feature, and modelling or classifying wheezes using the criteria of specific frequency and duration (Bahoura 2009). Methods for extraction involve spectral analysis techniques, such as; Fast Fourier Transform (FFT), autoregressive model or linear predictive coding, wavelet transform and cepstral analysis. Methods for classification include techniques such as Gaussian mixture models and artificial neural networks (Bahoura 2009). Subsequently, many studies created their algorithms to detect wheezes based on these two processes and then confirmed the sensitivity and specificity of their algorithms with the experts ((Taplidou et al. 2003; Taplidou & Hadjileontiadis 2007; Taplidou & Hadjileontiadis 2010) or the previous standard algorithms (Jain & Vepa 2008; Bahoura 2009; Riella et al. 2009).

Table 2:2: Summary of articles described wheezes detection algorithms

No.	Year/ Authors	Algorithm	Frequency range	Wheezes duration	Confirmation	Population	Area	Environment/ Method	Parameter to be detected	Results
1.	2010 Taplidou SA and Hadjileontiadis LJ	Continuous wavelet transform Wavelet bispectral analysis Wavelet bicoherence Instantaneous wavelet biamplitude and biphase curves	100-800 Hz	>150 ms	2 experts audiovisual inspection of all signals	393 wheezes 10 COPD 10 Asthma adults	Trachea Left and right axillae Posterior base of both lungs	Semi-quiet clinical laboratory Mouth piece with nose clip and airflow control at 1.5l/s through visual feedback	Characteristics of wheezes	Algorithm can differentiate COPD from Asthma
2.	2007 Taplidou SA and Hadjileontiadis LJ	Time-frequency analysis of breath sounds Present graph in frequency content evolves over time using short-time Fourier	100-1,000 Hz	>150 ms	2 experts audiovisual inspection of all signals	337 wheezes 6 COPD 3 Asthma 1 Pneumonia adults	Trachea Left and right axillae Posterior base of both lungs	Semi-quiet clinical laboratory Mouth piece with nose clip and airflow control in three categories: 0-0.25, 0.25-0.5 and 0.5-1.5 l/s through visual feedback	Wheezes versus no wheezes	Detectability 99.7 ± 1% Sensitivity 95.5% ± 4.8% Specificity 93.7 ± 9.3 %

No.	Year/ Authors	Algorithm	Frequency range	Wheezes duration	Confirmation	Population	Area	Environment/ Method	Parameter to be detected	Results
3.	2003 Taplidou S, Hadjileontiadis L, Penzel T, Gross V and Panas S	Wheezes –episode detector based on spectral analysis	100–800 HZ	>100 ms	Expert physician	14 asthmatic adults	Trachea, Posterior chest walls on 3rd and 7th and 8th intercostal spaces	Do not indicate	Wheezes and no wheezes	Detectability 93.45 ± 11.97 %
4.	2009 Bahoura M	The feature extraction algorithm such as Fourier transform, linear predictive coding or auto–regressive and Mel–frequency cepstral coefficients (MFCC) in combination with the classification methods based on vector quantification, Gaussian mixture models (GMM) and artificial neural networks	600–3000 Hz	Do not indicate	Other algorithms	Database from four different sources: south record on healthy and asthma, R.A.L.E database–CD, the ASTRA database–CD, various internet sites of laboratory working in this fields	Trachea	Do not indicate	Wheezes and no wheezes	Sensitivity 94.6% Specificity 91.9%

No.	Year/ Authors	Algorithm	Frequency range	Wheezes duration	Confirmation	Population	Area	Environment/ Method	Parameter to be detected	Results
5.	2009 Riella R, Nohama P and Maia J	Spectral analysis using Short time Fourier transform Fast Fourier transform Multi-layer perception artificial neural network	> 100 Hz	> 100 ms	3 experts	11,240 wheezes 72 no wheezes individuals ranging from newborn to 76 years old	Do not indicate	Do not indicate	Wheezes and no wheezes	Sensitivity 84.2% for isolated respiratory cycle and 92.86% for groups of respiratory cycles obtained from the same person
6.	2008 Jain A and Vepa J	Spectral analysis using Fast Fourier transform	225-800 Hz	≥ 250 ms	Previous algorithms	19 wheezes 21 normal lung sounds from lung sound database	Do not indicate	Do not indicate	Wheezes and other normal lung sounds	Sensitivity 84% Specificity 86%

2.7.2. 1. Automatic wheeze detection algorithms

A number of studies described in the literature (Table 2:2) have defined wheezes using spectral analysis and the detection of sinusoidal peaks of wheezes, with sufficient amplitude, based on specific frequency and duration criteria (Taplidou & Hadjileontiadis 2010; Taplidou & Hadjileontiadis 2007; Taplidou et al. 2003; Bahoura 2009; Riella et al. 2009; Jain & Vepa 2008).The spectrogram of wheezes showed the pattern of various types of wheezes episodes (high pitch and low pitch wheezes) over time (Jain & Vepa 2008) or presented the wheeze amplitude in the time domain (Taplidou & Hadjileontiadis 2007; Taplidou et al. 2003). It seems that spectral analysis of wheezes is currently agreed to be the optimal way to identify and classify wheezes in respiratory signals. The common spectral analysis methods to define wheezes are FFT, short time Fourier transform and wavelet transformation (Taplidou & Hadjileontiadis 2007; Taplidou et al. 2003; Riella et al. 2009; Jain & Vepa 2008; Taplidou & Hadjileontiadis 2010).

The criteria to verify wheezes vary in the published literature. The following have all been used: frequencies between 100 Hz and 1kHz and duration greater than 150 ms in Taplidou & Hadjileontiadis (2007), between 100 and 800 Hz with duration greater than 100 ms in Taplidou et al (2003), between 225 and 800 Hz with duration ≥ 250 ms in Jain & Vepa (2008) and greater than 100 Hz and duration greater than 100 ms in Riella et al (2009). This lack of consensus on the definition of a wheeze is likely to affect the value of sensitivity and specificity of the algorithm to verify wheezes. Therefore, it may be important to prescribe the frequency and duration ranges of wheezes in the study based on one of the standards of respiratory sound analysis: CORSA with frequencies equal or greater than 80 Hz and duration more than 100 ms (Sovijärvi et al. 2000b) or ATS with frequencies greater than 400 Hz and the duration of more than 250 ms (American Thoracic Society 1977).

The lung sound data, moreover, are also one of the factors influencing the sensitivity and specificity of wheeze detector algorithms. The study which recorded lung sounds from actual patients (Taplidou & Hadjileontiadis 2007, Taplidou & Hadjileontiadis 2003) presented higher values (95.5% ± 4.8% versus 84% for sensitivity and 93.7 ± 9.3 % versus 86% for specificity) than the one which used lung sound data from lung sound databases (Jain & Vepa 2008). It is possible that the researchers measuring lung sounds from patients followed

the standard method of lung sound recording, but those recording sounds for the database did not, which would account for the differences in sensitivity and specificity.

Additionally, the gold standard to be compared with the algorithms seems to affect the sensitivity and specificity to verify wheezes. The study which reported higher sensitivity and specificity verified their findings with the experts (Taplidou & Hadjileontiadis 2007; Taplidou & Hadjileontiadis 2003) while the study with lower sensitivity and specificity used previous algorithms as a confirmation (Bahoura 2009; Riella et al. 2008; Jain & Vepa 2008). This may be because the experts gain much experience to verify wheezes and usually examine this sound in their daily clinical practice.

According to the literature, it could not be determined which algorithm is better than another, because the creation of each algorithm is different in terms of the principles used, the criteria which were used to verify wheezes, the environment and method of lung sound recording and the population which was tested. In order to compare algorithms' ability to detect wheezes, all these conditions would need to be controlled. However, in order to maximise sensitivity and specificity of the algorithm to detect wheezes it is necessary to follow the standard of lung sound analysis based on CORSA or ATS which included the frequency and duration ranges of wheezes, the patients' preparation, the method of breathing, the recording procedures, and the environmental control. The method of breathing using airflow control may not be important because they do not consistently influence the sensitivity and specificity of the algorithm to detect wheezes (Taplidou & Hadjileontiadis 2007). Additionally, the method of recording lung sounds simultaneously with airflow control by pneumotachograph is probably not comfortable for patients (they have to wear nose clips and mouth pieces) and consequently is not suitable to apply in clinical settings. Therefore, in this research it was decided to use the simple method, but follow the standard method of recording lung sounds without airflow control, which is feasible for real clinical practice. The algorithms, which are designed to detect wheezes, were also tested against experts' opinion to optimise sensitivity and specificity.

In this research the algorithm that was used was written by Dr. Anna Barney and Professor Paul White, University of Southampton. This algorithm was developed following the CORSA recommendation (TEWA analysis for crackles and Fourier Transform for wheezes) (Charbonneau et al. 2000; Vannuccini et al. 1998) and Murphy Jr et al (1989)'s study (Murphy Jr et al. 1989). This is because Murphy and colleagues' explanation to describe crackle waveform seems to be the first objective way to indicate crackles and there is still no evidence to refute their theory. The algorithm used in this thesis was also tested in another study to provide good to excellent intra-class reliability to detect crackles in 54 patients (37 bronchiectasis and 17 cystic fibrosis) for lung sound recording in clinical environment (Marques et al. 2009) in which this research was conducted.

However, similar to other algorithms which have been applied to analyse sampled sound signals from CALSA, the algorithm used in this thesis presented a small error in the detection of the start and end points of the crackle signals, owing to the discrete nature of the sampled signal. The magnitude of the error depended on the sampling rate that was chosen, with a lower sampling rate leading to a larger potential error. This could potentially have affected whether a crackle was characterised as being coarse or fine, if the duration of the crackle was very close to the 10 ms boundary between the two types. Similar to other wheeze detection algorithms used in clinical practice (Beck et al. 2007; Guntupalli et al. 2008; Parthak et al. 2008), the algorithm applied in this study was also sensitive to high frequency sound from the clinical environment, such as ambulance sirens or speaking voices. Thus, the researcher had to confirm the presence of wheeze by listening to the recorded sound signal in the spectral analysis software and then delete any contaminating high frequency noises from the analysis. As this process is dependent on the hearing ability of the researcher, this inevitably resulted in an element of subjectivity in the analysis.

2.8. Characteristics of added lung sounds in COPD

COPD is an irreversibly progressive airflow limitation disease which results in pathological changes within the lung associated with chronic bronchitis and emphysema (Rabe et al. 2007). These changes develop when central airways are irritated (e.g. by tobacco smoke) and respond with mucus hyper-secretion

and mucous gland enlargement, resulting in an increase of secretion within the airways. The peripheral airways respond by becoming inflamed, leading to fibrosis and airway narrowing. In the lung parenchyma, the inflammation results in alveolar wall destruction which leads to hyperinflation and prolonged expiration times (Nagai 2002). Airway remodelling is common in COPD when many inflammatory cells infiltrate into the bronchial tissue and cause thickening of the airway walls (Turato et al. 2001). However, the relative contributions of these pathological changes of large airways, small airways and alveoli toward irreversible airflow limitation and clinical features vary between individuals. For example, morphologically, some patients show severe emphysema with or without bronchial wall thickening. On the other hand, some patients do not show any apparent emphysema (Kitaguchi et al. 2006).

The pathological changes which occur in the lungs of people with COPD may contribute to abnormal turbulent flow in the bronchial tree and hence give rise to added breath sounds.

2.8.1. Crackles in COPD

COPD is a chronic inflammatory disease of the lungs that results in progressive narrowing of the airways (Rabe et al. 2007). The main causes of narrowed airways in COPD are an increase of secretions from the hyper mucous glands which secrete into both peripheral and central airways and also the loss of cartilaginous support through inflammation (Piirila & Sovijarvi 1995). The mechanism to generate crackles is believed to be associated with the sudden reopening and reclosing of closed airways (Kiyokawa et al. 2001), therefore crackles in COPD are likely to be originated from the airways which become narrowed and hence are more likely to close and reopen.

To date, there have been few studies reporting characteristics of crackles in COPD and all of these studies compared the characteristic of crackles in COPD with other respiratory diseases. Piirilä et al (1991) reported that crackle 2CD was higher (lower frequency) in COPD and bronchiectasis than in patients with fibrosing alveolitis. Murphy (2008) also reported that average crackle frequencies were lower in COPD than in patients with interstitial pulmonary fibrosis. The number of crackles per cycle during inspiration has also been

found to be higher in fibrosing alveolitis and bronchiectasis than in COPD (Piirilä et al. 1991).This suggested that crackle 2CD and the number of crackle per breathing cycle could be used to differentiate between COPD and some other respiratory diseases. However, none of these studies document the changes to crackle characteristics caused by the pathological process of COPD. Therefore it is still unclear from the literature whether the characteristics of crackles will be changed during the course of COPD.

Crackles can be defined, using CALSA, by their frequency, duration, number and distribution per breath and timing in respiratory cycles. A recent study using lung sound analysis in a clinical setting has suggested that crackle 2CD is a more reliable parameter than IDW for measuring crackle duration in 52 patients with pulmonary inflammation conditions such as bronchiectasis and cystic fibrosis patients (Marques 2008). As COPD is also an inflammatory disease which affects both central and peripheral airways, crackles 2CD could possibly be a good indicator to characterise COPD. The frequency and duration of crackles are also believed to be associated with airway diameter, for example, high frequency crackles with duration less than 10 ms (fine crackles) are usually associated with peripheral airways while lower frequency crackles with duration more than 10 ms (coarse crackles) are associated with proximal airways (Piirila & Sovijarvi 1995). If the hypothesis that crackle frequency is related to airway diameter is accepted, then it is logical to hypothesise that any changes to the structure of the airways would result in changes to crackle parameters. COPD affects the more peripheral airways in the early stages of the disease process, so it would be anticipated that this would initially lead to an increase in high frequency crackles. As the disease progresses, the relationship of high to low frequency crackles would probably change.

The number of closed airways which reopen also directly affects the crackle number, as each reopening event causes one or more crackles. The pressure required to cause sudden reopening of closed airways has been shown to be related to the airway diameter (Alencar et al. 2001; Alencar et al. 2003), therefore it can be hypothesised that the characteristics of crackles may be related to the airway geometry. As COPD causes airway narrowing and narrower airways have been shown to result in changes of duration and number of crackles, it would be reasonable that these two parameters of crackles could be used to reflect changes of airways in COPD. Therefore,

crackle 2 CD and number of crackles per breathing cycles were chosen to be investigated in this study.

2.8.2. Wheezes in COPD

It was hypothesised by the researcher that the mechanism behind wheezes in COPD may be related to the damage of lung parenchyma and inflammation of central airways together, which induces an increase in pleural pressure in the tissue surrounding the airways and thus decreases the diameter of airways. Consequently, these cause the airways to collapse partially and decrease the expiratory flow, leading to a reduction in expiratory flow rate together with the reduction in critical velocity of airflow in these semi-collapsed airways. As a result, this may generate expiratory wheezing in COPD patients. There are few studies reporting the mechanism underlying wheezes in COPD. Fiz et al (2002) suggested that the airflow limitation or eddy-induced wall oscillations caused by intra-luminal and structural airway lesions could be the main mechanism (Fiz et al. 2002). Meslier et al also pointed out that any change of the mechanical properties of the lung during the process of COPD could result in the lower critical fluttering pressure and hence wheezes (Meslier et al. 1995). Therefore, it seems that the changes in lung structures during the course of COPD could give rise to airflow obstruction and decrease in critical velocity which could generate wheezes.

Wheezes can be characterised using CALSA by their mean frequency, number and the proportion of wheezes per respiratory cycle, duration and timing of wheezes (Baughman & Loudon 1984; Homs-Corbera et al. 2000; Sovijarvi et al. 2000a; Fiz et al. 2002; Fiz et al. 2006; Taplidou & Hadjileontiadis 2007; Murphy 2008). However, there is no formal publication studying the reliability or stability of each characteristic of wheezes in pulmonary diseases. So it is not known which characteristic should be used for either diagnostic or monitoring purposes. Many publications relate the number of wheezes to the severity of airway obstruction, as this number increases with more severity of airway obstruction (Baughman & Loudon 1984; Sovijarvi et al. 2000a; Fiz et al. 2006; Murphy 2008). Due to COPD being a progressive airflow limitation disease which results in progressively obstructed airways, it is reasonable to assume that the number of wheezes would relate to the severity of disease. Therefore

this study focused on the number of wheezes. The mean frequency, but not the timing, of wheeze has also been suggested to be able to differentiate between COPD and healthy controls (Fiz et al. 2002) and also between asthmatic patients and healthy controls (Fiz et al. 2006). Of particular interest to this thesis was the focus on wheezes in COPD and healthy controls where the mean frequency of wheezes seems to be relavant. Thus, the mean frequency of wheezes was also chosen to be investigated in this study.

2.9. CALSA as a measurement to detect airway obstruction

Airway obstruction is normally measured by spirometry but the figures used for its definition vary among institutes. The definition of airway obstruction by the European Respiratory Society depends on the values of FEV_1/slow vital capacity (VC) ratio as <88% of the predicted value in males and <89% predicted value in females (Siafakas et al. 1995) while the American Thoracic society define airway obstruction as FEV_1/ forced vital capacity (FVC) ratio <75% (Celli 1995). The British Thoracic Society define airway obstruction by both FEV_1<80% predicted and FEV_1/VC ratio of <70% (British Thoracic Society 1997). This inconsistency may lead to the non standard use of the data derived from spirometry in the diagnosis of COPD and may cause under detection of this disease among the general population. The use of CALSA as an additional measurement has been investigated by researchers in the last decade as an aid to diagnose both cardiology and pulmonary diseases (Murphy 2008). However, it is hypothesised by the researcher that CALSA may also be a sensitive measurement to detect obstructed airways. There is some literature (Table 2:3) to support this hypothesis.

According to the literature, CALSA could be used to detect changes after bronchodilator and bronchoconstrictor drug administrations in adult subjects; but only some variables of lung sounds (such as F50 and proportion of respiratory cycle occupied by wheezes) could be used to indicate the response (Baughman & Loudon 1984; Malmberg et al. 1994; Pasterkamp et al. 1997a). These studies generally had small samples and involved only asthma patients and healthy subjects (Baughman & Loudon 1984; Malmberg et al. 1994; Paterkamp et al. 1997a). This limits the validity and generalisability of their findings. Thus, there is still a need to study the use of CALSA to detect changes in the airway after bronchodilator and broncho-constrictor treatments

in a larger sample size and in other obstructive airway diseases such as COPD, to confirm these initial findings and establish if they are replicable in other patients with obstructive airway diseases.

It has also been reported that the use of CALSA provided useful additional information when applied with spirometry to detect early signs of lung abnormalities among high risk workers (Gavriely et al. 1994). They reported the sensitivity to identify lung abnormalities improved by 16% when they added CALSA information to spirometry data. This study involved 593 subjects. This suggests that CALSA could provide useful information to increase the ability of conventional measurements to detect obstructed airways. However, in this study the spectral analysis of lung sounds was applied to analyse only normal lung sounds in power spectra and the authors did not apply an automatic algorithm to detect any added lung sounds (crackles and wheezes), which may explain why 50% of the subjects in this study were found to present with normal lung sounds. It is hypothesised by the researcher that added lung sounds should also be analysed, to confirm the ability of CALSA to add information about early abnormalities among a population which presents with high risk of pulmonary disorders.

From the literature, lung sound data produced by using CALSA can be used to follow the responses of airways to both bronchodilators and bronchoconstrictors with a simpler method than performing maximal breathing manoeuvres in a spirometry test, which one study has suggested may itself lead to bronchial constriction (Gimeno et al. 1972). Recording lung sounds could be safer and more suitable for patients with less ability or willingness to co-operate (such as young children and elderly people) than conventional spirometry to represent airway responses in both bronchodilatation and bronchoconstriction.

Although the data are simple to collect, however, the method to analyse lung sounds is still complicated at present, and needs special algorithms or software to analyse and represent the data. The procedure is therefore not yet applicable for use in clinical practice. Spirometry data are harder to collect but easy to analyse and interpret. Consequently, pulmonary function test data from spirometry are currently more straightforward to communicate among

clinicians. Thus, it will be necessary to develop the analysis of lung sounds to make it easier and more convenient for applying in real clinical settings.

Table 2:3: Summary of articles compared CALSA with measurements to detect airway obstruction

No.	Year/ Authors	Design	Measurements/ standard to be compared	Variables to be detected/ compared	Population/ age	Results
1.	1994 Gavriely N, Nissan M, Cugell DW and Rubin AH	Cross-sectional study	Spirometry Questionnaire	Average power spectra of normal lung sounds, pulmonary function test and questionnaire	385 males and 108 females Age range 21-77 years	Normal lung sound data could increase the sensitivity to detect abnormalities from 71% to 87%
2.	1994 Malmberg LP, Sovijärvi AR, Paajanen E, Piirilä P, Haahtela T and Katila T	Cross-sectional study	Spirometry	Change of quartile frequencies of normal lung sounds in responses to broncho-constrictor when compared with FEV_1	12 asthmatic patients and 6 healthy controls Age range Asthmatic patients 16-61 years Healthy controls 22-31 years	Sensitivity and specificity of inspiratory F50 at chest wall to detect changes after broncho-constrictor = 83% and 100% Sensitivity and specificity of exspiratory F50 at chest wall to detect changes after broncho-constrictor = 75% and 100% F50 significantly correlated with FEV1 in asthmatic patients (r=-0.85)

No.	Year/ Authors	Design	Measurements/ standard to be compared	Variables to be detected/ compared	Population/ age	Results
3.	1997 Pasterkamp H, Araneta, RC, Yuns O, Holbrow J	Quasi-experiment	Response of normal lung sounds after broncho-constrictor	Intensity of normal lung sounds	12 asthmatic patients and 9 healthy controls Age range 9-16 years	Normal lung sound intensity at chest wall but not trachea decreased after bronchoconstrictor
4.	1984 Baughman RP and Loudon RG	Quasi-experiment	Response of wheezes after bronchodilator	Proportion of the respiratory cycle occupied by wheezes	20 asthmatic patients Median age = 43	The proportion of the respiratory cycle occupied by wheezes decreased from 86% to 31%

2.10. Comparing CALSA findings with anatomy of airways derived from imaging techniques to indicate airway obstruction

Although CALSA has been claimed to provide information about obstructed airways by many studies (Schreur et al. 1992; Gavriely et al. 1994; Schreur et al. 1994; Malmberg et al. 1995), there are currently no published studies comparing lung sounds to quantitative data on the geometry of the airways themselves. It is hypothesised by the researcher that lung sound data should be compared directly to the airway characteristics of each generation of the airway tree to reveal any relationship between lung sounds and airway obstruction.

In order to detect the geometry of the airways, HRCT has been claimed by Müller (Müller 2001) to be the gold standard of imaging technique with the highest sensitivity and specificity in identifying the small airway changes with a thin section CT of 1-to-2-mm collimation by applying a high spatial resolution reconstruction algorithm (Müller 2001). Although the development of HRCT is ongoing, much of the data extracted by HRCT is still subjective and qualitative, and requires a specialist to interpret the results. For example, an emphysema score (see section 2.12) has been derived to describe emphysema changes (Kawamura et al. 2003; Deveci et al. 2004; Dransfield et al. 2007; Patel et al. 2008). Other descriptors involve the presence or absence of specific features such as traction bronchiectasis, centribular nodules and ground glass appearance (Kawamura et al. 2003). Thus, there is still a need for objective data to describe the geometry of the airway and quantify any anatomical changes which are associated with obstructed airways.

There is only one published study which provides three dimensional data of the airway showing an image in coronal and sagittal planes of airway configurations including lengths, diameters and branching angles at which the airways divide from their parents (Sauret et al. 2002). These variables could be useful to describe progressive morphological changes of airways and consequently the mechanism of airflow changes in patients with obstructive diseases, but have not yet been studied in this context. It is hypothesised by the researcher that exploring any relationship between lung sound data and

the variables that can be extracted from three dimensional airway data may provide more information about the presence of airway obstruction.

Sections 2.5 to 2.10 have provided a description of lung sounds and a standardised method, of recording and analysing lung sounds (based on the CORSA project,). The development of CALSA to aid diagnosis of pulmonary disorders has also been presented. The main point of discussion is whether CALSA has potential to provide additional useful data to detect airway obstruction. At present the most sensitive measurement among imaging techniques to show airway obstruction is HRCT, which can display three dimensional views of the airway geometry. Thus, in order to confirm the ability of CALSA to indicate airway obstruction it was considered appropriate to use HRCT data as the gold standard. The next section will discuss issues relating to HRCT and its application in obstructive pulmonary diseases and also the possible correlation between airway dimensions measured by HRCT and characteristics of lung sounds measured by CALSA.

2.11. High resolution computed tomography (HRCT)

High resolution computed tomography (HRCT) has been reported to present the highest sensitivity and specificity among imaging techniques to differentiate focal and diffuse lung diseases (Müller 2001). The advances in HRCT technology make it possible to demonstrate the three dimensional anatomical structures of airways down to generation six with the inner diameter around 2.3±0.6 mm (Hasegawa et al. 2006). This is very useful to study the pathology of airways. The pathology of COPD mostly affects the small airways (with diameter less than 2 mm) but HRCT is not able to image with sufficient resolution to analyse these airways directly. However, there is evidence that large airways measured by HRCT (more clearly and accurately identified than small airways) have a significant relationship with small airways measured by histological methods in the same patients. Measures of larger airways could therefore theoretically be used to predict measurements in smaller airways (Nakano et al. 2005). This section will begin with the principles of operation of a computed tomography (CT) scanner followed by the history and development of HRCT. The parameters measured by HRCT in COPD will be discussed. Then previous research studies involving HRCT in COPD will be

reviewed and the possibility of comparing HRCT data with CALSA data will be evaluated.

2.11.1. Principle of CT scan working

A CT imaging system consists of a motorised table which can move the patients along with the system, an x-ray source and detectors (see appendix 3). When the table moves, the source of x-rays rotates within the circular opening and a set of detectors also move in the same direction on the far side of the patient. The source of x-rays generates a narrow, fan-shape beam with widths between 1 and 20mm. There are two types of CT, one is an axial CT in which the table is fixed while the x-ray source rotates, and then it moves concurrently with the next slice. This type is generally applied to scan the head region. Another type of CT is spiral or helical CT in which the table moves steadily while the x-ray source rotates to generate a spiral or helical scan (see appendix 3). It is commonly applied to scan the whole body. Another way to categorise CT is by the number of x-ray row sensors, as single row or multiple rows (Brenner & Hall 2007).

High Resolution Computed Tomography (HRCT) operates with the same basic principles as a normal CT scanner, except that a narrow slice width is chosen (1 – 2 mm) and a high spatial resolution image reconstruction algorithm used. Just as with conventional CT, the x-ray beams penetrate the body and are detected by a series of detectors that are positioned directly opposite the x-ray source (such that the patient occupies the space in the middle). The detectors quantify the intensity of the x-ray that they detect. As the x-ray pass through the various tissues in the body, they are attenuated according to the density of the tissue they are passing through. Thus, higher density tissues such as bone or tooth will results in greater attenuation (and hence lower x-ray intensity at the detector) than lower density tissues such as fat, muscle or lung. As the HRCT scanner rotates around the patient, it builds up an image of the distribution of tissue densities throughout the body. As this is repeated for a large number of thin slices, it becomes possible to reconstruct the 3D distribution of tissue densities (Brandt 2005).

HRCT generates transverse anatomical pictures where the value of each pixel refers to the x-ray attenuation of a defined volume of tissue (i.e. voxel) (see appendix 3). These x-ray attenuation values for each set of projections are recorded and processed by the computer in a matrix form. The picture matrix size and picture resolution are affected by the number of calculated pixels. This matrix size value is 512x512 pixels in normal clinical practice.

The degree of x-ray attenuation, or tissue density, is measured in Hounsfield units (HU) (see appendix 3) with a range between -1,000 HU, referring to the attenuation number of air, and 3,000 HU, with 0 HU referring to the attenuation number of water and 3,000 HU referring to bone tissue (Madani et al. 2001). Normal lung density on CT (-700 HU at functional residual capacity) is moderately higher than air (-1,000 HU) and is determined by the balance between air in the small airways and airspaces, and the soft tissues that have a higher density than air, but are not detectable as individual structures. The HU scale for scanning the lung which contains both air and water is between -950 and -550 HU (Kalender 2000).Increased attenuation patterns occur when the density of the lung parenchyma increases. Increased density occurs when air is reduced (e.g. smaller volumes), air is replaced by tissue/cells, there are increased blood volumes, or thickening of airway walls. Decreased attenuation patterns occur when the density decreases. Density decreases when there is decreased blood flow, increased air trapping or destruction of lung tissue.

2.11.2. History and development of HRCT

Chest x-rays provide limited information because they only image in one plane, whereas CT scans image in more than one plane. CT imaging uses x-rays in conjunction with computing algorithms to image the body. Although radiographs provide higher spatial resolution, CT can detect more subtle variations in attenuation of x-rays. Since 1975, CT scans for the whole body have been available to examine gross lung structures. Conventional 8-10 mm collimation (see appendix 3) scans have been claimed to be better in the assessment of pulmonary parenchyma than chest x-ray (Müller 2001). The original CT scan, however, was not suitable for the thorax because of the low resolution leading to large partial volume effects and the large difference in attenuation between tissue and air. Consequently, small lesions are very

difficult to identify using standard CT (Sluimer et al. 2006). Also, because the fine parenchymal detail cannot be imaged, standard CT cannot be used to detect interstitial and airway diseases (Remy-Jardin et al. 1991). Thus, HRCT was introduced in 1985 to overcome this limitation. It became possible to generate slices of 1 mm thickness, which could provide anatomical detail of the lungs similar to that available from gross pathological specimens (Sluimer et al. 2006). This type of CT provided a significant advance in increasing understanding of pathology and pathophysiology of diffuse lung diseases. Müller believed that HRCT has the highest sensitivity and specificity among other imaging techniques in detecting the focal and diffuse lung diseases because it could identify the small airway changes with a thin section CT of 1-to-2-mm collimation by applying a high spatial resolution algorithm (Müller 2001).

The fact that spiral or helical CT, a continuous CT scanner, leads to high quality data of imaging in airways, pulmonary vessels, systemic vessels and lung nodules has been well defined. This multi-detector CT scanner provides multi-planar reconstructions of the pictures and three dimensional images of structures and visualisation of pulmonary and systemic vessels, which in detail can compare to conventional angiography findings. Additionally, with graphical software programs, this CT is able to delineate the luminal surface of the airways with pictures similar to those of bronchoscopy (virtual bronchoscopy) or bronchography (virtual bronchography) (Müller 2001).

Despite the advantages offered by the introduction of HRCT, scanner speed limitations at the time meant that a 1-cm gap between slices was necessary to cover the entire thorax while avoiding breathing artefacts. In the last ten years, multi-detector-row scanners have been developed to fill this gap between slices. These scanners provide up to 64 1-mm slices simultaneously per rotation, and run each rotation in less than a second (Sluimer et al. 2006). It is now therefore possible to allow isotropic acquisition of the complete thorax with sub-millimetre resolution within a single breath-hold, thereby removing breathing artefacts. Thus, current scanners improve both temporal and spatial resolution (see appendix 3), cover more anatomical areas, increase continuously contrast material and allow better quality reconstruction compared to a single-detector row CT (Siegel 2003).

The advance of this multi-slice x-ray computed tomography (MSCT) together with HRCT and spiral CT make it possible to reconstruct a 3 dimensional image of the lung and the bronchial tree down to the sub-segmental level (Weinheimer et al. 2008). The quality of this method was also higher than that of single-detector row CT in detecting central airways in animal models even when the low dose of x-ray beam was applied (Boiselle et al. 2003). Additionally, Hoffman et al claimed that the multi-detector row CT can correctly elicit the lung to the fifth through seventh generation bronchi in ten seconds or less and provide regional lung density, texture, ventilation and perfusion. Therefore, it is appropriate for detecting the pathological processes of interstitial lung diseases (Hoffman et al. 2003).

CT lung research focuses mainly on evolving and testing the models that accurately define anatomical pictures of bronchial trees and accurately provide functional images in many lung conditions. Additionally, there has been some effort to quantify the variables of CT in clinical practices to help clinicians in applying surgical treatments and pharmacotherapeutic trials (Madani et al. 2001).

2.12. HRCT parameters for characterising COPD

Airway remodelling is common in COPD when many inflammatory cells infiltrate into the bronchial tissue and cause thickening of the airway walls (Turato et al. 2001). Thus, airway wall thickness measured by HRCT has been used to relate to this inflammation process and to the degree of airflow obstruction in COPD (Tiddens et al. 1995). The parameters that indicate airway wall thickness which can be detected by HRCT are wall area (WA =airway external-airway internal: mm^2), square root of wall area ($\sqrt{WA}$, internal area (IA: π (internal diameter)^2: mm^2), percentage of wall area (WA% = airway external-airway internal x 100/airway external), the ratio of airway wall thickness to total airway diameter (T/D ratio = external diameter-internal diameter/2) the ratio of wall area to internal airway (WA/IA) (Deveci et al. 2004) and the summation of WA to summation of IA ($\Sigma WA/\Sigma IA$) to determine normalised airway wall thickness (Berger et al. 2005).

Another parameter which can be calculated from HRCT is an emphysema score of both lungs to classify the percentage of emphysematous tissue in the lungs. The most commonly used score is based on that developed for the National Emphysema Treatment Trial (NETT) and is assessed by visual inspection which is dependent on the radiologist's opinion and hence still be subjective. The score has 5 levels i.e. no trace development, trivial involvement of emphysematous tissue <5%, mild involvement of emphysematous tissue 5 - <25%, moderate involvement of emphysematous tissue 25-50% and severe involvement of emphysematous tissue >50% (Patel, et al., 2008). The emphysema scoring is based on a threshold of the Hounsfield units of the data. The severity of emphysema can also be quantified, by using free open-source software to quantify the percentage of the lung field occupied by low attenuation areas (LAA%). Emphysema is defined as LAAs with specific Hounsfield unit thresholds (Dransfield et al 2007). There are other parameters created by new software which are also used to characterise COPD, such as volume fraction of emphysema (V_{950}), mean lung density and CT air trapping index (Lee et al. 2008).

However, in addition to airway wall thickness, modern CT can provide three dimensional data which can be used to create images in coronal and sagittal planes of airways configuration, including airway length, airway diameter and airway angle (Sauret et al. 2002). The branching angles at which the airways divided from their parents could be useful to describe progressive morphological changes of airways and consequently the mechanism of airflow changes in COPD patients. These parameters have not yet been studied. Thus, the researcher believes that research to explore any relationships between airway dimensions and the mechanisms of airflow in COPD is needed in order to provide useful data to describe the pathological changes in COPD.

2.13. Research studies involving HRCT and COPD

HRCT has been studied in COPD to show abnormalities of airways and lung parenchyma which are believed to be a result of chronic airflow obstruction (Reilly 2006). HRCT is believed to be the most sensitive measurement among imaging techniques, at present, to measure peripheral airway dimensions and emphysematous areas of COPD lungs (Müller 2001). The following section will

evaluate the research studies of HRCT in COPD in relation to 1) the precision of HRCT software in quantifying airway dimensions 2) the ability of HRCT to differentiate among healthy people, smokers and phenotypes of COPD 3) the possible correlation between HRCT and CALSA in pulmonary studies.

2.13.1. The precision of HRCT software in quantifying airway dimensions

In order to quantify HRCT data in an objective way, it is essential to develop software which is automated and validated to measure airway dimensions both in vitro and in vivo. Validation studies usually involve the use of phantom airways (i.e. simulated airway models constructed from Plexiglass/Silicon etc). Although these may not be truly representative of in vivo human lungs, they provide useful models for assessing the ability of software to produce consistent results. There are several publications which study the accuracy of HRCT software to measure airway dimensions (King et al. 2000; Berger et al. 2005; Dame Carroll et al. 2006; Hasegawa et al. 2006; Montaudon et al. 2007).

Most of these studies made their measurements using phantom airways or the airways of animals which may not mimic human airways. Although the surrounding pressure was controlled in these studies to inflate lungs before excising and scanning them by HRCT, there may be some shrinkage of lung tissue which prevents the findings being representative of an *in vivo* study. Nevertheless, using excised lungs provides the possibility to study the effect of volume averaging, since it can include irregularities in the adventitial and mucosal borders, varying airway orientation, varying thinness of the airway wall and adjacent blood vessels which cannot be duplicated by phantom studies (King et al. 2000). Additionally, recent advances in tissue conservation technology provide the *in vivo* condition which mimic the human lung as closely as currently possible (Montaudon et al. 2007).

To test the precision of software to detect airway dimensions, many gold standards have been used, such as an optical micrometer (King et al. 2000), callipers (Dame Carroll et al. 2006) and manual methods by experts (Berger et al. 2005; Montaudon et al. 2007). Despite the variety of standards used, most studies reported a similar small error of airway wall thickness measurement (mean ± SE =0.17±0.32 mm^2 (King et al. 2000) and 95% limit of agreement = ±4.3 mm^2 (Dame Carroll et al. 2006) and airway lumen (mean ± SE =

0.52±0.24 mm^2 (King et al. 2000) and 95% limit of agreement = ±3.2 mm^2 (Dame Carroll et al. 2006) between HRCT software and the standard (optical micrometer, calipers).

Additionally, there was high agreement between manual measures by experts and HRCT software for internal area (IA) (r=0.953–0.986) and wall area (WA) (r=0.859–0.883) and this value was still high when repeated measurements were made over time (r=0.999 for IA and r=0.998 for WA (Berger et al. 2005). Therefore, it seems to be generally accepted that HRCT software can be used to represent the values of airway dimensions for both in vitro and in vivo lungs. Nonetheless, some authors have found an overestimation in both airway wall area (King et al. 2000; Dame Carroll et al. 2006) and lumen area (Dame Carroll et al. 2006) and it has been reported that the accuracy seems to decrease as airways become smaller (King et al. 2000; Hasegawa et al. 2006; Montaudon et al. 2007). Thus, the software may not be entirely accurate in verifying the dimensions of peripheral airways, which are the ones that are primarily affected in COPD. However, Nakano et al (2005) have found that large airways with internal diameter of 7.5 mm may be used to predict the mean dimensions of small airways with an internal diameter of 1.27 mm with moderate association (r^2=0.57, p<0.01) when they compared HRCT with histological measurements in human lung biopsy (Nakano et al. 2005). If their findings prove to be reproducible, this may provide a rationale for the application of large airway data (which HRCT can measure with greater accuracy) to represent the dimensions of small airways, in order to reflect the underlying pathology and differentiate phenotypes within COPD.

2.13.2. The ability of HRCT to differentiate among healthy subjects, smokers and different phenotypes of COPD patients

Berger et al (2005) applied an algorithm to HRCT data from 24 subjects and found that it was possible to differentiate among three groups that are smokers with COPD, smokers without COPD and non smokers. Normalised airway wall thickness was significantly larger in smokers with COPD than it was in either smokers or non-smokers without COPD. Deveci et al (2004) also explored the lung morphology of smokers, non smokers and COPD patients, comparing HRCT data to pulmonary function test data in 10 healthy non-smokers, 10 healthy smokers, 17 COPD stage 2 (moderate disease) and 5

COPD stage 3 (severe disease) (Deveci et al. 2004). Airway dimensions among the four groups were compared using airway wall thickness (T) to total diameter (D) ratio (T/D) and the percentage of wall area (WA%). The results showed that mean WA% and mean T/D were significantly higher in COPD than smokers and non smokers in both small and large airways ($p<0.01$) while mean WA% was significantly higher in smokers than non smokers in only large airways ($p<0.01$). As would be expected, FEV_1 was significantly lower in COPD and smokers than non smokers ($p<0.005$). However, WA% and T/D were not different between moderate and severe COPD. There was a negative correlation between airway wall thickness and FEV_1 %predict ($r= -0.735$ for WA% and -0.713 for T/D) but there was a positive correlation between airway wall thickness and cumulative smoking history ($r=0.608$, $p<0.001$ for WA%, 0.624, $P<0.001$ for T/D). They suggested that WA% may be used to differentiate between smokers and non smokers. They also suggested that HRCT data (which provided both small and large airways dimensions) would be useful for measuring lung function in COPD and smokers (Deveci et al. 2004).

There are some limitations in this study as the sample size of the severe COPD group is small (5 patients) which may be a reason for the lack of a statistically significant difference in airway wall thickness between moderate and severe COPD. Additionally, all the COPD patients were male making the results less generalisable for the whole population. Nevertheless, this study was able to investigate both small and large airways and found a statistically difference between smokers and healthy controls in large airways, which may have been caused by responses to toxic stimulation (cigarette smoke). It is not known if the size of this difference was also clinically relevant. However, Hasegawa et al (2006) suggested that airflow limitation in COPD was more closely related to the dimensions of the distal (small) airways than proximal (large) airways. They developed new software for measuring airway dimensions using curved multiplanar reconstruction (see appendix 3, p172). They used this new software in 52 patients with clinically stable COPD to measure airway dimensions and correlated them with FEV_1 percent predicted. The results showed that there was a positive correlation between airway luminal area (Ai) and FEV_1% and a negative correlation between WA% and FEV_1%. Additionally, the correlation coefficient of those variables was higher for smaller airways from the third to sixth generation (Ai and FEV_1%: r=0.26, 0.37, 0.58 and 0.64 for

right upper lobes (B1) and 0.60, 0.65, 0.63 and 0.73 for anterior basal bronchus of right lower lobes (B8) respectively; WA% and FEV_1%: r=-0.224, -0.260, -0.477 and -0.552 for B1 and -0429, -0.485, -0.488 and -0.547 for B8). Thus, the study concluded that airflow limitation in COPD was more closely related to the changes in small airway dimensions than large airway dimensions (Hasegawa et al. 2006). This study may be the first published study to show the values of airway dimensions from generation 3 to 6 by HRCT.

HRCT has also been used to determine the independent contribution of airway wall thickness and emphysema score to airflow limitation in COPD. Patel et al (2008) compared airway wall thickness and a qualitative emphysema score with pulmonary function tests in 519 COPD patients (FEV_1<60% predicted and FEV_1/FVC<90% predicted, age (mean± SD) 58.0±5.3 years with smoking history > 5 pack-years) and 640 individuals related to those with COPD (FEV_1/FVC<90% predicted, FEV_1/FVC <0.7, with smoking history > 5 pack-years, age (mean± SD) 57.2±9 years). They measured airway wall thickness by plotting lumen perimeter (Pi) against the square root of wall area for all airways with a lumen perimeter >6mm and calculated the square root of the wall area at a Pi of 10 mm and 20 mm for each subject. A qualitative emphysema score measured by the radiologist was also used to classify the severity of emphysema (as mention before in section 2.12).They found that FEV_1 was independently associated with airway wall thickness and emphysema. Additionally, both emphysema and airway disease present independent aggregation within the families of individuals with COPD (Patel et al. 2008). However, this study assessed airways larger than 2 mm, and these airways may not reflect small airways where most airflow limitation occurs in COPD. Also the emphysema score is defined by the radiologist's opinion which is subjective and hence less reliable.

According to the literature, HRCT is able to show the different values of airway dimensions among healthy people, smokers and COPD. Therefore, this technique may be sensitive enough to detect early changes in airway geometry and emphysematous changes during the early onset of COPD, and may also be useful to identify those at risk of developing COPD.

2.14. The possible correlation between HRCT and CALSA

CALSA has recently become of interest to many researchers as an aid to diagnosis in many pulmonary disorders (Murphy 2008). It is believed that the quality of lung sounds depends on the anatomy of the airways (Pasterkamp et al. 1997b), which can now be displayed in three dimensional images using HRCT. There are only a few publications comparing lung sound data with HRCT findings, and these have presented only qualitative data (e.g. visually assigned emphysema scores) which do not reflect the specific geometry of airways which affect the mechanism of airflow and hence lung sounds. Al Jarad et al (1993) compared clinical auscultation, CALSA, HRCT and chest radiography to detect early signs of asbestosis. Fifty-three asbestos subjects (51 men and 2 women; age (mean± SD) 61±7 years) were divided into two groups using the International Labour Office scale (ILO) (Intemational Labour Office 1980). This is a classification system for grading the quality of chest x-rays and describing and coding abnormalities. Using this system to examine the chest x-rays, they identified 21 subjects with an ILO score of > 1/0 (indicating the presence of asbestosis) and 32 with an ILO $\leq$ 1/0 (indicating no evidence of asbestosis). The score represents changes in parenchymal tissue of the lung from chest x-ray with the first number indicates the profusion (concentration) of small opacities (0=absence, 1= presence) and the second number indicates the profusion seriously considered as an alternative (0=not serious, 1=serious). Standard auscultation was performed by two clinicians at the posterior chest wall and lung sounds were recorded at the same area. HRCT was performed from lung apex to lung bases in full inspiration up to total lung capacity. The results showed that CALSA could detect crackles in 14 patients with ILO $\leq$ 1/0 (44%) while standard clinical auscultation could detect 7 patients (22%). All but two patients with evidence of abnormal interstitial diseases or gravity dependent subpleural line measured by HRCT had crackles detected by CALSA. Both auscultation and CALSA revealed mid to late inspiratory crackles in patients with ILO >1/0.They concluded that CALSA can detect abnormalities in asbestos subjects with greater sensitivity than auscultation or chest radiography when using HRCT as the gold standard to be compared (Al Jarad et al. 1993). As the majority of the subjects were male, the results from this study may not reflect the values among a general population. Although the inter-observer agreement for ILO score is low (as mentioned in discussion of this

article but they do not report an actual number), it is only used to screen normal and abnormal lungs before measuring the subject by HRCT, standard stethoscope and CALSA. Thus, this seems to not affect the results and conclusion of the study.

Piirila et al (2000) also compared lung sound data with HRCT and pulmonary function tests in 64 patients with asbestos-related pleural disease with or without pulmonary diseases. They measured fibrosis and emphysema score by HRCT, a quartile frequency of power spectrum of lung sounds (F25, F50) and frequency of maximum intensity (Fmax) by CALSA, diffusing capacity and pulmonary function tests by spirometry and found a positive correlation between fibrosis score and inspiratory crackles counts (r=0.625, p=0.0001) and a positive correlation between fibrosis score and expiration F50 (r=0.406, p =0.0009). There was also a positive correlation between diffusing capacity with inspiration Fmax (r=0.529, p=0.024), F25 (r=0.473, p=0.047) and RMS (r=0.481, p=0.043). Additionally, the emphysema score was negatively correlated with expiratory F25 (r=0.478, p=0.045) and F50 (r=-0.482, p=0.043). Moreover, high sound frequency was associated with fibrotic changes of the lungs while low sound frequency was associated with pulmonary emphysema. They concluded that lung sounds were significantly correlated with HRCT findings of pulmonary tissue in asbestos-exposed subjects. Thus, CALSA may provide complementary clinical information to evaluate asbestos-related pulmonary disease (Piirila et al. 2000).

Only one study has been identified which explored the relationship between crackle parameters and subjective HRCT findings (Kawamura et al. 2003). In Kawamura et al's study, 18 patients with interstitial pneumonia and 23 with non-interstitial pneumonia (26 men and 15 women; median age 65.2 years) were recruited. Two radiologists analysed HRCT data independently and were blinded from crackle findings. The kappa coefficient values between the two radiologists (a statistical measure of inter-observer agreement for categorical items) showed 'moderate' to 'good' association at 0.62-0.89. The results showed that crackle IDW was significantly shorter in the group with marked increase in lung attenuation on expiratory CT than in the group without it (0.93 ms vs 1.33 ms). Crackle 2CD was significantly shorter in the group with traction bronchiectasis (4.71 ms) and longer in the group with emphysematous

change (7.08 ms) than in the group without them (6.80 ms, 4.92 ms). In non-interstitial pneumonia patients, there was no statistically significant association between crackle IDW or 2CD and any of the HRCT findings. The researchers concluded that abnormal respiratory sounds were associated with some HRCT findings. Thus, they suggest that acoustic properties may be attributable to pathological disease changes and crackle characteristics may be described by HRCT findings (Kawamura et al. 2003). However, the amount of difference in crackle durations between groups in this study is not enough to differentiate between coarse and fine crackles which can be used to provide information to differentiate between some pulmonary diseases (Piirila 1991, Piirila and Sovijarvi 1995). Additionally, their conclusion is based on a comparison between objective crackle data and subjective HRCT findings. The latter are open to interpretation which provides a potential source of bias.

2.15. Conclusion

This chapter has presented the background knowledge of CALSA and HRCT and their application in COPD. The literature reviews lead to the conclusion that CALSA has a potential to be a useful measurement to detect airway obstruction and HRCT is able to display airway dimensions with minimal error and is also claimed to be the most sensitive measurement among imaging techniques available at present. Moreover, HRCT data can be used to differentiate among healthy people, smokers and non smokers. However, with its cost and difficulty of access, mass screening with HRCT is impractical. CALSA, in contrast, is more accessible, cheap and can be applied in all age ranges. Additionally, it can be an alternative measurement to detect obstruction in the airways. Despite there being some documents reporting the association between qualitative HRCT findings and lung sound data, there is no published study relating objective HRCT findings as a three dimensional picture of airways and lung sound parameters in normal and pathological lungs. If lung sounds are found to have a significant relationship with HRCT, it may be possible to use CALSA to detect airway obstruction. Thus, the researcher believes that research in these aspects should be conducted to lead to new knowledge, and provide novel data for the diagnosis, classification and monitoring of COPD. The next chapter will present research questions, objective and method.

Chapter 3 : Method

3.1. Introduction

This chapter will explain the methods used in this study. An overview of the protocol performed in this research will be presented. Aspects of the method are described as follows: research questions, research objectives, study design, research governance, sample population, data collection procedure, data analysis and conclusion.

Although the aim of this research was to compare lung sound data with HRCT data, the researcher was aware that with limited funding it would not be possible to design and conduct her own HRCT study. It was therefore decided to collaborate with ongoing HRCT studies that were suitable.

The research presented in this thesis is therefore an addition to a larger, externally-funded HRCT study (appendix 4) which involved 4 groups of subjects; COPD stage 1 and 2 patients, healthy-non smokers and healthy smokers. The research questions were therefore formulated with consideration to the available groups of research subjects for the funded study.

3.2. Research questions

This study was designed to answer two main research questions:

- Are there statistically significant relationships between the characteristics of added lung sounds and measures of airway geometry, airway wall thickness and percentage of wall area in healthy non-smokers, healthy smokers, or patients with COPD?
- Are there any cut off points using added lung sound variables measured by CALSA and airway wall variables and emphysema scores measured by HRCT to differentiate between healthy subjects and COPD patients?

3.3. Research objectives

The main objective of this study was to explore whether there are any significant relationships between added lung sound characteristics measured

by CALSA and the geometry of airway measured by HRCT. The specific aims were:

- To investigate the relationship between crackle 2CD and airway diameter, length, internal perimeter, branching angles, airway wall thickness and percentage of wall area in healthy non-smokers, healthy smokers, COPD stage 1 and COPD stage 2.
- To investigate the relationship between the number of crackles per breathing cycle (NCpB) and airway diameter, length, internal perimeter, branching angles, airway wall thickness and percentage of wall area in healthy non-smokers, healthy smokers, COPD stage 1 and COPD stage 2.
- To investigate the relationship between the number of wheezes per breathing cycle (NWpB) and airway diameter, length, internal perimeter, branching angles, airway wall thickness and percentage of wall area in healthy non-smokers, healthy smokers, COPD stage 1 and COPD stage 2.
- To investigate the relationship between wheeze frequency and airway diameter, length, internal perimeter, branching angles, airway wall thickness and percentage of wall area in healthy non-smokers, healthy smokers, COPD stage 1 and COPD stage 2.
- To predict the number of crackle 2CD, NCpB, NWpB and wheeze frequency based on the number of airway diameter, length, internal perimeter, branching angles, airway wall thickness and percentage of wall area in healthy non-smokers, healthy smokers, COPD stage 1 and COPD stage 2.
- To predict the airway diameter, length, internal perimeter, branching angles, airway wall thickness and percentage of wall area based on the number of crackle 2CD, NCpB, NWpB and wheeze frequency in healthy non-smokers, healthy smokers, COPD stage 1 and COPD stage 2.
- To evaluate the ability of CALSA and HRCT to differentiate COPD from healthy subjects.

3.4. Study design

A quantitative observational design was suitable for this study as no experiment was required to explore the relationships between lung sound parameters and airway dimension parameters.

3.5. Research governance

3.5.1. Ethical approval

This research was conducted under ethical approval for the main study "An Investigation into the Application of Imaging to the Characterisation of the Phenotypes of Chronic Obstructive Pulmonary Disease". The Southampton and South West Hampshire local research ethics committee approved the study (LREC number: 09/H0502/91, appendix 4) and the Southampton University Hospital Trust Research and Development department sponsored the study. The full protocol for this study can be found in the appendix 4. The researcher wrote the lung sound data collection and analysis section in the protocol of the large study that was submitted for ethical approval, but was not responsible for the ethical approval process.

3.5.2. Ethical consideration

Verbal and written explanation was given to all subjects including the objectives in measuring lung sounds and an information sheet (appendix 4, p208-223) regarding the whole study was also given. The subject consent was obtained by a written consent form (appendix 4, p 205-207) and subjects were reminded that they had the right to withdraw from the study at any time if they wanted without giving any reason. The researcher is a qualified physiotherapist and received additional training in operating the digital stethoscope to a safe standard. There are no known side effects from recording lung sounds by digital stethoscope.

3.5.3. Health and safety

The Southampton University Hospital Trust (SUHT) Hand Hygiene Policy and Infection Control Policy were followed. The sterile technique was applied

before and after making physical contact with the subjects by washing hands with an antiseptic solution. The 70% alcohol wipes were used to clean the stethoscope before and after every recording. The laptop computer was disconnected from the mains power supply during patient contact to remove the risk of electrical shock.

3.6. Sample population

The main study originally intended to recruit a sample of 20 subjects for healthy (10 healthy smoker and 10 healthy non-smoker) and 20 subjects (10 COPD stage 1 and 10 COPD stage 2) for COPD. The lung sounds part of the study was exploratory and so no formal power calculation was appropriate. To preserve resources and decrease the impact on the subjects, historical screening data that had been obtained less than a year prior to the date of inclusion into this study were accepted. The inclusion and exclusion criteria were dictated by the needs of the larger HRCT study (appendix 4, p186-188) within which this study was embedded.

3.6.1. Inclusion criteria

- Age 40-70 years

GOLD Stage 1 COPD patients

- Current or ex smokers
- Physiological evidence of obstructive airway disease (FEV_1/FVC ratio of <70%)
- FEV_1 of ≥ 80% predicted
- Incomplete disease reversibility (<10% improvement with 400 ug inhaled salbutamol)
- Symptoms of breathlessness, cough or sputum production
- No evidence of exaggerated diurnal variation to suggest asthma
- Normal exhaled NO (exclude asthma)

GOLD Stage 2 COPD patients

- Current or ex smokers

- Physiological evidence of obstructive airway disease (FEV_1/FVC ratio of <70%)
- FEV_1 of 50-79% predicted
- Incomplete disease reversibility (<10% improvement with 400 ug inhaled salbutamol
- Symptoms of breathlessness, cough or sputum production
- No evidence of exaggerated diurnal variation to suggest asthma
- Normal exhaled NO (exclude asthma)

Smoking control

- Current smokers
- Normal spirometry
- Normal exhaled NO
- PC_{20} (Histamine) >8 mg/ml (non-cumulative: exclude asthma)

Healthy control

- Life- long non-smoker
- No history suggestive of airway disease
- Normal spirometry
- Normal exhaled NO
- PC_{20} (Histamine) >8 mg/ml (non-cumulative: exclude asthma)

3.6.2. Exclusion criteria

- Pregnancy or breast feeding
- Significant co-morbidity (e.g. ischaemic heart disease)
- Current acute lung infection or acute exacerbation of COPD, investigation will be deferred until the infection is treated
- Inability to understand directions for doing and study assessment
- Inability to be contacted in case of emergency
- Past or present tuberculosis, systemic lupus erythematosis or multiple sclerosis

- Any clinically significant neurological, renal, endocrine, gastrointestinal, hepatic or haematological abnormalities uncontrolled with standard treatment
- History of psychiatric, medical or surgical disorders which may interfere with the study

3.6.3. Recruitment process

The details of subject recruitment followed the protocol of the main study and are presented in appendix 4, p184-185. The subjects were contacted via many sources such as clinical appointments at the respiratory centre at the Southampton University Hospital Trust (SUHT), out-patient appointments at SUHT and possibly from clinical and outpatient settings at other hospitals in the region, the existing three Division database, hospital and community pulmonary rehabilitation classes, primary care organisations with includes General Practices, advertisement and other nearly finished existing studies which can ensure that the twelve week 'wash-out' period is observed. There is no subject involved from the other nearly finished studies.

3.7. Data collection procedure

The data collection was performed at the Southampton University Hospital Trust. Subjects had made an appointment in advance with the radiologist for HRCT imaging, and lung sound data were collected on the same day. After the study had been explained to the subject, demographic and basic anthropometric data were collected from the medical chart and by interview, together with clinical history such as diagnosis, past history, medication and smoking habits. Variables such as age, gender, height and weight were also collected because these have been reported to affect the characteristics of lung sounds (Pasterkamp et al. 1997b).

3.7.1. Lung sound measurement

The location for data collection was the waiting area in the HRCT room and was the same area for all measurements of the study. To measure the level of background noise, the researcher placed the digital stethoscope head against the surface of the bed (which was considered to be approximately equivalent

to placing the stethoscope against the chest wall during lung sound recording with zero airflow and no heartbeat) (see section 2.5.1.) and recorded the sound for 25 seconds. This sound signal was then analysed in Matlab to find out if the amplitude of the background noise was at least 10 times lower than that of the lung sound signals detected from the chest wall of a breathing patient. This test was performed for every lung sound recording session. The subjects were asked to lie on their backs in the same position as when they were measured by HRCT with their arms slightly abducted. A digital stethoscope (The ds32a+ Stethoscope, Thinklabs, USA) was connected to a laptop (Toshiba Satelline, Intel Centrio) installed with Matlab (Mathworks, MA, USA) software. The stethoscope was placed, according to CORSA guidelines, in six chest locations (excluding the trachea because the intention of the study was to compare added lung sounds over the chest wall and the airway dimensions under the chest walls) : anterior right region of chest wall and anterior left region of chest wall (second intercostal space, mid-clavicular line); lateral right region of chest wall and lateral left region of chest wall (fourth to fifth intercostal space, mid-axillary line); posterior right region of chest wall and posterior left region of chest wall (five centimetres from the paravertebral line) and seven centimetres below the scapular angle of both sides (Figure 3:1). The recording for each anatomical location lasted twenty five seconds (Rossi et al. 2000). This time is dictated by the stethoscope and cannot be altered. Subjects were asked to take slightly deeper than normal breaths through their mouths because the airway resistance in the oropharynx is lower than those of the airflow in the nasal passage (Moussavi et al. 2000). The subjects were also asked to breathe at their normal rate because the intention of the study was to apply CALSA in normal clinical practice condition.

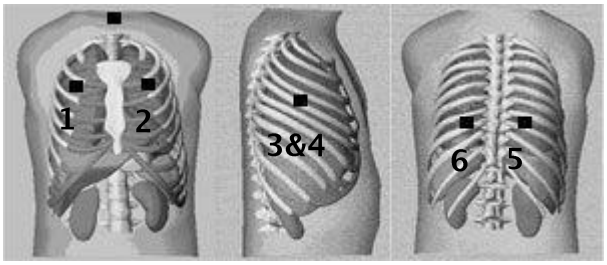


Figure 3:1: Diagram of the six auscultation locations of the chest used in this research: 1– anterior right, 2 – anterior left, 3 – lateral right, 4 – lateral left, 5 – posterior right, 6 – posterior left (Adapted from Marques 2008 with permission)

Airflow control was not used because it would have required the application of a mouthpiece or facemask (Figure 3:2). Recording without airflow control has been reported to give high sensitivity and specificity in detecting wheezes (Taplidou & Hadjileontiadis 2007; Parthak et al. 2008) and crackles (Murphy et al. 2004). Appropriate rest was provided between recordings to prevent over-breathing leading to dizziness or other symptoms of hypocapnia (low carbon dioxide). The input from the stethoscope microphone was connected via an integral amplifier to the sound card of a laptop with customised software, suitable for data acquisition and analysis, written in Matlab (version 7.1). A sampling frequency of 44.1 kHz was adopted for all recordings.

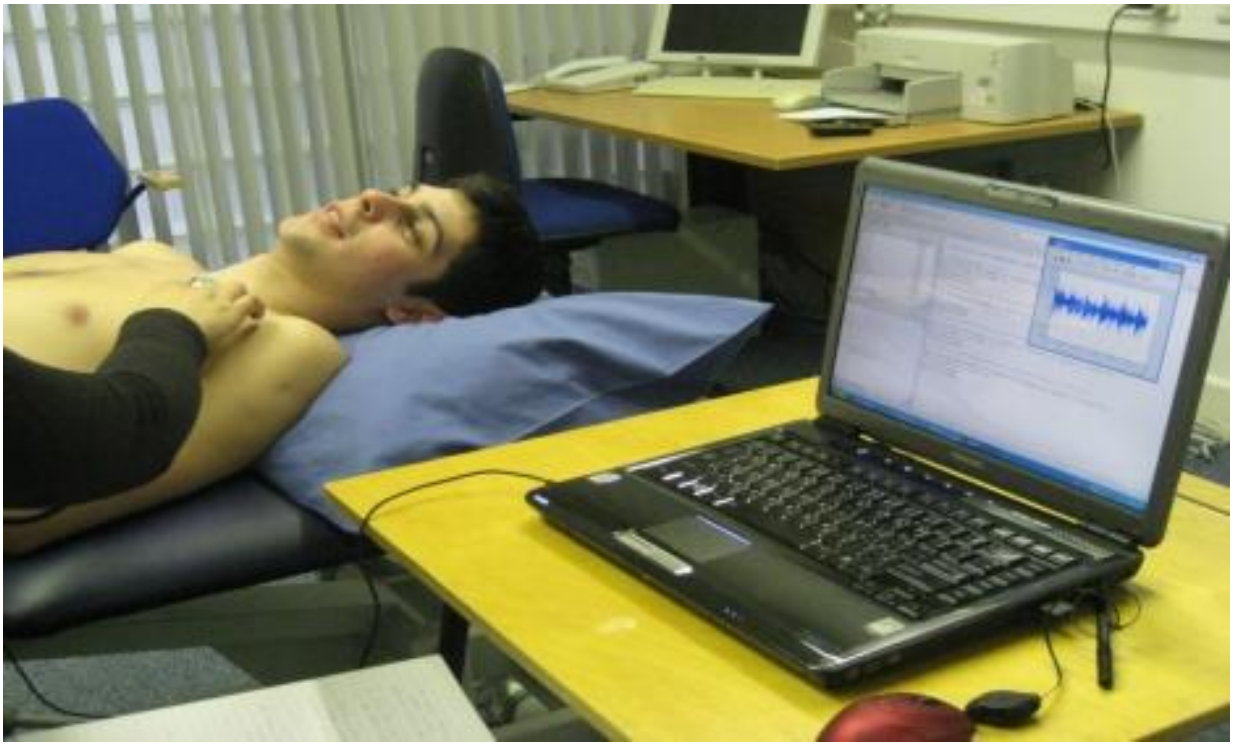


Figure 3:2: Lung sound recording using digital stethoscope

3.7.2. HRCT imaging and processing

The HRCT scan was performed on a Siemens Sensation 64 CT scanner (Siemens Medical Solutions Erlangen, Germany) using a high resolution algorithm, with detector aperture 0.6 mm, pitch 1, effective mAs 90, 120 kV (see appendix 3). The effective dose for this procedure was approximately 3.8 milli-sievert (mSv) to get the best visualization of the airway tree (Coxson 2008). Images were captured with subjects in suspended full inspiration and on full expiration (Figure 3:3). The combined radiation dose of the inspiratory and expiratory scans was approximately 7 mSv. The expiratory CT scan required a lower radiation dose because the lung fields were smaller. The sequence of data collection is demonstrated in diagram 1.

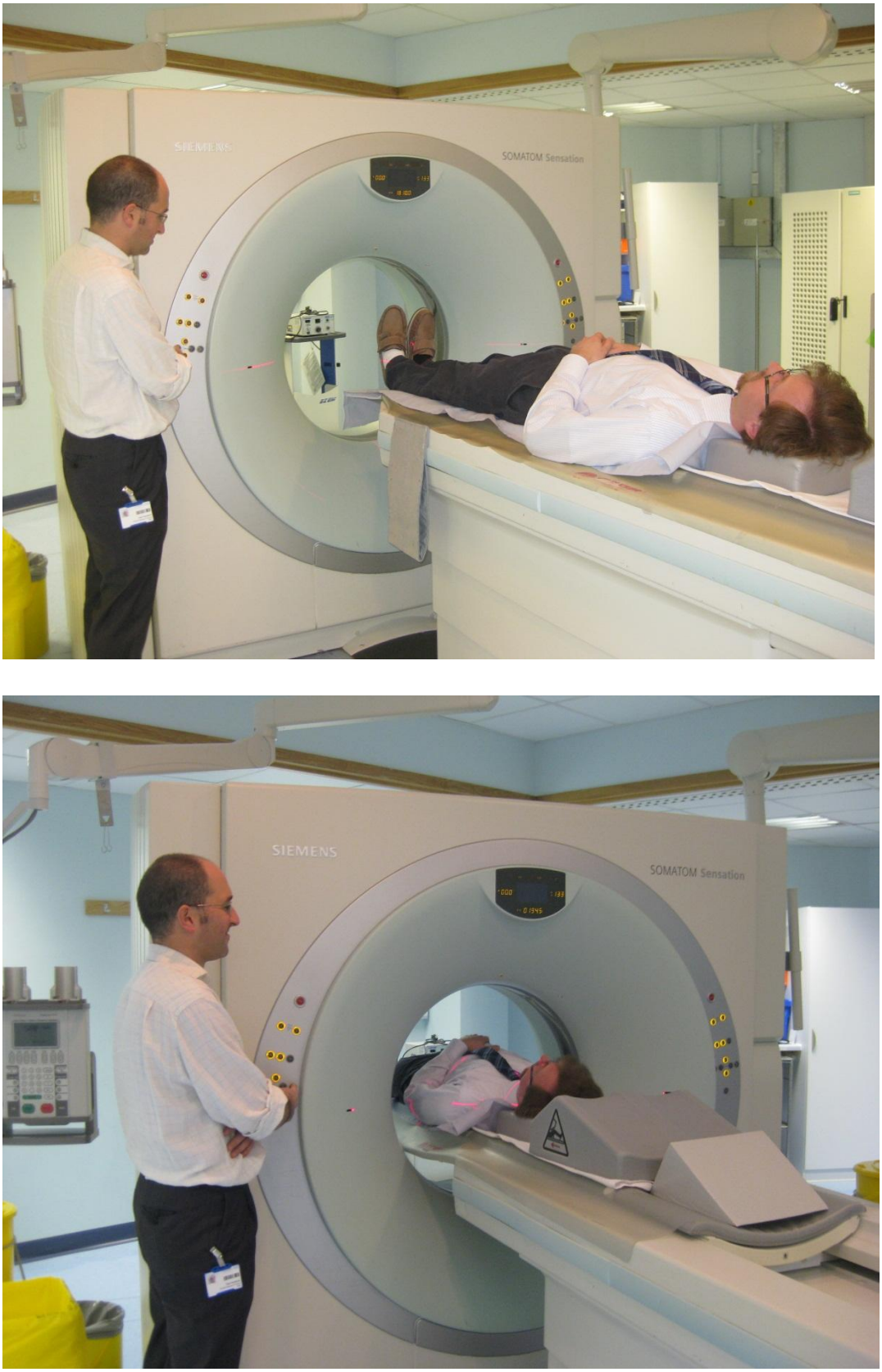


Figure 3:3: HRCT scanning

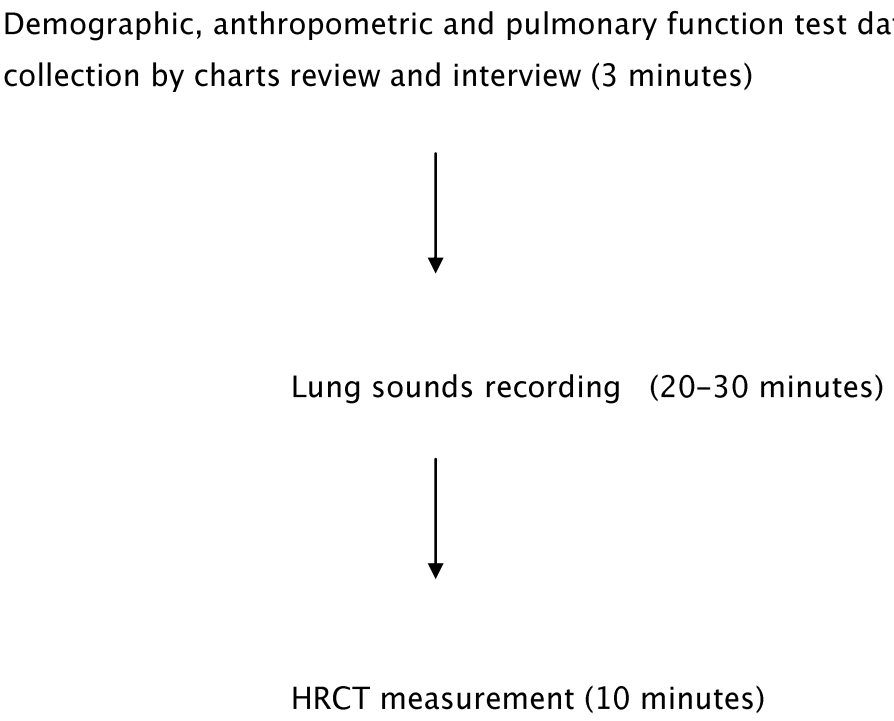


Diagram 1: Process of data collection

3.8. Data analysis

3.8.1. Processing the lung sound files

All lung sound files from the six areas of the chest wall were processed using an algorithm written in Matlab by Dr. Anna Barney and Professor Paul White (University of Southampton). This software allowed the operator to determine the breathing cycle detection manually by listening to the .wav file. The researcher had to pick the starting and ending points of the breathing cycles manually in the MATLAB program, by listening to lung sounds. She used the criterion that these two points should contain a complete cycle (inspiration and expiration) of breathing. The software automatically identified the number of crackles and wheezes per respiratory cycle, the duration of the initial deflection width (ms) and the duration of the two cycles deflection (ms) of each crackle, the type of wheezes, the duration of wheezes (ms) and the frequency of each wheeze (ms) which were then imported and saved in the Excel files. Each Excel file contained 8 columns for crackle analysis with the following information:

Method

1. First column indicates when the breathing cycle begins (seconds);
2. Second column indicates when the breathing cycle ends (seconds);
3. Third column indicates the duration of the breathing cycle (seconds);
4. Fourth column indicates the moment in the breathing cycle when each crackle occurs (seconds);
5. Fifth column indicates the IDW value (ms) of each crackle;
6. Sixth column indicates the 2CD value (ms) of each crackle;
7. Seventh column indicates the moment where the crackles occurs but in percentage of the breathing cycle;
8. Eighth column indicates the percentage of the breathing cycle occupied by crackles.

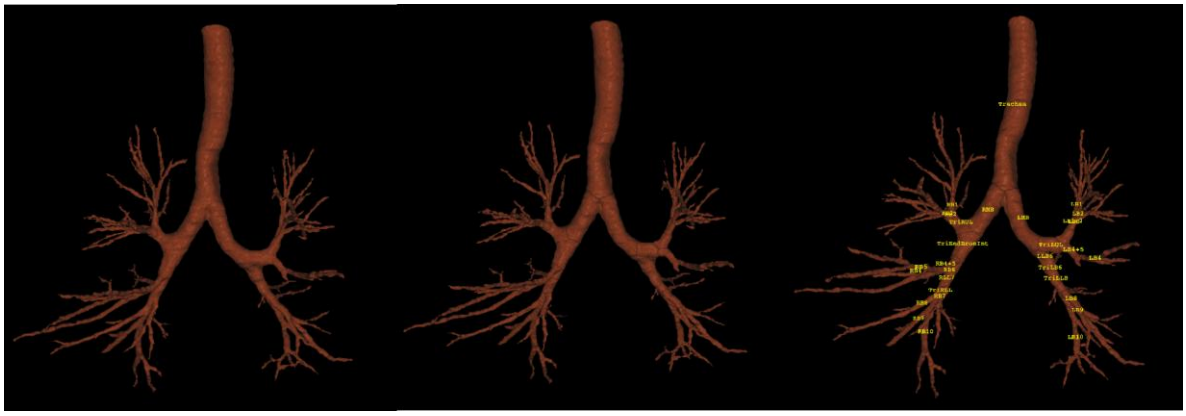
Another Excel file which indicates wheezes also contained 8 columns as follows:

1. First column indicates when the breathing cycle begins (seconds);
2. Second column indicates when the breathing cycle ends (seconds);
3. Third column indicates the duration of the breathing cycle (seconds);
4. Fourth column indicates the moment in the breathing cycle when each wheeze occurs (seconds);
5. Fifth column indicates the duration (ms) of each wheeze;
6. Sixth column indicates the frequency (Hz) of each wheeze;
7. Seventh column indicates the moment where the wheeze occurs but in percentage of the breathing cycle;
8. Eighth column indicates the percentage of the breathing cycle occupied by wheezes.

3.8.2. Processing HRCT data

The images of HRCT were initially reconstructed using a slice thickness of 0.75 mm, a reconstruction increment of 0.5 mm and the Siemens B35f reconstruction algorithm (appendix 3). These parameters were fixed to determine the settings that provided the optimal visualization of the airways recommended imaging protocol for VIDA Pulmonary Workstation 2 algorithm. The Digital Imaging and Communication in Medicine (DICOM) images were transferred from the CT scanner to a dedicated imaging workstation. The

Pulmonary Workstation 2 (PW2) software (VIDA Diagnosis, Iowa City, USA) was used to segment the airway from the HRCT data and to analyse the results to give measures of airway diameter, internal perimeter, length, branching angles, airway wall thickness, percentage of wall area and emphysema score. After importing the HRCT data to VIDA software, Dr. Michael Bennett (the image processing specialist) had to check for the quality of the image and the missing areas of lungs and also the artefacts in pictures to make sure the imaging data were of sufficient standard. Then he picked the starting point called 'a seed point' near the top of the trachea to be analysed. After that, the PW2 software ran a region growing technique to segment the airway tree from that point down to around airway generation 4 or 5 of both lungs. Before the PW2 software did any analysis, he had to choose algorithms such as airway segmentation (a) to separate airway from other data of the lungs, airway skeletonisation (b) to split the segment airway into individual branch, airway labelling (c) to label each branch (Figure 3:4, Figure 3:5) and airway measurement to measure the geometry of the airway in each branch. Then the PW2 software automatically ran and generated the data of the airway tree and reported the result of airway geometry, such as diameter, internal perimeter, length, branching angles, airway wall thickness and percentage of wall area and in both excel file and PDF file.



(a) (b) (C)

Figure 3:4: Airway segmentation- airway voxels are indentified (a), Airway Skeletonisation- The airway voxels are assigned to individual branches (b) and Airway labelling – Each airway branch is labelled (Data from Vida Diagnostic, Pulmonary Workstation 2)

In some cases a degree of user intervention was required to ensure that the PW2 software had segmented the airway tree properly. Firstly, it was necessary to inspect the results of the airway segmentation to ensure that all of the branches were labelled and that the labels were assigned correctly, as shown in Figure 3:5. Secondly, it was sometimes necessary to 'grow' the airways manually to ensure that as much of the airway was segmented as possible, as shown in Figure 3:6 where the airway highlighted in green has been manually added.

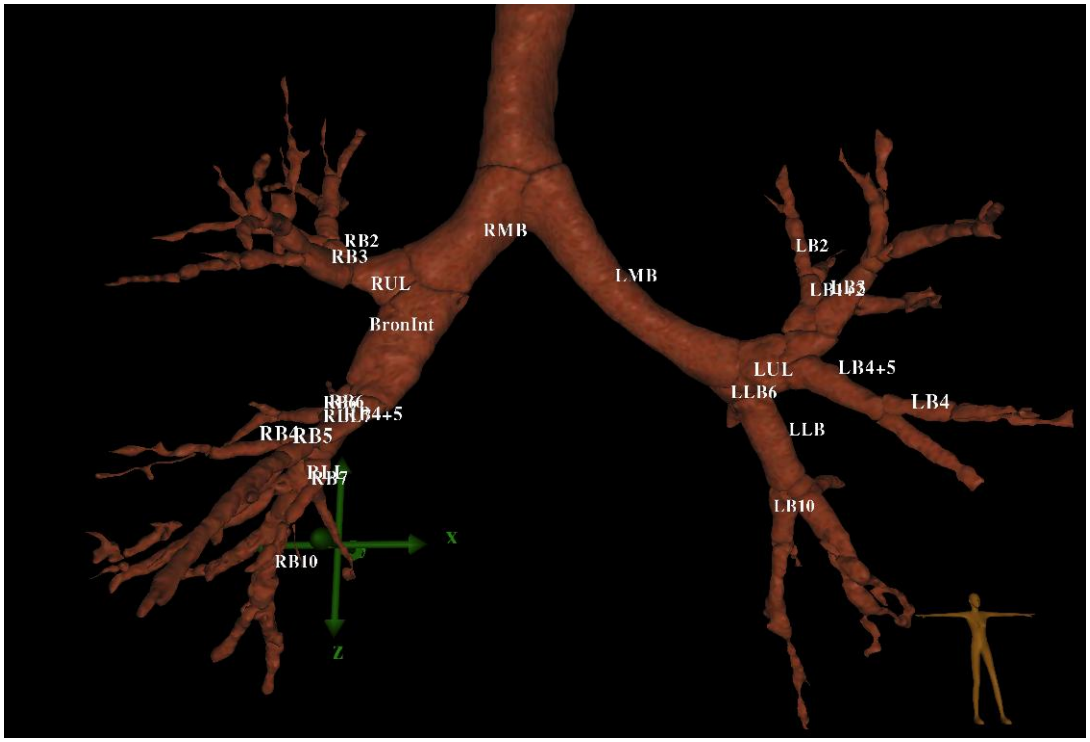


Figure 3:5: Airway trees after labelling

(Data from Vida Diagnostic, Pulmonary Workstation 2)

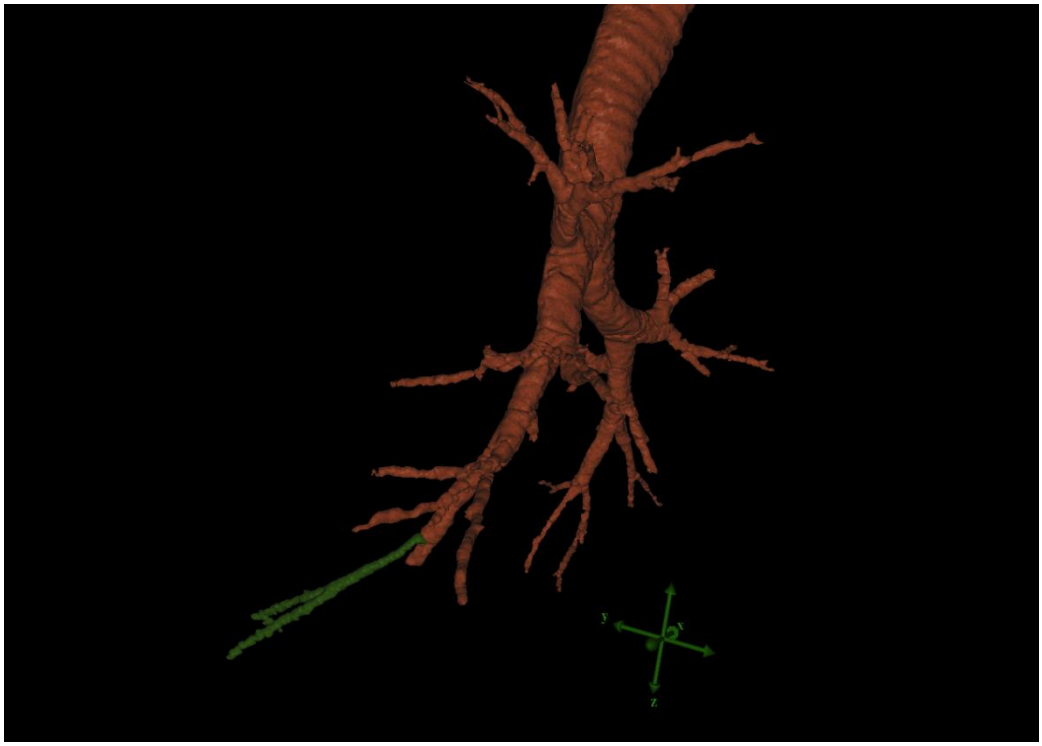


Figure 3:6: A successful manual branching at generation 4 of right lower lobe (The branch is complete construction: Data from Vida Diagnostic, Pulmonary Workstation 2)

3.8.3. Analysis of demographic and pulmonary function test variables, added lung sound data and HRCT findings

Variables such as age, gender, height, weight, forced vital capacity (FVC), the forced expiratory volume within the first second (FEV_1), the forced expiratory volume within the first second percentage predicted (FEV_1 pp), the FVC percentage predicted values (FVC pp) and the percentage of peak expiratory flow (PEF) were transferred into SPSS (SPSS version 16) to be checked for normal distribution of each variable by:

1) Descriptive with Skew and Kurtosis values

2) Frequency distribution histograms

3) Normalities plots (Stem and leaf and box plot)

4) Kolmogorov-Smirnov and Shapiro- Wilk test

The appropriate descriptive statistics (mean ± SD or median and IQR) were used to summarise the sample depending on the spread of these variables. The comparison between groups was also analysed with the appropriate statistics depending on the nature of the data.

The lung sound data were treated first as dependent and then independent variables, and comprised the crackle 2CD, NCpB, NWpB and wheeze frequency. The number of crackles and wheezes detected were divided by the number of breathing cycles for each subject at each of the 6 anatomical locations, to give the NCpB, NWpB at each location. The crackle 2CD and wheeze frequency were also averaged from all crackles and wheezes in all recorded breathing cycles for each subject of the 6 anatomical locations (anterior left region of chest wall, anterior right region of chest wall, lateral left region of chest wall, lateral right region of chest wall, posterior left region of chest wall and posterior right region of chest wall). These numbers were then averaged across the group and the comparison of lung sound data at each site between groups was then analysed using the appropriate statistics depending on the nature of the data.

The HRCT data were treated first as independent and then dependent variables (only airway wall thickness and percentage of wall area which indicate airway wall thickening), and comprised airway diameter, length, internal perimeter branching angles, emphysema score, airway wall thickness and percentage of wall area. These variables at the same generation at each lobe were also averaged to represent the airway geometry at generation 2, 3, 4 and 5 at upper, middle (lingula for left lung) and lower lobe. Then they were averaged across the group and the comparison between groups of airway variable data at each generation of each lobe of the lung was also analysed using the appropriate statistics depending on the nature of the data.

In order to explore any relationship between the characteristics of crackles and/or wheezes and airway variables, bivariate correlation using Pearson's correlation was applied to test the degree of correlation. Multiple regression tests using stepwise method (forward and backward methods together) were also applied to predict the dependent variables from the independent variables. The assumption of multiple regression (regression diagnostics) was tested by:

1) Checking the normalities of independent variables

2) Checking in the variance of each residual (ε) to obtain the constant value or homoscedasticity

3) Checking the mean value of the error term is zero: that is E (ε) = 0.

Then the equation of each dependent variable was predicted by the independent variables.

3.8.4. The proposed model to predict added lung sound variables from airway variables

Currently, there is insufficient evidence to indicate exactly where added lung sounds detected at one area of the chest wall originated. The sounds could originate from the airway at the same lung area as the recording sensor, or be transmitted from the airway at different lobes or even the other lung. In order to explore these possible phenomena, the researcher proposed six different approaches to analyse and report data, based on hypotheses about the origin of lung sounds heard at the chest wall.

Lung sound (acoustic) data correlated with HRCT (geometric data)

In the following description of the 6 approaches for analysis, acoustic data refers to added lung sound data (crackle 2CD, NCpB, NWpB and wheeze frequency) and geometric data refers to HRCT data (airway diameter, length, internal perimeter branching angles, airway wall thickness and percentage of wall area). The detail of six approaches for acoustic and airway geometry analysis is summarised in Table 3:1.

Approach 1: For any given airway generation the acoustic data for each chest location were correlated with the geometric data for each lobe of each lung (number of correlations = 90 times 4 acoustic variables, and times 6 geometric variables).

Approach 2i: For any given airway generation the acoustic data for each left sided chest location were correlated with the geometric data for each left lung – averaged across the lobes (number of correlations = 9 times 4 acoustic variables, and times 6 of geometric variables).

Approach 2ii: For any given airway generation the acoustic data for each right sided chest location were correlated with the geometric data for each right lung – averaged across the lobes (number of correlations = 12 times 4 acoustic variables, and times 6 of geometric variables).

Approach 3: For any given airway generation the acoustic data for each chest location were correlated with the geometric data for a given lobe averaged across both lungs (number of correlations = 24 times 4 acoustic variables, and times 6 of geometric variables).

Approach 4: For any given airway generation the acoustic data for each chest location averaged across both lungs (for example anterior right and anterior left) were correlated with the geometric data averaged across all lobes for both lungs together (number of correlations = 12 times 4 acoustic variables, and times 6 of geometric variables).

Approach 5: For any given airway generation the acoustic data for each chest location averaged across both lungs (for example anterior right and anterior left) were correlated with the geometric data averaged across each lung separately (number of correlations = 21 times 4 acoustic variables, and times 6 of geometric variables).

Approach 6: For any given airway generation the acoustic data averaged across all chest locations for both lungs were correlated with the geometric data averaged across all lobes for both lungs together (number of correlations = 4 times 4 of acoustic variables and 6 of geometric).

Table 3:1: Summary of six approaches of data analysis to explore relationships between crackles and airway variables

		Airway variables																								
		LUL	LUL	LUL	LIN	LIN	LLL	LLL	LLL	RUL	RUL	RML	RML	RLL	RLL	RLL	WLL	WLL	WLL	WRL	WRL	WRL	WRL	BL	BL	BL	BL
Approach	Acous.Area	G2	G3	G4	G3	G4	G2	G3	G4	G2	G3	G3	G4	G3	G4	G5	G2	G3	G4	G2	G3	G4	G5	G2	G3	G4	G5
1	AL	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X											
	LL	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X											
	PL	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X											
	AR	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X											
	LR	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X											
	PR	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X											
2i	AL																X	X	X								
	LL																X	X	X								

Airway variables

		LUL	LUL	LUL	LIN	LIN	LLL	LLL	LLL	RUL	RUL	RML	RML	RLL	RLL	RLL	WLL	WLL	WLL	WRL	WRL	WRL	WRL	BL	BL	BL	BL
Approach	Acous.Area	G2	G3	G4	G3	G4	G2	G3	G4	G2	G3	G3	G4	G3	G4	G5	G2	G3	G4	G2	G3	G4	G5	G2	G3	G4	G5
2i	PL																X	X	X								
2ii	AR																			x	x	x	x				
	LR																			x	x	x	x				
	PR																										
3	AL																							X	X	X	X
	LL																							X	X	X	X
	PL																							X	X	X	X
	AR																							X	X	X	X
	LR																							X	X	X	X

		Airway variables																								
		LUL	LUL	LUL	LIN	LIN	LLL	LLL	LLL	RUL	RUL	RML	RML	RLL	RLL	RLL	WLL	WLL	WLL	WRL	WRL	WRL	WRL	BL	BL	BL	BL
Approach	Acous.Area	G2	G3	G4	G3	G4	G2	G3	G4	G2	G3	G3	G4	G3	G4	G5	G2	G3	G4	G2	G3	G4	G5	G2	G3	G4	G5
	PR																							X	X	X	X
4	AL+AR																							x	x	x	x
	LL+LR																							x	x	x	x
	PL+PR																							x	x	x	x
5	AL+AR																x	x	x	x	x	x					
	LL+LR																x	x	x	x	x	x					
	PL+PR																x	x	x	x	x	x					

Airway variables

		LUL	LUL	LUL	LIN	LIN	LLL	LLL	LLL	RUL	RUL	RML	RML	RLL	RLL	RLL	WLL	WLL	WLL	WRL	WRL	WRL	WRL	BL	BL	BL	BL
Approach	Acous.Area	G2	G3	G4	G3	G4	G2	G3	G4	G2	G3	G3	G4	G3	G4	G5	G2	G3	G4	G2	G3	G4	G5	G2	G3	G4	G5
6	AL+AR +AL+AR +AL+AR																							x	x	x	x

Acous.Area= acoustic areas, AL= anterior left region of chest wall, LL=lateral left region of chest wall, PL=posterior left region of chest wall, AR= anterior right region of chest wall, LR=lateral right region of chest wall, PR=posterior right region of chest wall, LUL=left upper lobe, Lingula= lingula lobe, LLL=left lower lobe, RUL=right upper lobe, RML=right middle lobe, RLL=right lower lobe, G=generation, WLL=whole left lung, WRL=whole right lung, BL= both lungs

3.8.5. The prediction of airway wall thickness variables by added lung sounds

In order to explore the prediction of static airway thickness variables at specific anatomical areas of the lung based on dynamic added lung sounds detected at different areas across the lung. Airway wall thickness and percentage of wall area were analysed separately in each generation (2, 3, 4 and 5 (only right lung) of each lobe (upper, middle (lingual for left lung) lower) of the lung to represent these variables at generation in each lobe. The relationship between airway variables at each generation of each lobe of each lung (left lung or right lung) and added lung sound variables at six auscultation areas were then tested by bivariate correlation using Pearson's correlation test. The crackle 2CD, NCpB, NWpB and wheeze frequency which had significant correlation with airway wall thickening variables were included into the equation as an independent variable into the multiple regression equation using step-wise method to predict the airway (dependent) variables. Then the equation of each dependent variable was predicted by the independent variables (characteristics of added lung sounds).

3.9. The ability of HRCT and CALSA to differentiate healthy subjects from COPD patients

In order to differentiate between healthy subjects and COPD patients, the variables which indicate COPD (emphysema score and percentage of wall area) were plotted in scatter plots among four groups of subjects to find out whether there is a cut off point of these numbers between healthy subjects and COPD patients. The crackle 2CD, NCpB, NWpB and wheeze frequency were also plotted in the same way in order to find out the cut off point of these figures between groups.

3.10. Conclusion

This chapter has described the methodology of this research and the analysis methods to:

1) Test whether any relationship exists between the crackle 2CD and airway diameter, length, internal perimeter, branching angles, airway wall thickness and percentage of wall area. This was done in four different groups of subjects, different areas, three to four different generations of the airway tree and seven different approaches.

2) Test whether there are any relationships between NCpB, NWpB and airway diameter, length, internal perimeter, branching angles, airway wall thickness and percentage of wall area. This was done in four different groups of subjects, different areas, three to four different generations of the airway tree and seven different approaches.

3) Test whether there are any relationships between wheeze frequency and airway diameter, length, internal perimeter, branching angles, airway wall thickness and percentage of wall area. This was done in four different groups of subjects, different areas, three to four different generations of the airway tree and seven different approaches.

4) Test whether there are any predictions of crackle 2CD, NCpB, NWpB and wheeze frequency by airway diameter, length, internal perimeter, branching angles, airway wall thickness and percentage of wall area. This was done in different areas and three or four different generations of the airway tree.

5) Test whether there are any predictions of airway wall thickness and percentage of wall areas by crackle 2CD, NCpB, NWpB and wheeze frequency.

6) Test whether there is a cut off point for both HRCT and lung sound data to differentiate between healthy subjects and COPD patients.

The next chapter will present the main findings of the analysis.

Chapter 4 : Results

4.1. Introduction

This chapter will present the results of the data analysis. As there were too small numbers of subjects in each COPD group (5 mild and 3 moderate COPD) to run the statistical analysis to compare mean differences between these two groups, it was decided to collect mild and moderate COPD into one group. Thus, those data will be presented from three groups: healthy-non smokers, healthy smokers and COPD. Demographic and pulmonary function test data for the three groups of the sample population will be presented first, followed by the lung sound data and the geometry of the airways, percentage of wall area and emphysema score. Then the relationships between the characteristics of the added lung sounds and airway variables and the predictive ability of crackles for airway wall thickness are reported. Finally, the ability of CALSA and HRCT to differentiate between healthy subjects and COPD patients are presented.

4.2. Sample, demographic and pulmonary function test data

Data collection for the lung sounds research took place between March 2010 and December 2011. During this period, 120 people took part in a screening test for eligibility, 36 passed the screening and volunteered for the main HRCT study, and of these 27 agreed to take part in the lung sound recordings. From these, 26 full sets of data (including lung sounds and HRCT data) were obtained.

Demographic data with mean± standard deviation and median (range) from 26 subjects (8 COPD (3 moderate, 5 mild), 9 smokers and 9 non-smokers) are presented in Table 4:1. These data included gender, age, weight, height and body mass index (BMI) which was calculated from the equation BMI =weight (kg)/height^2 (m^2). The values of the pulmonary function tests at baseline are also shown in Table 4:1. An analysis of variances showed significant differences in FEV_1 (F (2, 23) = 9.38, p = 0.001), FVCpp (F (2, 23) = 3.85, p = 0.036), FEV_1pp (F (2, 23) = 23.61, p = 0.001), FEV_1/FVC (F (2, 23) = 29.03, p =

0.001 and PEFR *(F* (2, 23) = 10.88, *p* = 0.001) among three groups. Post hoc analyses using the Bonferroni post hoc criterion for significance indicated that FEV_1 was significant lower in COPD patients compared to that of healthy non-smokers while FVCpp was also significant lower in COPD patients compared to that of healthy smokers. Bonferroni post hoc comparison between three groups also indicated that FEV_1pp, FEV_1/FVC and PEFR were significant lower in COPD patients compared to those of healthy smokers and healthy-non smoker.

The Bonferroni post hoc comparison between three groups (appendix 7) also showed that FEV1/FVC was significant lower in healthy smokers compared to that of healthy-non smokers. The detailed demographic and pulmonary function test data of all subjects are in appendix 5.

Table 4:1: Demographic data for the sample population

Variables	COPD (8)	Healthy Smokers (9)	Healthy Non-smokers (9)
Gender (n)	F(2) M(6)	F(7) M(2)	F(3)M(6)
Age (year)	59.38±3.25	53.56±7.55	49.78±9.16
Weight‡ (Kg)	77.0 (42.0)	69.9 (44.0)	82.3 (50.0)
Height (m)	1.69±0.08	1.66±0.07	1.72±0.08
BMI‡ (Kg/m^2)	26.02 (8.15)	25.98 (13.94)	29.67 (12.82)
FVC (l)	3.59±0.98	3.89±0.85	4.33±0.53
FEV_1 (l)	2.13±0.75*	2.87±0.76	3.54±0.49
FVC pp (%)	98.79±15.52#	117.93±13.64	113.84±15.38
FEV_1pp (%)	71.13±12.54**	102.44±11.77	107.90±11.09
%FEV_1/FVC	59.89±7.28**	73.21±5.56*	80.24±3.39
PEFR (%)	68.24±13.63**	107.18±18.06	99.17±21.04

F= Female, M= Male, BMI= Body mass index

ŧValues are not normal distribution and shown as median (range)

**Significantly different from healthy smokers and healthy non-smokers (*Bonferroni post hoc comparison test*, $p<0.05$)

*Significantly different from healthy non-smokers (*Bonferroni post hoc comparison test*, $p<0.05$)

#Significantly different from healthy smokers (*Bonferroni post hoc comparison test*, $p<0.05$)

4.3. Lung sound data

4.3.1. Crackles

The characteristics of crackles such as the NCpB and crackle 2CD recorded from six areas of the lungs (anterior left region of chest wall (AL), anterior right region of chest wall (AR), lateral left region of chest wall (LL), lateral right region of chest wall (LR), posterior left region of chest wall (PL), posterior right region of chest wall (PR)) are presented in Table 4:2-Table 4:3. An analysis of variances showed that the NCpB was not significantly different between the three groups in six areas of the lung. Crackle 2CD was also not significant different among the three groups in AL, AR, LR, PL and PR. However, there was significant difference in this number at LL between the three groups (F (2, 23) = 4.06, p = 0.030). The Bonferroni post hoc comparison between the three groups showed that crackle 2CD in lateral left region of chest wall (appendix 8) was significantly lower in COPD (M= 13.97, SD = 1.75) than that in healthy non-smokers (M= 15.81, SD = 1.30, t (25) = 2.59, p = 0.048). The detailed crackle data of all subjects are in appendix 6.

Table 4:2: Average NCpB in six areas of the lung within each group of subjects

Area	COPD (n=8)	Healthy Smokers (n=9)	Healthy Non-smokers (n=9)
AL	3.75±1.77	3.52±1.28	2.75±1.43
LL	4.62±1.96	4.25±1.27	5.43±3.03
PL	3.35±1.44	4.78±2.22	4.36±1.63
AR	2.96±1.29	3.71±1.68	4.03±2.19
LR	3.61±1.17	5.09±1.48	3.47±2.64
PR	3.84±3.23	4.54±1.70	4.67±2.37

AL = anterior left region of chest wall, AR = anterior right region of chest wall, LL =lateral left region of chest wall, LR= lateral right region of chest wall, PL= posterior left region of chest wall, PR= posterior right region of chest wall

Table 4:3: Average crackle 2CD in six areas of the lung within each group of subjects

Area	COPD (8)	Healthy Smokers (9)	Healthy Non-smokers (9)
AL	15.74±1.02	15.63±0.83	16.00±1.42
LL	13.97±1.75*	15.64±1.31	15.81±1.30
PL	13.62±1.61	14.89±1.48	13.27±2.97
AR	15.76±1.44	16.39±0.95	16.40±1.43
LR	15.59±1.06	14.51±1.00	15.42±1.75
PR	13.87±2.12	15.00±1.02	13.48±2.13

AL = anterior left region of chest wall, AR = anterior right region of chest wall LL =lateral left region of chest wall, LR= lateral right region of chest wall, PL= posterior left region of chest wall, PR= posterior right region of chest wall

*Significant different from healthy non-smokers (*Bonferroni post hoc comparison test, $p<0.05$*).

4.3.2. Wheezes

Wheezes were found in 11 subjects (6 COPD, 2 healthy smokers and 3 healthy non-smokers). The wheezes number per breathing cycle ranged between 0.10 and 1.50 in COPD, 0.75-1.20 in healthy smokers and 0.20-0.85 in healthy non-smokers Table 4:4. However, the number of subjects who presented with wheezes in at least 2 areas of chest wall in COPD seemed to be higher than those of healthy smokers and healthy non-smokers. The detailed of wheeze data of all subjects are in appendix 6.

Table 4:4: Range of wheezes number per breathing cycle and mean frequency of wheezes within each group of subjects

Variable	COPD (8)	Healthy Smokers (9)	Healthy Non-smokers (9)
Number	0.10 -1.50	0.75-1.20	0.20-0.85
Mean frequency of wheezes (Hz)	377.60-556.64	302.73-539.50	292.97-672.74
Number of subjects with wheezes ≥2 auscultation areas over chest wall	4	1	2

4.4. The geometry of airway, percentage of wall area and emphysema score data

The measurements relating to the geometry of the airway in the three groups such as diameter, internal perimeter, length and branching angles are presented in appendix 9. There are some statistically significant differences in these variables between the three groups. The average airway wall thickness and percentage of wall area which indicate airway wall thickening (section 2.12.) were showed in Table 4:5. An analysis of variance showed that there were significant differences in percentage of wall area at generation 3 of left upper lobe (G3LUL, F (2, 23) = 4.33, p = 0.025), generation 4 of left lower lobe (G4LLL, F (2, 23) = 3.92, p = 0.034), generation 2 (G2RUL, F (2, 23) = 5.12, p = 0.015) and 3 of right upper lobe (G3RUL, F (2, 23) = 4.12, p = 0.030) and generation 3 (G3RLL, F (2, 23) = 3.92, p = 0.034), generation 4 (G4RLL, F (2, 23) = 4.20, p = 0.028) and generation 5 (G5RLL, F (2, 23) = 3.72, p = 0.040) of right lower lobe between the three groups. Post hoc analyses using the Bonferroni post hoc criterion for significance indicated that percentage of wall area at G3LUL, G4LLL, G3RUL, G3RLL and G5RLL were significant higher in COPD than those of healthy smokers. The Bonferroni post hoc test also showed that there were significant differences in percentage of wall area at G2RUL and G4RLL between COPD and healthy non-smokers. Emphysema score presented a wide range in all groups, ranging from 0.04 to 48.71% (Table 4:6). The emphysema score was generally higher in COPD than healthy non-smokers and healthy smokers.

Table 4:5: Percentage of wall area

Area	COPD (8)	Healthy Smokers (9)	Healthy Non-smokers (9)
LMB	43.43±3.09	41.69±3.85	42.98±2.62
RMB	39.66±3.25	41.11±2.74	40.50±2.70
G2LUL	48.81±2.09	46.97±3.10	48.81±2.43
G3LUL	64.56±2.75#	61.45±2.89	64.22±1.39
G4LUL	63.50±2.93	60.67±4.93	64.11±2.47
G3LGL	57.26±4.72	54.71±4.54	57.18±2.97
G4LGL	64.44±2.27	57.26±4.72	57.26±4.72
G2LLL	49.74±2.63	48.07±2.30	47.17±5.66
G3LLL	57.85±3.56	53.96±4.94	55.05±6.11
G4LLL	63.10±2.62#	59.57±3.42	62.14±1.84
G2RUL	52.11±3.47*	45.29±5.17	45.28±5.92
G3RUL	63.92±3.61#	60.13±2.03	60.51±3.13
G3RML	61.51±3.72	53.75±7.05	56.46±2.80
G4RML	63.52±1.65	61.24±2.44	62.48±1.73
G3RLL	58.95±2.70#	54.79±3.24	56.10±3.31
G4RLL	62.42±2.32*	59.00±2.63	58.59±3.68
G5RLL	63.90±1.86#	60.78±2.52	61.72±2.69

G= generation, LUL=left upper lobe, LGL=lingula lobe, LLL=left lower lobe, RUL=right upper lobe, RML= right middle lobe and RLL= right lower lobe, *Significant difference from healthy non-smokers (*Bonferroni post hoc comparison test, $p<0.05$*), # Significant difference from healthy smokers (*Bonferroni post hoc comparison test, $p<0.05$*).

Table 4:6: Emphysema score (%) in each lobe of the lungs

Area	COPD (8)	Healthy Smokers (9)	Healthy Non-smokers (9)
LUL	2.16-25.55	0.51-15.73	0.61-4.88
LLL	3.04-21.91	0.14-7.77	0.27-7.40
RUL	0.38-48.71	0.04-5.49	0.38-3.78
RML	1.05-21.91	0.12-9.05	2.21-11.32
RLL	2.12-17.94	0.06-7.56	0.46-4.42

LUL=left upper lobe, LLL=left lower lobe, RUL=right upper lobe, RML= right middle lobe and RLL= right lower lobe

4.5. The relationship between characteristics of added lung sounds and airway variables

This study was the first study to explore any potential relationships between added lung sound characteristics and the three dimensional airway geometry and airway wall thickness in human lungs, derived from HRCT. Currently, there is insufficient evidence to indicate exactly where added lung sounds detected at one area of the chest wall originated. The sounds could originate from the airway at the same lung area or be transmitted from the airway at different lobes or even the other lung. In order to explore these possible phenomena, the author proposed six different approaches (section 3.8.4 and Table 3:1) to analyse and report data in 26 subjects (9 healthy non-smokers, 9 healthy smokers and 8 COPD). It was decided not to present the results from wheezes because the number detected in the subjects was too small to run the statistical analysis.

4.5.1. Approach 1 Six areas of lung sounds versus airway variables from each lobe of both lungs

The results of multiple regressions which followed the American Psychological Association guideline (Field 2009) in the first approach are presented in Table 4:7–Table 4:16. Data relating to the lobes of the left lung are presented first in Table 4:7, Table 4:8, Table 4:9,Table 4:10 then these data of the right lung are presented in Table 4:11, Table 4:12, Table 4:13, Table 4:14, Table 4:15 and Table 4:16. The example of detail for correlation and multiple regression tests of the first model were presented in appendix 10.

Multiple regression results of left lung

There were four equations of multiple regressions when the airway variables (which present significant relationships with crackle characteristics) were entered using the stepwise (forward and backward methods together) technique into SPSS to predict the crackle 2CD and NCpB (Table 4:7–Table 4:10).

Table 4:7: Multiple regression equation to predict crackle 2CD at anterior left region of chest wall by airway wall thickness at generation 3 of right lower lobe and percentage of wall area at generation 2 of left lower lobe

	b	SE b	β
Step 1			
Constant	8.42	1.82	
Airway wall thickness at generation 3 of right lower lobe	4.44	1.09	.65*
Step 2			
Constant	15.15	2.39	
Airway wall thickness at generation 3 of right lower lobe	4.38	0.89	.64 **
Percentage of wall area at generation 2 of left lower lobe	-0.14	0.04	-.46***

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.42, R^2 change for step 2 = 0.22,*p=0.001, **p=0.001 and ***p=0.002

Table 4:8: Multiple regression equation to predict crackle 2CD at lateral left region of chest wall by airway wall thickness at generation 3 of left lower lobe and internal perimeter at generation 3 of right lower lobe

	b	SE b	β
Step 1			
Constant	8.70	2.49	
Airway wall thickness at generation 3 of left lower lobe	3.90	1.49	.47*
Step 2			
Constant	5.26	2.83	
Airway wall thickness at generation 3 of left lower lobe	3.32	1.41	.40**
Internal perimeter at generation 3 of right lower lobe	0.21	0.1	.37***

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.22, R^2 change for step 2 = 0.13,*p=0.015, **p=0.028 and ***p=0.043

Table 4:9: Multiple regression equation to predict NCpB at lateral left region of chest wall by branching angle at generation 4 of left upper lobe and length at generation 2 of left upper lobe

	b	SE b	β
Step 1			
Constant	-16.36	7.73	
Branching angle at generation 4 of left upper lobe	0.14	0.05	.51*
Step 2			
Constant	-21.75	7.24	
Branching angle at generation 4 of left upper lobe	0.15	0.05	.53**
Length at generation 2 of left upper lobe	0.34	0.13	.42***

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.26, R^2 change for step 2 = 0.17,*p=0.012, **p=0.004 and ***p=0.019

Table 4:10: Multiple regression equation to predict NCpB at posterior left region of chest wall by airway wall thickness at generation 4 of left lower lobe, percentage of wall area at generation 3 of right middle lobe and branching angle at generation 4 of left upper lobe

	b	SE b	β
Step 1			
Constant	1.24	1.18	
Airway wall thickness at generation 4 of left lower lobe	1.94	0.71	.50*
Step 2			
Constant	9.41	3.22	
Airway wall thickness at generation 4 of left lower lobe	1.71	0.64	.44**
Percentage of wall area at generation 3 of right middle lobe	-0.14	0.05	-.44***
Step 3			
Constant	-2.60	5.39	
Airway wall thickness at generation 4 of left lower lobe	1.18	0.60	.30#
Percentage of wall area at generation 3 of right middle lobe	-0.16	0.05	-.52##
Branching angle at generation 4 of left upper lobe	0.10	0.04	-.41###

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.25, R^2 change for step 3 = 0.14, *p=0.012, **p=0.014, ***p=0.014, #p=0.063, ##p=0.002, ###p=0.016

Results

The following Tables present the relationships within the lobes of the right lung.

Multiple regression results of right lung

There were six equations of multiple regressions when entered the airway variables (which present significant relationships with crackle characteristics) using the stepwise (forward and backward methods together) technique into SPSS to predict the crackle 2CD and NCpB (Table 4:11–Table 4:16).

Table 4:11: Multiple regression equation to predict crackle 2CD at anterior right region of chest wall by branching angle at generation 4 of left upper lobe and airway diameter at generation 3 of right lower lobe

	b	SE b	β
Step 1			
Constant	4.15	3.87	
Branching angle at generation 4 of left upper lobe	0.08	0.03	.57*
Step 2			
Constant	2.97	3.53	
Branching angle at generation 4 of left upper lobe	0.07	0.03	.45**
Airway diameter at generation 3 of right lower lobe	0.56	0.23	.41***

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.32, R^2 change for step 2 = 0.15,*p=0.005, **p=0.016 and ***p=0.026

Table 4:12: Multiple regression equation to predict crackle 2CD at lateral right region of chest wall by length at generation 2 of left upper lobe, airway wall thickness at generation 3 of left upper lobe and airway wall thickness at generation 2 of left upper lobe

	b	SE b	β
Step 1			
Constant	11.79	1.09	
Length at generation 2 of left upper lobe	0.27	0.09	.54*
Step 2			
Constant	5.30	2.69	
Length at generation 2 of left upper lobe	0.24	0.08	.47**
Airway wall thickness at generation 3 of left upper lobe	4.70	1.81	.41***
Step 3			
Constant	6.83	2.61	
Length at generation 2 of left upper lobe	0.21	0.07	.42#
Airway wall thickness at generation 3 of left upper lobe	4.37	1.70	.38##
Airway wall thickness at generation 2 of left upper lobe	-0.31	0.15	-.31###

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.29, R^2 change for step 3 = 0.09, *p=0.004* *p=0.006, ***p=0.016, #p=0.010,##p=0.017, ###p=0.045

Table 4:13: Multiple regression equation to predict crackle 2CD at posterior right region of chest wall by branching angle at generation 4 of left lower lobe and branching angle at generation 3 of lingula lobe

	b	SE b	β
Step 1			
Constant	47.21	11.49	
Branching angle at generation 4 of left lower lobe	-0.21	0.07	-.52*
Step 2			
Constant	35.34	10.90	
Branching angle at generation 4 of left lower lobe	-0.18	0.07	-.45 **
Branching angle at generation 3 of lingula lobe	0.06	0.02	.45***

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.27, R^2 change for step 2 = 0.20,*p=0.009, **p=0.010 and ***p=0.011

Table 4:14: Multiple regression equation to predict NCpB at anterior right region of chest wall by airway diameter at generation 4 of right middle lobe and length at generation 2 of right upper lobe

	b	SE b	β
Step 1			
Constant	-3.10	2.19	
Airway diameter at generation 4 of right middle lobe	1.55	0.49	.56*
Step 2			
Constant	-0.70	1.94	
Airway diameter at generation 4 of right middle lobe	1.73	0.41	.62**
Length at generation 2 of right upper lobe	-0.21	0.06	-.50***

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.31, R^2 change for step 2 = 0.24, *p=0.005, **p=0.001 and ***p=0.003

Table 4:15: Multiple regression equation to predict NCpB at lateral right region of chest wall by branching angle at generation 3 of left upper lobe

	b	SE b	β
Step 1			
Constant	26.48	9.08	
Branching angle at generation 3 of left upper lobe	-0.15	0.06	-.47*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.22, adjusted R^2 = 0.18, *p=0.022

Table 4:16: Multiple regression equation to predict NCpB at posterior right region of chest wall by percentage of wall area at generation 2 of right upper lobe

	b	SE b	β
Step 1			
Constant	13.91	3.54	
Percentage of wall area at generation 2 of right upper lobe	-0.20	0.07	-.49*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.24, adjusted R^2 = 0.20, *p=0.012

As shown in Table 4:11-Table 4:16, this approach generated 10 predictive equations.

4.5.2. Approach 2 Six areas of lung sounds versus airway variables from the whole left or whole right lung

The reports of multiple regression of the third approach are presented in Table 4:17-Table 4:23. Data from the left lung are presented first in Table 4:17 and Table 4:18 followed by the right lung in Table 4:19, Table 4:20, Table 4:21, Table 4:22 and Table 4:23.

Multiple regression results of the left lung

There were two equations of multiple regressions when entered the airway variables (which present significant relationships with crackles characteristics) using the stepwise (forward and backward methods together) technique into SPSS to predict the crackle 2CD and NCpB (Table 4:17-Table 4:18).

Table 4:17: Multiple regression equation to predict crackle 2CD at anterior left region of chest wall by airway diameter at generation 2 of the left lung

	b	SE b	β
Step 1			
Constant	12.79	1.09	
Airway diameter of generation 2 of the left lung	0.31	0.11	.50*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.25, adjusted R^2 = 0.22, *p=0.010

Table 4:18: Multiple regression equation to predict crackle 2CD at lateral left region of chest wall by airway wall thickness at generation 3 of the left lung

	b	SE b	β
Step 1			
Constant	5.36	3.95	
Airway wall thickness at generation 3 of the left lung	6.22	2.49	.46*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.21, adjusted R^2 = 0.17, *p=0.021

The following Tables present the relationships of the right lung.

Multiple regression results of the right lung

There were five equations of multiple regressions when entered airway variables (which present significant relationships with crackles characteristics) using the stepwise (forward and backward methods together) technique into SPSS to predict the crackle 2CD and the NCpB (Table 4:19–Table 4:23).

Table 4:19: Multiple regression equation to predict crackle 2CD at anterior right region of chest wall by branching angle of right main bronchus and internal perimeter at airway generation 2 of the right lung

	b	SE b	β
Step 1			
Constant	37.29	6.17	
Branching angle at right main bronchus	-0.14	0.04	-.57*
Step 2			
Constant	34.87	5.67	
Branching angle at right main bronchus	-0.13	0.04	-.53*
Internal perimeter at generation 2 of the right lung	0.03	0.01	.38**

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.32, R^2 change for step 2 = 0.14, *p=0.002 and **p=0.020

Table 4:20: Multiple regression equation to predict crackle 2CD at lateral right region of chest wall by airway wall thickness at generation 5 of the right lung

	b	SE b	β
Step 1			
Constant	10.27	1.84	
Airway wall thickness at generation 5 of the right lung	3.24	1.21	.48*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.23, adjusted R^2 = 0.20, *p=0.013

Table 4:21: Multiple regression equation to predict NCpB at anterior right region of chest wall by percentage of wall area at generation 2 of the right lung

	b	SE b	β
Step 1			
Constant	10.72	2.58	
Percentage of wall area at generation 2 of right lung	-0.15	0.05	-.49*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.24, adjusted R^2 = 0.21, *p=0.01

Table 4:22: Multiple regression equation to predict NCpB at lateral right region of chest wall by airway wall thickness at generation 5 of the right lung

	b	SE b	β
Step 1			
Constant	10.73	2.70	
Airway wall thickness at generation 5 of the right lung	-4.41	1.77	-.45*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.21, adjusted R^2 = 0.17, *p=0.020

Table 4:23: Multiple regression equation to predict NCpB at posterior right region of chest wall by percentage of wall area at generation 2 of right lung

	b	SE b	β
Step 1			
Constant	13.91	3.53	
Percentage of wall area at generation 2 of the right lung	-0.20	0.74	-.49*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.24, adjusted R^2 = 0.20, *p=0.012

As shown in Table 4:17-Table 4:23, this approach generated 7 predictive equations.

4.5.3. Approach 3 Six areas of lung sounds versus airway variables of both lungs

The results of multiple regressions in this approach are presented in Table 4:24–Table 4:32.

Multiple regression results of both lungs

There were nine equations of multiple regression when entered airway variables (which present significant relationships with crackles characteristics) using the stepwise (forward and backward methods together) technique into SPSS to predict crackle 2CD and NCpB (Table 4:24–Table 4:32).

Table 4:24: Multiple regression equation to predict crackle 2CD at anterior left region of chest wall by airway wall thickness at generation 3 of both lungs

	b	SE b	β
Step 1 Constant	6.31	2.88	
Airway wall thickness at generation 3 of both lungs	5.97	1.81	.56*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.31, adjusted R^2 = 0.28, *p=0.003

Table 4:25: Multiple regression equation to predict crackle 2CD at posterior left region of chest wall by branching angle at generation 3 of both lungs

	b	SE b	β
Step 1			
Constant	-11.57	10.64	
Branching angle at generation 3 of both lungs	0.18	0.08	.44*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.19, adjusted R^2 = 0.16, *p=0.024

Table 4:26: Multiple regression equation to predict crackle 2CD at anterior right region of chest wall by branching angle of right main bronchus and internal perimeter at generation 2 of both lungs

	b	SE b	β
Step 1			
Constant	42.20	5.95	
Branching angle of right main bronchus	-0.19	0.04	-.67*
Step 2			
Constant	36.25	5.36	
Branching angle of right main bronchus	-0.16	0.04	-.58*
Internal perimeter at generation 2 of both lungs	0.07	0.02	.43**

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.44, R^2 change for step 2 = 0.18, *p=0.001 and **p=0.004

Table 4:27: Multiple regression equation to predict crackle 2CD at lateral right region of chest wall by airway wall thickness at generation 5 of both lungs

	b	SE b	β
Step 1			
Constant	10.27	1.84	
Airway wall thickness at generation 5 of both lungs	3.24	1.21	.48*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.23, adjusted R^2 = 0.20, *p=0.013

Table 4:28: Multiple regression equation to predict crackle 2CD at posterior right region of chest wall by branching angle at generation 3 and airway wall thickness at generation 2 of both lungs

	b	SE b	β
Step 1			
Constant	-8.21	9.05	
Branching angle at generation 3 of both lungs	0.16	.07	.45*
Step 2			
Constant	-7.58	7.88	
Branching angle at generation 3 of both lungs	0.17	.06	.48**
Airway wall thickness at generation 2 of both lungs	-0.80	.27	-.47***

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.20, R^2 change for step 2 = 0.20, $*p=0.021$, $**p=0.007$ and $***p=0.007$

Table 4:29: Multiple regression equation to predict NCpB at lateral left region of chest wall by internal perimeter at generation 2 of both lungs

	b	SE b	β
Step 1			
Constant	0.54	1.84	
Internal perimeter at generation 2 of both lungs	0.13	.06	.44*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.19, adjusted R^2 = 0.16, *p=0.024

Table 4:30: Multiple regression equation to predict NCpB at posterior left region of chest wall by percentage of wall area at generation 2 of both lungs

	b	SE b	β
Step 1			
Constant	18.74	5.63	
Percentage of wall area at generation 2 both lungs	-0.30	.12	-.47*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.22, adjusted R^2 = 0.19, *p=0.016

Table 4:31: Multiple regression equation to predict NCpB at anterior right region of chest wall by percentage of wall area at generation 2 of both lungs

	b	SE b	β
Step 1			
Constant	20.47	5.05	
Percentage of wall area at generation 2 of both lungs	-0.35	0.11	-.57*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.32, adjusted R^2 = 0.29, *p=0.003

Table 4:32: Multiple regression equation to predict NCpB at lateral right region of chest wall by airway wall thickness at generation 5 of both lungs

	b	SE b	β
Step 1			
Constant	10.73	2.70	
Airway wall thickness at generation 5 of both lungs	-4.41	1.77	-.45*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.21, adjusted R^2 = 0.17, *p=0.020

As shown in Table 4:24-Table 4:32, this approach generated 9 predictive equations.

4.5.4. Approach 4 Take lung sounds from average level of both sides versus airway variables of both lungs

The results of multiple regressions in this approach are presented in Table 4:33-Table 4:37.

Multiple regression results

There were five equations of multiple regressions when entered the airway variables (which present significant relationships with crackles characteristics) using stepwise (forward and backward methods together) technique into SPSS to predict crackle 2CD and the NCpB (Table 4:33-Table 4:37).

Table 4:33: Multiple regression equation to predict crackle 2CD at anterior level of chest wall by internal perimeter at generation 2 of both lungs and branching angle of both main bronchi

	b	SE b	β
Step 1			
Constant	13.13	0.71	
Internal perimeter at generation 2 of both lungs	0.09	.02	.65*
Step 2			
Constant	25.05	4.50	
Internal perimeter at generation 2	0.08	.02	.57**
Branching angle at both main bronchi	-0.08	.03	-.38***

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.42, R^2 change for step 2 = 0.14, *p=0.001, **p=0.001 and ***p=0.014

Table 4:34: Multiple regression equation to predict crackle 2CD at lateral level of chest wall by airway wall thickness at generation 3 of both lungs

	b	SE b	β
Step 1			
Constant	7.11	3.43	
Airway wall thickness at generation 3 of both lungs	5.08	2.16	.43*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.19, adjusted R^2 = 0.15, *p=0.027

Table 4:35: Multiple regression equation to predict crackle 2CD at posterior level of chest wall by branching angle at generation 3 of both lungs

	b	SE b	β
Step 1			
Constant	-9.88	8.58	
Branching angle at generation 3 of both lungs	0.17	.06	.50*

Note R^2 = 0.25, adjusted R^2 = 0.21, *p=0.011

Table 4:36: Multiple regression equation to predict NCpB at posterior level of chest wall by percentage of wall area at generation 2 of both lungs

	b	SE b	β
Step 1			
Constant	17.42	5.69	
Percentage of wall area at generation 2 of both lungs	-0.27	0.12	-.43*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.18, adjusted R^2 = 0.15, *p=0.030

Table 4:37: Multiple regression equation to predict NCpB at anterior level of chest wall by percentage of wall area at generation 2 of both lungs and length of both main bronchi

	b	SE b	β
Step 1			
Constant	-0.82	1.56	
Internal perimeter at generation 4 of both lungs	0.28	0.10	.49*
Step 2			
Constant	-4.88	2.27	
Percentage of wall area at generation 2 of both lungs	0.27	0.09	.47**
Length of both main bronchi	0.11	0.05	.38***
Step 3			
Constant	4.96	4.77	
Internal perimeter at generation 4 of both lungs	0.12	0.11	.22#
Length of both main bronchi	0.13	0.04	.47##
Percentage of wall area at generation 2 of both lungs	-0.18	0.08	-.43###
Step 4			
Constant	9.00	3.28	
Length of both main bronchi	0.14	0.04	.51$
Percentage of wall area at generation 2 of both lungs	-0.24	0.06	-.56$$

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.14, R^2 change for step 4 = -0.03, *p=0.011,**p=0.009, ***p=0.030, #p=0.26,##p=0.006, ###p=0.032, $$p$=0.003 and $$$p$=0.003

As shown in Table 4:33-Table 4:37, this approach generated 5 predictive equations.

4.5.5. Approach 5 Take lung sounds from average level of both sides versus airway variables from each side of the lung

The results of multiple regressions in this approach for the left side are presented first in Table 4:38-Table 4:41 then these data of the right are presented in Table 4:42-Table 4:46.

Multiple regression results of the left lung

There were four equations of multiple regressions when entered airway variables (which present significant relationships with crackle characteristics) using stepwise (forward and backward methods together) technique into SPSS to predict crackle 2CD and NCpB (Table 4:38-Table 4:41).

Table 4:38: Multiple regression equation to predict crackle 2CD at anterior level of chest wall by airway diameter at generation 2 of the left lung and branching angle at left main bronchus

	b	SE b	β
Step 1			
Constant	13.26	0.98	
Airway diameter at generation 2 of the left lung	0.28	0.09	.50*
Step 2			
Constant	21.49	3.98	
Airway diameter at generation 2 of the left lung	0.24	0.109	.40**
Branching angle at left main bronchus	-0.06	0.03	-.36***

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.25, R^2 change for step 2 = 0.13, *p=0.009, **p=0.019 and ***p=0.040

Table 4:39: Multiple regression equation to predict crackle 2CD at lateral level of chest wall by airway wall thickness at generation 3 of the left lung

	b	SE b	β
Step 1			
Constant	7.23	2.83	
Airway wall thickness at generation 3 of the left lung	5.02	1.79	.50*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.25, adjusted R^2 = 0.22, *p=0.010

Table 4:40: Multiple regression equation to predict crackle 2CD at posterior level of chest wall by branching angle at generation 3 of the left lung

	b	SE b	β
Step 1			
Constant	-2.67	7.04	
Branching angle at generation 3 of the left lung	0.12	0.05	.44*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.19, adjusted R^2 = 0.16, *p=0.026

Table 4:41: Multiple regression equation to predict NCpB at anterior level of chest wall by internal perimeter at generation 4 of the left lung

	b	SE b	β
Step 1			
Constant	-0.08	1.26	
Internal perimeter at generation 4 of the left lung	0.23	0.08	.48*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.23, adjusted R^2 = 0.20,*p=0.012

The following Tables present the relationships of the right lung.

Multiple regression results of the right lung

There were five equations of multiple regressions when entered airway variables (which present significant relationships with crackles characteristics) using stepwise (forward and backward methods together) technique into SPSS to predict crackle 2CD and NCpB (Table 4:42-Table 4:46).

Table 4:42: Multiple regression equation to predict crackle 2CD at anterior level of chest wall by airway wall thickness at generation 3 of the right lung

	b	SE b	β
Step 1			
Constant	8.27	2.78	
Airway wall thickness generation 3 of the right lung	4.85	1.73	.50*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.25, adjusted R^2 = 0.21, *p=0.010

Table 4:43: Multiple regression equation to predict crackle 2CD at posterior level of chest wall by branching angle at generation 3 of the right lung

	b	SE b	β
Step 1 Constant	-7.42	8.91	
Branching angle at generation 3 of the right lung	0.16	0.07	.45*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.19, adjusted R^2 = 0.16, *p=0.024

Table 4:44: Multiple regression equation to predict NCpB at anterior level of chest wall by percentage of wall area at generation 2 of the right lung

	b	SE b	β
Step 1 Constant	7.49	1.83	
Percentage of wall area at generation 2 of the right lung	-0.09	0.04	-.41*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.17, adjusted R^2 = 0.14, *p=0.036

Table 4:45: Multiple regression equation to predict NCpB at lateral level of chest wall by length of generation 3 of the right lung

	b	SE b	β
Step 1			
Constant	1.19	1.44	
Length of generation 3 of the right lung	0.21	0.09	.43*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.18, adjusted R^2 = 0.15, *p=0.030

Table 4:46: Multiple regression equation to predict NCpB at posterior level of chest wall by percentage of wall area at generation 2 of the right lung

	b	SE b	β
Step 1			
Constant	12.73	2.51	
Percentage of wall area at generation 2 of the right lung	-0.18	0.05	-.57*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.32, adjusted R^2 = 0.29, *p=0.002

As shown in Table 4:38-Table 4:46, this approach provides 9 predictive equations.

4.5.6. Approach 6 Take lung sounds from six areas as one area versus airway variables of both lungs

The results of multiple regressions in this approach are presented in Table 4:47.

Multiple regression result of both lungs

There was only one equation of multiple regressions when entered airway variables (which present significant relationships with crackles characteristics) using stepwise (forward and backward methods together) technique into SPSS to predict crackle 2CD and NCpB (Table 4:47).

Table 4:47: Multiple regression equation to predict NCpB from both lungs by percentage of wall area at generation 2 of both lungs

	b	SE b	β
Step 1 Constant	12.79	3.91	
Percentage of wall area at generation 2 of both lungs	-0.18	0.08	-.42*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.17, adjusted R^2 = 0.14, *p=0.035

As shown in Table 4:47, this approach generated 1 predictive equation.

4.6. The predictive ability of crackles for airway wall thickening variables

In the same idea as section 4.5, it was decided not to run the statistical analysis and also present the results from wheezes due to the number detected in the subjects were too small. Thus, only crackles were analysed to test whether these variables measured by the cheaper CALSA can predict the airway wall thickening variables (airway wall thickness and percentage of wall

area) measured by the more expensive HRCT. The results of multiple regressions are presented in Table 4:48-Table 4:55. Data for the left side are presented in Table 4:48, Table 4:49 and data for the right side are presented in Table 4:50-Table 4:55.

Multiple regression results of the left lung

There were two equations of multiple regression when entered crackle variables (which present significant relationships with airway wall thickening variables) using the stepwise (forward and backward methods together) technique into SPSS to predict the airway wall thickening variables (Table 4:48-Table 4:49).

Table 4:48: Multiple regression equation to predict airway wall thickness at generation 3 of left upper lobe by crackle 2CD at lateral right region of chest wall and crackle 2CD at anterior left region of chest wall

	b	SE b	β
Step 1			
Constant	0.84	0.23	
Crackle 2CD at lateral right region of chest wall	0.04	0.015	.49*
Step 2			
Constant	-0.89	0.33	
Crackle 2CD at lateral right region of chest wall	0.05	0.013	.53**
Crackle 2CD at anterior left region of chest wall	0.06	0.016	.51***

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 for step 1= 0.24, R^2 change for step 2 = 0.26,*p=0.012, **p=0.002 and ***p=0.002

Table 4:49: Multiple regression equation to predict airway wall thickness at generation 3 of left lower lobe by crackle 2CD at lateral left region of chest wall

	b	SE b	β
Step 1 Constant	0.79	0.33	
Crackle 2CD at lateral left region of chest wall	0.06	0.022	.47*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.22, adjusted R^2 = 0.19, *p=0.015

The following Tables present the relationships within the lobes of the right lung.

Multiple regression results of the right lung

There were six equations of multiple regression when entered crackle variables (which present significant relationships with airway wall thickening variables) using the stepwise (forward and backward methods together) technique into SPSS to predict the airway wall thickening variables (Table 4:50–Table 4:55).

Table 4:50: Multiple regression equation to predict airway wall thickness at generation 3 of right middle lobe by crackle 2CD at anterior left region of chest wall

	b	SE b	β
Step 1			
Constant	0.89	0.24	
Crackle 2CD at anterior left region of chest wall	0.05	0.02	.52*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.28, adjusted R^2 = 0.24, *p=0.006

Table 4:51: Multiple regression equation to predict airway wall thickness at generation 3 of right lower lobe by crackle 2CD at anterior left region of chest wall

	b	SE b	β
Step 1			
Constant	0.28	0.49	
Crackle 2CD at anterior left region of chest wall	0.09	0.03	.51*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.26, adjusted R^2 = 0.23, *p=0.008

Table 4:52: Multiple regression equation to predict airway wall thickness at generation 5 of right lower lobe by crackle 2CD at lateral right region of chest wall

	b	SE b	β
Step 1			
Constant	0.43	0.40	
Crackle 2CD at lateral right region of chest wall	0.07	0.03	.49*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.23, adjusted R^2 = 0.20, *p=0.013

Table 4:53: Multiple regression equation to predict percentage of wall area of right main bronchus by crackle 2CD at anterior right region of chest wall

	b	SE b	β
Step 1			
Constant	55.68	6.72	
Crackle 2CD at anterior right region of chest wall	-0.94	0.41	-.42*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.18, adjusted R^2 = 0.14, *p=0.03

Table 4:54: Multiple regression equation to predict percentage of wall area at generation 2 of right upper lobe by NCpB at anterior right region of chest wall

	b	SE b	β
Step 1			
Constant	53.19	2.32	
NCpB at anterior right region of chest wall	-1.62	0.58	-.49*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.24, adjusted R^2 = 0.21, *p=0.010

Table 4:55: Multiple regression equation to predict percentage of wall area at generation 3 of right middle lobe by NCpB at posterior left region of chest wall

	b	SE b	β
Step 1			
Constant	63.61	2.51	
NCpB at posterior left region of chest wall	-1.56	0.55	-.50*

Note b= the regression coefficient, SE= standard error, β =standardized betas, R^2 = the coefficient of determination, R^2 = 0.25, adjusted R^2 = 0.22, *p=0.009

As shown in Table 4:48-Table 4:55, this approach generated 8 predictive equations.

4.7. The ability of HRCT and CALSA to differentiate healthy subjects from COPD patients

Because there was a small number of subjects in each group (9 healthy, 9 smokers and 8 COPD), it was decided to present all the data frequency from five (for emphysema score) or six (for crackle 2 CD and NCpB) areas of the lung in each subject. In the same idea, the data frequency of the percentage of wall area of each generation from six lobes of the lung in each subject were also be presented by the scatter plots. This makes the number of data for emphysema score to be 45 in healthy-non smokers and smokers and 40 in COPD patients and 54 for crackle 2 CD, NCpB and percentage of wall areas at generation 2, 3, 4 and 5, in healthy non-smokers and healthy smokers but 48 in COPD patients. The scatter plots of these variables were presented in Figure 4:1, Figure 4:2, Figure 4:3, Figure 4:4, Figure 4:5, Figure 4:6 and Figure 4:7. The cut off point of emphysema score (Figure 4:1) in percentage between healthy people and COPD patients is 10% and is illustrated by the red line. The results showed that these variables could not be used to differentiate between healthy subjects and COPD patients.

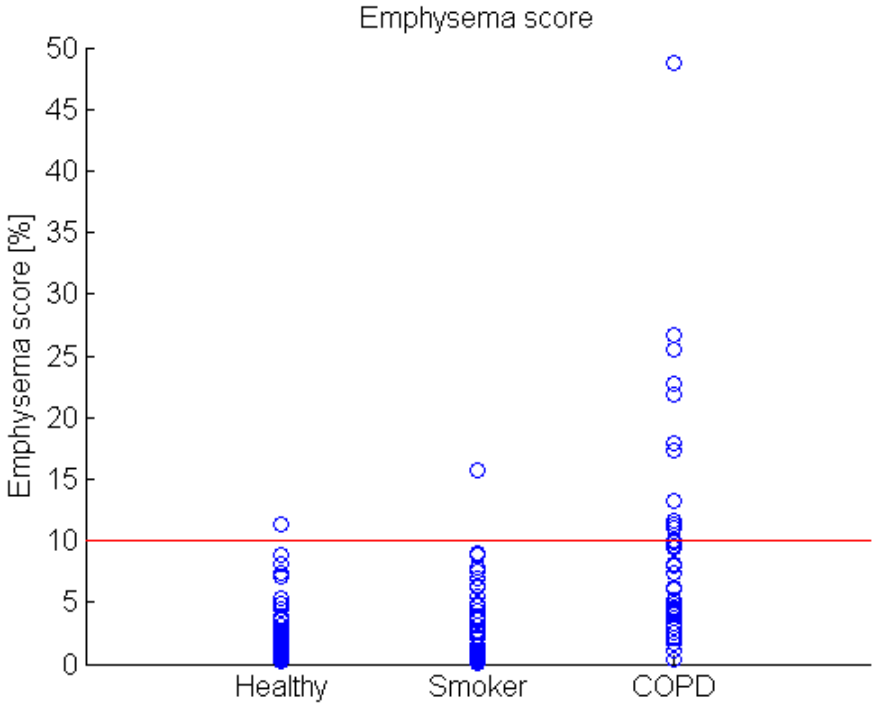


Figure 4:1: Emphysema score

Figure 4:2: Percentage of wall area at generation 2

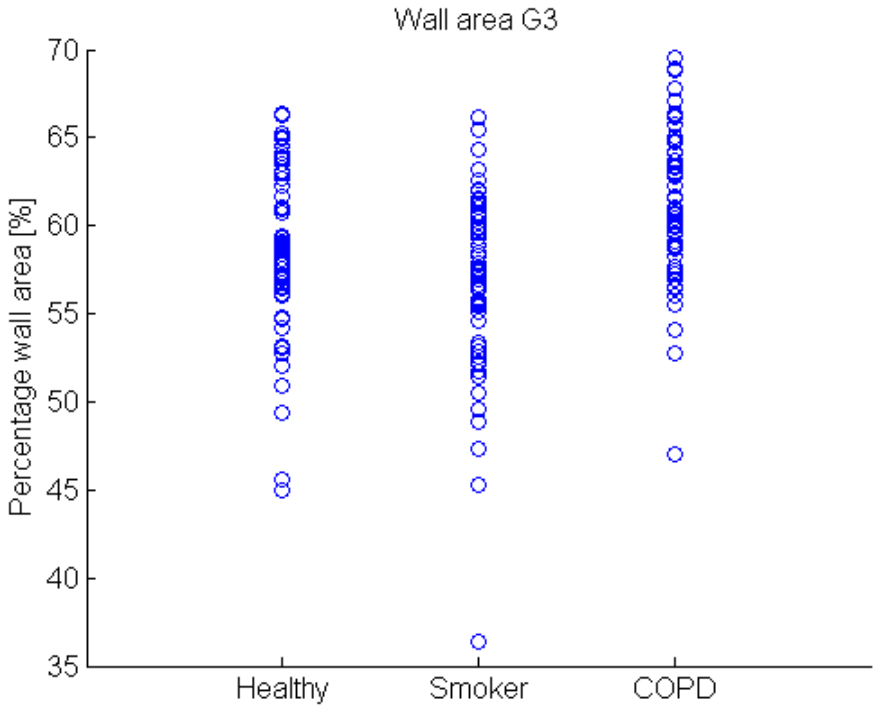


Figure 4:3: Percentage of wall area at generation 3

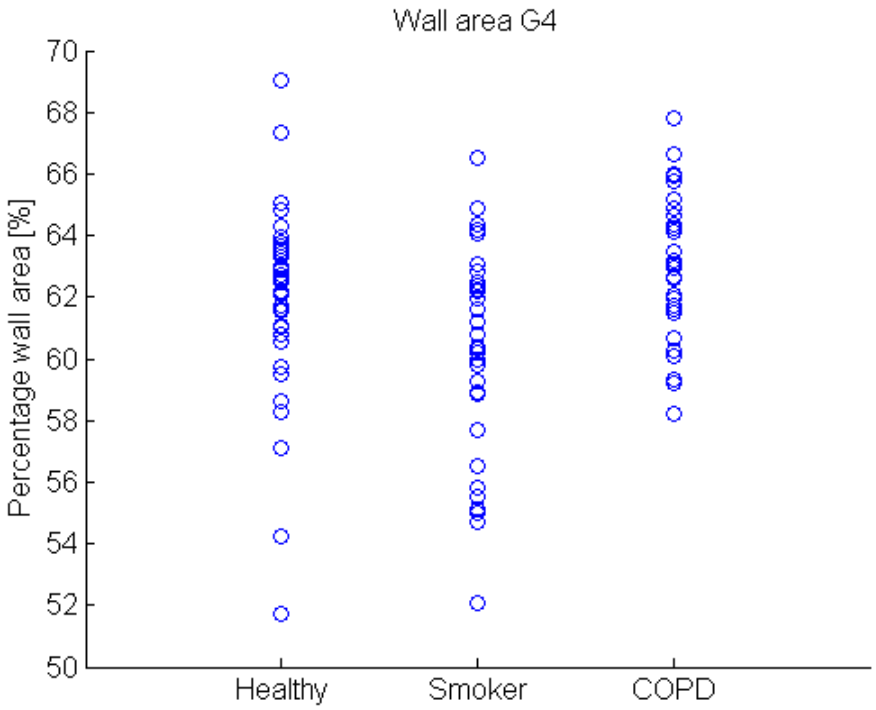


Figure 4:4: Percentage of wall area at generation 4

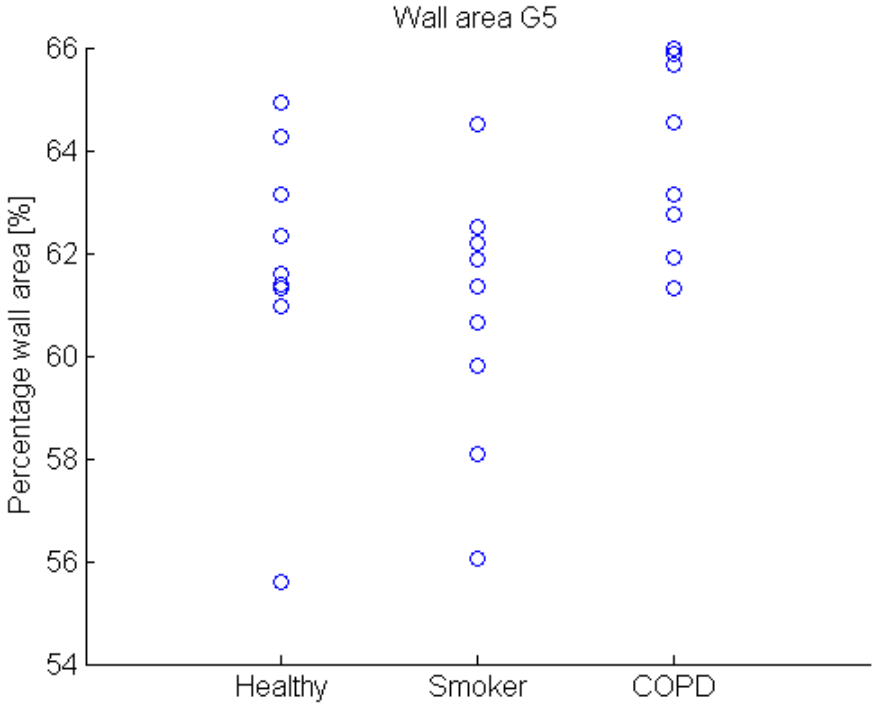


Figure 4:5: Percentage of wall area at generation 5

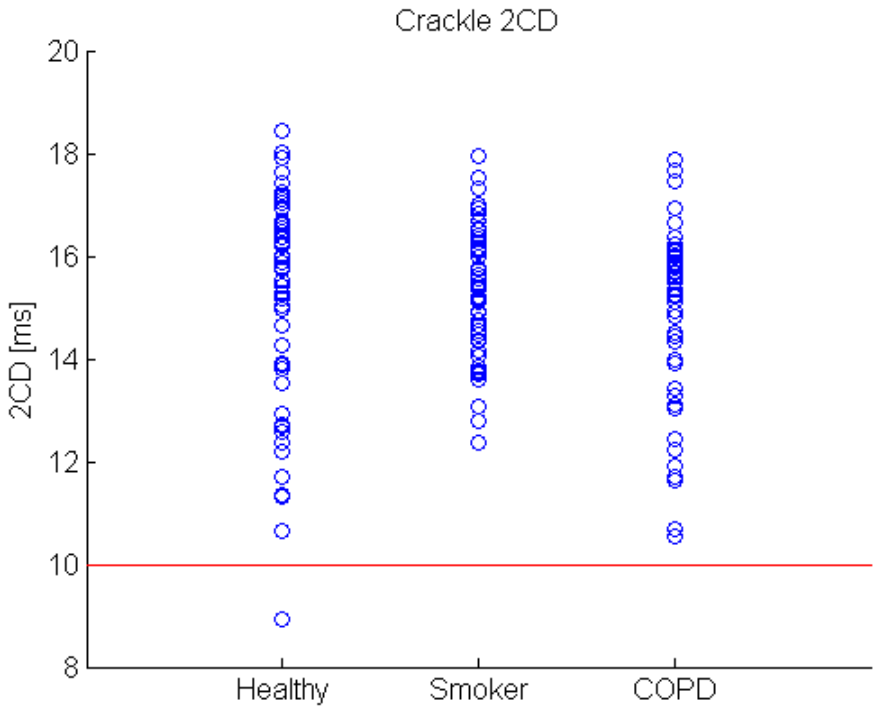


Figure 4:6: Crackle 2 CD

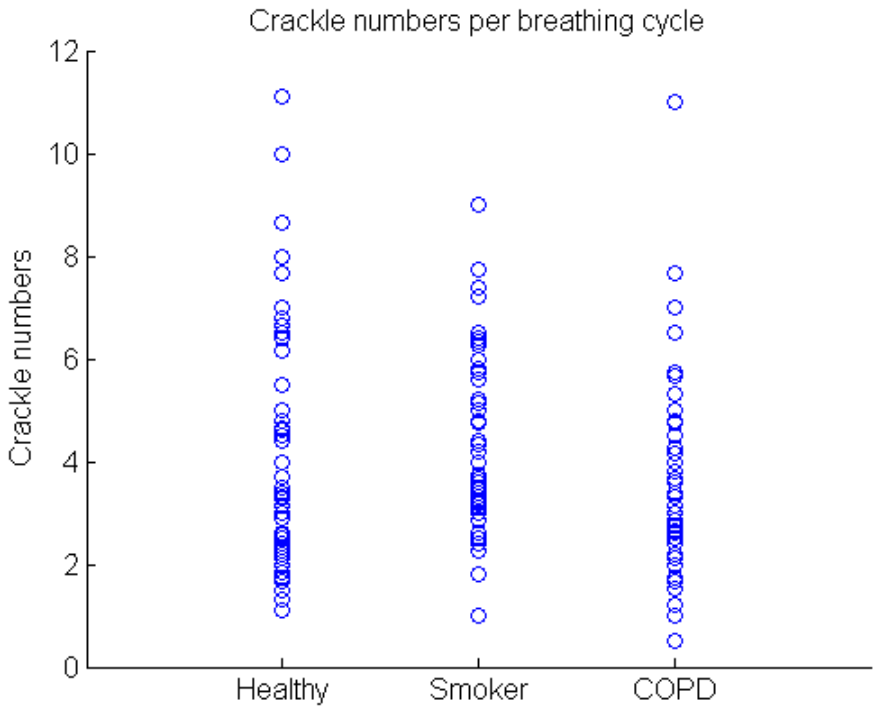


Figure 4:7: Crackle numbers per breathing cycle

4.8. Conclusion

This chapter presented the results from the analysis of 26 subjects including 9 healthy non-smokers, 9 healthy smokers and 8 COPD patients. Among these three groups, COPD was the oldest and had significantly lower FEV_1, FVC_{PP}, FEV_{1PP}, $\%FEV_1/\%FVC$ and PEFR when compared with healthy non-smokers and healthy smokers. A high BMI was found in all groups. There were no significant differences in terms of crackle 2CD, NCpB, WNpB and mean frequency of wheezes among the three groups. However, the number of subject with wheezes ≥ 2 auscultation areas was slightly higher in the COPD group.

The percentage of wall area was significantly higher in COPD than healthy smokers at generation 3 of left upper lobe, generation 4 of left lower lobe, generation 3 of right upper lobe, generation 3 of right lower lobe and generation 5 of right lower lobe.This number was also higher in COPD than in healthy non-smokers at generation 2 of right upper lobe and generation 4 of right lower lobe. There were no significant differences in the percentage of wall area between healthy non-smokers and healthy smokers at any generation or lobe of the lungs. Emphysema score showed a wide range in all groups and was generally higher in COPD than healthy-non smokers and healthy smokers, as expected. The results of relationships between characteristics of crackles and airway variables have been presented in section 4.5. In terms of the degree of correlation, approach 4 gave the highest correlation coefficient (0.65) for the relationship between crackle 2CD at anterior level of the chest wall and airway diameter at generation 2 of both lungs and internal perimeter at generation 2 of both lungs (Table 4:33, p119). The adjusted R^2 (0.29) and also the regression coefficient (b=-0.35) were highest in approach 3 for the equation to predict NCpB at anterior right region of chest wall and percentage of wall area at generation 2 of both lungs (Table 4:31, p117).

However, the common results from the analysis in the six different approaches indicated that there was a significantly positive correlation between crackle 2CD and airway wall thickness at generation 3 and 5. Other variables which significantly correlated with crackle 2CD were the branching angles at the main bronchus (negatively correlated) and at generation 3 (positively correlated). Crackle 2CD was also significantly positive correlated with airway diameter and internal perimeter at generation 2. Additionally, there was also a significantly

negative correlation between the NCpB and percentage of wall area at generation 2 and airway wall thickness at generation 5.

The results from the analysis of the prediction of airway wall thickening variables from crackles have been presented in section 4.6. Crackle 2CD at anterior left region of chest wall could significantly be used to predict the airway wall thickness at generation 3 of left upper lobe, airway wall thickness at generation 3 of right middle lobe and airway wall thickness at generation 3 of right lower lobe. Crackle 2CD at lateral left region of chest wall could also significantly be used to predict the airway wall thickness at generation 3 of left lower lobe while this number at lateral right region of chest wall could significantly be used to predict the airway wall thickness at generation 5 of right lower lobe. Crackle 2CD at anterior right region of chest wall could significantly be used to predict the percentage of wall area at right main bronchus. Additionally, the NCpB at anterior right region of chest wall could significantly be used to predict the percentage of wall area at generation 2 of right upper lobe. This variable at posterior left region of chest wall could significantly be used to predict the percentage of wall area at generation 3 of right middle lobe. The results to test the ability of HRCT and CALSA to differentiate between healthy subjects and COPD patients have been presented in Figure 4:1–Figure 4:7. The figures expressed that emphysema score and percentage of wall area measured by HRCT and crackle 2CD and NCpB measured by CALSA could not be used to differentiate between healthy subjects and COPD patients due to the overlapping distribution of these variables, hence it was not possible to define a cut off point between the groups from these variables.

The next chapter will present the discussion regarding to the main findings.

Chapter 5 : Discussion

5.1. Introduction

The aim of this study was to explore the relationship between the characteristics of added lung sounds measured by CALSA and airway dimensions measured by HRCT. The chapter will begin with a discussion of the demographic and pulmonary function test findings, followed by the lung sound, emphysema score and percentage of wall area results in relation to previous studies. Then the discussion of the relationship between the characteristics of crackles and airway geometry will be proposed. The predictive ability of crackles for airway wall thickening variables will also be explored. Next, the ability of HRCT and CALSA to differentiate between healthy subjects and COPD patients will be discussed. Finally, the limitations of this study will be reviewed followed by some ideas for future research in this area.

5.2. Demographic and pulmonary function test findings

In this study the COPD subjects were on average older (59.38±3.25 years) than healthy non-smokers (49.78±9.16 years) and smokers (53.56±7.55 years) (Table 4:1, p 90). This might be explained by the fact that COPD is a slowly progressive disease which is often undiagnosed in the early stages. Thus, it could be suspected for many years until clinically apparent and moderately advanced (Celli et al. 2004). Additionally, the pathology of COPD presents a large variation of pulmonary changes between individuals e.g. some suffer from chronic bronchitis while others experience emphysema or both (Makita et al. 2007) which means that the standard clinical evaluation may not be able to specify COPD at the early stage. Pulmonary function test results showed a decrease in pulmonary function in COPD subjects compared to healthy non-smokers and smokers. This finding indicates airflow limitation which is found commonly in COPD. The mechanisms which cause airflow limitation in COPD involve the increase in airway resistance and loss of elastic recoil pressure of the lung from chronic inflammation and parenchyma destruction (Turato et al. 2001).

5.3. Lung sound, emphysema and percentage of wall area results

The lack of statistically significant difference between crackle 2CD and NCpB among the three groups (Table 4:2, Table 4:3, p 92) can be explained by three reasons. Firstly, the COPD subjects included in this study were in a stable condition and they received bronchodilators and also anti inflammatory drugs as prescribed by their GP which can keep their lungs in the stable state so they may not have many collapsed airways. Secondly, COPD causes airflow limitation while crackles are generated by the reopening of the collapsed airways, a process which requires a sufficient flow of air to achieve (Piirila & Sovijarvi 1995; Pasterkamp et al. 1997b; Alencar et al. 2003; Majumdar et al. 2009). Therefore, in COPD subjects, it is likely that there will be fewer crackles because of the reduced airflow. The crackles in this study are coarse and only a small number of them (2-4 per breathing cycle) are detected. These findings are consistent with the study by Piirilä et al (1991) which studied the same age range of subjects (50-60 years old) and also found that crackles in COPD were coarse with the number around 2.9±1.5 (Piirilä et al. 1991). Although the number of crackles per breathing cycle was higher (4±6) in the study by Murphy (2008), their study did not show the age of subjects and they used multiple-channel CALSA to detect lung sounds while this study applied single channel CALSA. This may result in a different number of crackles being detected. Finally, the sample size in each group is small leading to a potential lack of power to detect differences in crackle variables (Field 2009).

Unexpectedly, wheezes were rarely found in COPD subjects in this study. This might be explained by the fact that they still took their medications (both bronchodilators and anti- inflammatory drugs) to maintain their stable status in order to be recruited into the study. Additionally, the number of subjects may be too small to show significant difference between groups. However, the number of subjects who presented wheezes in two or more auscultation areas was higher in the COPD group. This means that COPD subjects still presented more areas of airflow limitation than healthy non-smokers and healthy smokers. Moreover, wheezes are usually found during forced exhalation when the airflow velocity reaches a critical point, which is called the flutter velocity, to oscillate the airway walls (Grotberg & Gavriely 1989). However, the method

of breathing used in this study was slightly deeper than normal (based on the standard method of CORSA) which may not generate a sufficient air flow velocity to create wheezes. Besides that, although wheezes are commonly found in airflow obstruction as apparent in COPD, this situation can exist without giving rise to wheezes (Grotberg & Gavriely 1989).

Several author have also reported the same findings; that the percentage of airway wall area was higher in COPD patients (Table 4:5, p 95) compared with healthy people (Deveci et al. 2004; Berger et al. 2005; Orlandi et al. 2005; Hasegawa et al. 2006; Patel et al. 2008).This variable indicates airway wall remodelling in COPD which is caused by chronic inflammation usually resulting from chronic bronchitis (Mair et al. 2010). Although the pathology of COPD mainly affects the small airways, the large airways have been reported to be involved (Tiddens et al. 1995; Turato et al. 2001). This study also found airway wall thickening in the large airways among COPD subjects, but could not show the changes in small airways due to the limited spatial resolution of HRCT that could only provide accurate airway wall data until the fifth generation. However, large airway walls measured by HRCT (more clearly and accurately identified than small airways) had a significant relationship with small airway walls measured by histological methods in the same patients and could be used to predict changes of the wall in small airways (Nakano et al. 2005). Thus, changes of percentage of wall area in the large airways of COPD subjects from our study are also likely to reflect changes in the small airways. As expected, the emphysema score was generally higher in COPD compared with healthy non-smokers and healthy smokers. This suggests that there is an inflammation in the lung parenchyma which results in alveolar wall destruction and leads to hyperinflation (Nagai 2002) that can be detected by decreased attenuation from HRCT (De Jong et al. 2005). According to the results in this study which found both large airway wall thickening and high percentage of emphysema in COPD subjects, the type of COPD in this study seems to be mixed between emphysema and chronic bronchitis and probably (as assumed) small airway diseases.

5.4. Relationship between crackles and airway variables

In this study, the author created six different approaches (section 3.8.4, Table 3:1, p82) to look at the data in order to explore the possible relationship between characteristics of crackles and airway variables, based on the hypothesis that sound detected over the chest wall can originate from any airway generation of any lobe from either lung. Approach 1 was developed to test whether added lung sounds detected at each area (from six areas) over the chest wall correlated with airway variables at each generation from each lobe of each lung. Approach 2 was developed to average the airway variables from the same generation of each lobe to present airway variables from each generation from each side of the lung then test the relationship between airway variables at each generation of each side with added lung sounds at each area of the lung. Approach 3, airway variables from the same generation of both sides of the lung were averaged to present airway variables at each generation from both lungs and then compared to the added lung sounds at each area of the chest wall. Approach 4 was developed to average each characteristic of added lung sounds at the same level into one number to represent the added lung sound data of each level (anterior, lateral and posterior) and then compared to airway variables at each generation of both lungs. In the same way of approach 4 which group added lung sounds into each level, approach 5 was developed to explore the relationship of each level of added lung sounds, but with the airway variables at each generation of each side of the lung. Finally, approach 6, added lung sounds were averaged to represent one number of their characteristics from both lungs and compared with the airway variables at each generation of both lungs.

Among these approaches, approach 3 provided the highest value of adjusted R^2 at 0.29 for the equation to predict NCpB at anterior right region of chest wall and percentage of wall area at generation 2 of both lungs (Table 4:31, p117). In approach 3, the crackles at each anatomical site from six different areas were compared with airway variables at each generation from both lungs. This means that crackles detected at specific sites correlated more with specific airway generations, regardless of lobe or side. This result also indicates that crackles detected over the chest wall may represent changes in airways at specific generations rather than specific lobes or sides of the lungs.

This finding can be supported by the study in excised canine lungs (Suki et al. 1994) which found that airways reopen in cascades or avalanches activated from the overcoming of a hierarchy of critical opening pressures along the airway tree (Suki et al. 1994). The avalanche model proposes that at the starting point, where all airways are closed, the intra-bronchial pressure is lower than the opening threshold pressure. After inflation, the intra bronchial pressure increases until reaching the threshold in the parent airways then the daughter airways further down the trees (deep down in generation level) which have lower opening thresholds are reopened in cascade and cause crackle sounds (Suki et al. 1994; Suki et al. 2000; Alencar et al. 2001). Thus, it is reasonable, according to the cascade phenomenon that crackles should be correlated more with airways in the generation level, regardless of lobes and side. Therefore, clinicians who examine lung sounds using a standard stethoscope, which is similar to the single channel digital stethoscope used in this study, to detect crackles over specific areas or levels of the chest wall should be aware that any crackles they hear may originate from specific generations of the airways, rather than a specific lobe or side of the lung. This means that crackles heard over a part of the chest wall associated with overlying the lower lobe, for example, may arise from elsewhere; or that crackles heard on the left chest wall may originate from the right lung. According to the transmission of lung sounds from their origin usually present time delay when they are recorded over the chest wall (Pasterkamp et al. 1997b), the transmission of crackles also present this time delay from the reopening airways at specific generations to the single microphone of digital stethoscope. Therefore, it is unlikely to locate the exact origin of crackles in this study.

In terms of the degree of correlation, approach 4 gave the highest correlation coefficient (0.65) for the relationship between crackle 2CD at anterior level of the chest wall and airway diameter at generation 2 of both lungs and internal perimeter at generation 2 of both lungs (Table 4:33, p119). The adjusted R^2 was highest in approach 3 (0.29) for the equation to predict NCpB at anterior right region of chest wall and percentage of wall area at generation 2 of both lungs (Table 4:31, p117). It may be speculated that it is reasonable to group the data of airways at the same generation from both lungs because they still provide the highest relationship and highest predictive equation with crackles

even when compared with only one area at anterior right region of chest wall or the combination of both anterior left and anterior right region of chest wall. According to the value of adjusted R^2, the percentage of wall area at generation 2 accounts for 30% of the variation in NCpB at anterior right region of chest wall with another 70% to predict this number coming from other variables e.g. lung volume, velocity of airflow, airway opening pressure and surface tension, which are not investigated in this study.

According to the common results from the six different approaches, there was a significantly positive correlation between crackle 2CD and airway wall thickness at generation 3 and 5 (r=0.45–0.56) with an increase in crackle 2CD between 3–6 ms for every difference in 1 mm of airway wall thickness. The definition of coarse crackles by CORSA is that the 2CD are more than 10 ms while the definition of fine crackles is that the 2CD are less than 10 ms (Sovijarvi et al. 2000a). A difference in airway wall thickness of 1 mm may cross the threshold of crackles and differentiate between types. The positive relationship between crackle 2CD and airway wall thickness can be explained by the currently accepted theories about the mechanism to generate crackles, which are believed to originate from the reopening of collapsed airways (section 2.4). The collapsed airways in COPD may be caused by the film collapse mechanism (see section 2.4) rather than the compliant walls. The chronic inflammation in the airway lumen causes a large amount of secretion in the airways which increases the fluid pressure along the tube and consequently closes the airway by the film collapse mechanism. Additionally, the destruction of lung parenchyma could result in the loss of support by the external (pleural) pressure which makes the airway tend to close. Moreover, the chronic inflammation of airway wall which causes airway thickening can also decrease the diameter of the lumen, which makes the airway fluid move closer together and hence close the airway by the film collapse mechanism. In order for the airflow to pass through these collapsed airways, it has to overcome the fluid bridge to break the occlusion and this generates the crackle sounds heard at the chest wall. The results of this study showed that thicker airway walls generated longer 2CD. This may be explained by the pathology of COPD that the thicker the airway walls the more severe the airflow obstruction. It has been reported that crackle generation depends on the airflow, with low airflow generating longer 2CD of crackles (Jones et al. 2000). This may explain the

results of this study; that decreased airflow in COPD caused by the increased thickening of airway walls tends to generate longer 2CD crackles. Another possible mechanism is the decrease of airway distensibility (change of airway diameter/change of volume) in COPD (Diaz et al. 2011) which results in it being difficult to reopen closed airways. The time to reopen and generate crackles may be lengthened and hence give rise to longer 2CD.

The study also found a positive correlation between crackle 2CD and both airway diameter and internal perimeter at generation 2, with an increase in crackle 2CD between 0.24 and 1.75 ms for every 1 mm difference of airway diameter and 0.3–2.3 ms for every difference of 10 mm of internal perimeter. This means that the larger the airway, the longer the 2CD (i.e. the coarser the crackles). This finding is consistent with a previous study by Piirilä and Sovijärvi (1995), which claimed that large airways (airway generation 2) are believed to generate longer duration crackles, and can be confirmed by the three dimensional data of the airway of human in vivo study. However, the HRCT in this study can only provide accurate data for the airway down to generation 5, which are still large airways, thus, the theory that small airways generate fine crackles could not be explored in this study.

Other variables which were significantly correlated with crackle 2CD were the branching angles at the main bronchus (negatively correlated) and at generation 3 (positively correlated) with a decrease in crackle 2CD between 0.6 and 1.8 ms and an increase between 0.6 and 1.8 ms for every difference of 10 degrees of the angle at main bronchi and generation 3 respectively. This means that the wider the branching angle of the main bronchi, the shorter the duration of crackles (i.e. higher frequency), while the wider the branching angle at generation 3, the longer the duration of crackles (i.e. lower frequency). This may be because of the different anatomy of the main bronchi, which are at the first bifurcation, and the airways at generation 3, which are at the third bifurcation, leading to a differences in volume of air within the airway, and the mechanism of airflow in the airways which are two main factors to generate crackles. However, there is no evidence to report differences of branching angle during the course of airway diseases, thus 10 degree difference of branching angles are not likely to occur. Also the 10 degree difference in branching angle between bronchi and generation 3 in this study only predicted 0.6–1.8 ms differences in crackle 2CD. Consequently, this finding can not

provide any differentiation between coarse and fine crackles. Thus, this relationship is unlikely to provide useful data for clinical diagnosis.

The results also showed a negative correlation between NCpB and the percentage of wall area at generation 2 with a decrease in 0.1 and 1.75ms for every 5% difference of percentage of wall area (adjusted R^2 between 0.14 and 0.29). This means that the thicker the airways are, the fewer number of crackles are recorded. The theory behind this may be explained by the basic mechanics of forces which act on the airway wall (section 2.4). The less compliant (thicker) airways pull the airway open and make it unlikely to collapse (even if there exists secretion blocking or loss of support from parenchymal destruction). As crackles are generated by the reopen of collapsed airways, it could be expected that fewer crackles would be generated under this condition. Additionally, the generation of crackles has been reported to depend on airflow; with low airflow resulting in fewer crackles being detected, even if collapsed airways exist (Jones et al. 2000). COPD causes airflow limitation while crackles are generated by the re-opening of the collapsed airways, a process which requires a sufficient flow of air to achieve (Piirila & Sovijarvi 1995; Pasterkamp et al. 1997b; Alencar et al. 2003). Therefore, in COPD subjects, it is likely that there will be fewer crackles because of the reduced airflow through obstructed airways.

In summary, the results relating crackle characteristics with airway dimensions showed that a thicker airway wall was observed to be associated with a smaller number of crackles. The generation of crackles is believed to be dependent on the airflow (Jones et al. 2000), so this finding would make sense because a thicker airway wall will lead to a narrower lumen and hence a reduction in the flow of air though the airway. If airways with thicker walls generate crackles, they give rise to crackles with a longer 2CD due to the decrease of airway distensibility (change of airway diameter/change of volume) in COPD (Diaz et al. 2011) which results in it being more difficult to reopen the closed airways. The time to reopen and generate crackles may be lengthened and hence give rise to a longer 2CD. Thus, the data presented here support the theoretical models reported in the literature regarding the generation of crackles.

5.5. The predictive ability of crackles for airway wall thickening variables

Crackle 2CD recorded at the anterior left region of chest wall could be used to predict the airway wall thickness at generation 3 of the left upper lobe, airway wall thickness at generation 3 of the right middle lobe and airway wall thickness at generation 3 of the right lower lobe, with an increase in 0.04–0.09 mm of airway wall thickness for every difference in 1 ms of crackle 2CD (adjusted R^2 = 0.21–0.24, r=0.47–0.52). Crackle 2CD at anterior right region of chest wall could significantly be used to predict the percentage of wall area at right main bronchus, with a decrease in percentage of wall area at 0.94% for every difference in 1 ms of crackle 2CD (adjusted R^2 =0.14, r=–0.42). Crackle 2CD at lateral left region of chest wall could also be used to predict the airway wall thickness at generation 3 of left lower lobe while this number at lateral right region of chest wall could be used to predict the airway wall thickness at generation 5 of right lower lobe, with an increase in 0.06–0.07 mm of airway wall thickness for every difference in 1 ms of crackle 2CD (adjusted R^2 = 0.19–0.20, r=0.47.0.49).

Additionally, the NCpB at anterior right region of chest wall could be used to predict the percentage of wall area at generation 2 of right upper lobe, with a decrease in the percentage of wall area at 1.62 % (adjusted R^2=0.15, r=–0.42) for every 1 number difference of crackle numbers per breathing cycle. This variable at posterior left region of chest wall could be used to predict the percentage of wall area at generation 3 of right middle lobe, with a decrease in the percentage of wall area at 1.56 % (adjusted R^2=0.15, r= 0.42) for every 1 number difference of crackle number per breathing cycle. From these results it may be speculated that crackle 2CD and NCpB detected at some areas of the chest wall may be used to predict the airway wall thickening variables at generation 2, 3 and 5.

It was found that fewer and longer of crackles detected at some areas of the chest wall could indicate that the airway walls are thicker. This can be explained by:

1) Under normal mechanism of the lung, the thicker airway wall can pull out the airway to open and make it less likely to close, hence less likely to re-open and generate crackles.

2) In disease, the thicker the wall the greater the severity of chronic obstruction of the airflow. Due to the dependence of the generation of crackles on the airflow, it is possible to generate fewer crackles although the airways are in the collapsed condition.

3) Once the thicker wall airways reopen, they take longer time to reopen due to loss of distensibility and hence lead to longer crackles.

5.6. The ability of HRCT and CALSA to differentiate between healthy subjects and COPD patients

The results to test the ability of HRCT and CALSA to differentiate between healthy subjects and COPD patients have been presented in Figure 4:1-Figure 4:7 (p135-138). The Figures showed that the emphysema score and the percentage of wall area measured by HRCT and crackle 2CD and NCpB measured by CALSA could not be used to differentiate between healthy subjects and COPD patients due to the overlapping distribution of these variables, hence it was not possible to define a cut off point between the groups from these variables. These findings are not surprising for several reasons. Firstly, because of the large variations in the pathology of COPD which are very complex (Patel et al. 2008) and also the overlapping between emphysematous and chronic bronchitis types among COPD patients (Mair et al. 2010). Secondly, HRCT in this study could not accurately show the airways with the diameter less than 2 mm which are the ones which would mostly be affected in COPD. Thirdly, crackles alone may not be sufficient to indicate the presence of COPD, because they have been reported by others to be few in this disease (Piirila & Sovijarvi 1995; Murphy 2008).

The decrease in intensity of normal lung sounds resulting from chronic airflow obstruction which is commonly detected (Schreur et al. 1992; Piirila & Sovijarvi 1995; Mair et al. 2010) in COPD may therefore be more relevant for the diagnosis of COPD than crackle characteristics. However, the lung sound

analysis algorithm used in this study does not analyse normal lung sounds, so it was not possible to test this theory within this PhD.

5.7. Summary

This is the first study to explore the relationship between characteristics of crackles measured by CALSA and airway variables measured by HRCT in vivo human lungs. The findings from this study have supported the hypothesis that there are significant relationships between characteristics of crackles and airway variables. Crackles detected at specific sites or levels seem to correlate more with specific airway generations, regardless of lobe or side. This meant that crackles detected over chest wall may originate from airways at a specific generation rather than a specific lobe or side of the lungs.

The crackle 2CD and NCpB could be used to predict the airway wall thickness which is a change that occurs in chronic inflammatory disease and is found in COPD. According to the value of adjusted R^2, the crackle 2CD and NCpB could be used to explain around 20% of airway wall thickness and percentage of wall area while the other 80% to predict these variables comes from other variables e.g. biomarkers from histological study of airway walls, which were not investigated in this study. Neither HRCT nor CALSA in this study were able to differentiate between healthy subjects and COPD patients. This is because the limitation of HRCT to provide data about the small airways and also crackles measured by CALSA may not be the best indicator for the presence of COPD.

5.8. Limitations and suggestions for future research

There are some limitations in this study regarding the methods of CALSA and HRCT measurements, the HRCT algorithm, the number of participants, the cost of HRCT and the lung sound analysis algorithm. Firstly, the HRCT provided three dimensional pictures of the airway during full inspiration while lung sound recording by CALSA provided data in both inspiration and expiration leading to the difference in lung volume between the two measurements. This also results in a comparison of airways from static snapshot pictures of HRCT with lung sound data from dynamic respiration. However, the methods of HRCT and CALSA followed standard methods for both measurements which

can provide diagnostic data in clinical practice, thus it was reasonable to compare these two measurements to find out the relationship.

Secondly, the findings from the six different approaches support the theory that crackles detected from one area of the chest wall could represent airway remodelling from specific generations, regardless of lobe and side of the lung but could not specify the exact origin of crackles. This may be because the single microphone used in this study could not provide any data on the location of the source of crackles. An array of microphones could be used to provide such location information. Thus, a future study could be done using arrays of microphones placed over the chest wall in both anterior and posterior sides and then compared with the three dimensional data of airway geometry measured by HRCT.

Another limitation was the small number of participants in this study which resulted in a low goodness of fit of the equations to predict lung sounds from airway variables and vice versa. According to the number of predictors, which mostly were two and three in this study, 40 participants were needed to achieve the large effect size (r=0.5-1.0) and 80% power of test (Field 2009). However, subject recruitment was dependent on recruitment to the larger HRCT study which took a longer time than expected. The lung sound analysis algorithm in this study could analyse added lung sounds, but not the intensity of normal lung sounds, which have been reported to be more affected during the course of COPD (Schreur et al. 1992) than added lung sounds. Thus, the lung sound analysis algorithm in future studies should include normal lung sound analysis, especially when studying a COPD group, in order to explore the specific changes and then compare them with airway geometry.

In this study, wheezes were found in only a small number of participants meaning that a statistical analysis was not possible. Due to wheezes indicating the severity of airflow limitation and seeming to relate to the mechanics of the airway wall, it would be very interesting to explore their relationship with airway wall variables. Future studies could be conducted in other lung pathology with more wheezes, e.g. asthmatic patients or more severe COPD patients.

Finally, as this was the first study of this nature, multiple correlations were conducted to explore the potential relationships, with the inherent limitations of multiple testing; and therefore further research needs to be conducted to confirm the clinical significance of these correlations.

The final chapter presents the main conclusions of this research and a summary of the main priorities, in the PhD student's opinion, for further work.

Chapter 6 : Conclusions

6.1. Introduction

There were two research questions in this study; 1) Are there statistically significant relationships between the characteristics of added lung sounds and measures of airway geometry, emphysema score, airway wall thickness and percentage of wall areas in healthy non-smokers, healthy smokers, or patients with COPD? 2) Are there any cut off points using added lung sound variables measured by CALSA and airway wall variables and emphysema scores measured by HRCT to differentiate between healthy subjects and COPD patients?. The observational study has been conducted to answer these research questions in vivo of 26 pairs of human lungs. However, only crackle characteristics were compared with measures of airway geometry, emphysema score, airway wall thickness and percentage of wall area due to the small number of wheezes which were detected in the subjects. Therefore, the results from the study can answer both research questions but only for crackle sounds. Next, the conclusion and summarise of the main areas for future works will be presented.

6.2. Conclusion

This study was the first study to look at the possible relationships between lung sounds measured by CALSA and airway geometry measured from HRCT in vivo of human lungs.

The HRCT airway wall and emphysema measurements were found to be unable to differentiate between the subject groups. Similarly, CALSA measurements of crackle 2CD and NCpB were unable to differentiate between the groups. However, some significant correlations were found between the HRCT airway wall measurements and the crackle 2CD and NCpB measurements.

Six different approaches were investigated to explore the possible correlations, but all of these indicated that there were significant relationships between crackle 2CD and airway wall thickness at generation 3. Similar relationships

were also observed between the NCpB and the percentage of wall area at generation 2.The results indicate that correlations with the lung sounds exist at the generation level, regardless of the side of the lung (left or right) or the lobe.This means that the clinicians who examine lung sounds using a stethoscope to detect crackles should be aware that crackles may indicate changes in airway generation level regardless of lobes and side of the lung.

Lung sound analysis by CALSA is cheap, safe and can easily be done at the bedside while HRCT scans are very expensive and involve exposure to ionising radiation. In the case of severe sickness or injury, the benefits of HRCT often outweigh the risks. However, in the case of COPD this is unlikely to be the case and so the routine scanning of COPD patients by HRCT is not feasible in clinical practice. The significant relationship between crackles measured by CALSA and airway wall variables measured by HRCT in this study indicated that CALSA measurement was able to add useful information to aid diagnosis of airway thickening diseases, e.g. COPD.

6.3. Summary of the main areas for further work

The following areas could be considered for future work:

- Research designed to confirm the relationship between crackles and airway wall thickness variables. This will provide the new knowledge to describe the anatomical changes of airway walls related to the changes of lung sounds and also the clinical application of crackles measured by CALSA to add useful information to aid diagnosis of diseases which result in increased airway wall thickness. The research would require the collection of data from various pulmonary diseases with crackles, e.g. bronchiectasis, cystic fibrosis.
- Research designed to investigate the relationship between wheezes and airway variables. This will provide the new knowledge to understand the mechanism of wheezes regarding to the anatomy of the airways and also the application of wheezes measured by CALSA to diagnose respiratory diseases. This would require the collection of data from wheezy population, e.g. asthmatic patients.
- Research designed to develop normal lung sound analysis algorithm which could be used to detect changes in COPD patients and then

compared with airway variable data from HRCT. The study will provide the algorithm which sensitively detect changes in lung sounds of COPD patients and also provide the clinical application of normal lung sounds measured by CALSA to diagnose COPD.

- Research designed to locate the origin of crackles. This will provide the better understanding of crackles transmission to the chest wall and also specify the location of collapsed airways which are useful for aid diagnosis and the application of drug delivery to the lung. The research would involve the application of an array of microphones to detect lung sounds and then compared with airway variable data from HRCT.
- Research designed to explore any relationship between lung sounds and ventilation/ perfusion data at specific bronchopulmonary segments from Single Photon Emission Computed Tomography (SPECT) scans. The research will provide measurements of regional ventilation of the lungs which influence the mechanism to generate crackles, hence reveal the mechanism inside the physiology of the lung and characteristics of the sounds at specific anatomical sites.

Appendices

Appendix 1: Parts of this study that have been presented in conferences

Conference A: The Health Sciences Postgraduate Research Conference 17 June 2011 (oral presentation), University of Southampton

And

Conference B: Breaking Boundaries III: Innovation& Enterprise in Translational Medicine, 15 November 2011, Southampton Hospital University Trust (poster presentation)

An investigation into the relationship between airway geometry and lung sounds

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Introduction

Computer aided lung sound analysis (CALSA) has been proposed as a new objective tool to record and analyse lung sounds. Lung sounds generated in the lungs are believed to relate to the size and shape of the airways. High resolution computed tomography (HRCT) is considered to have the highest sensitivity of imaging measurement methods, and is capable of showing three dimensional pictures of the large and medium airways in which lung sounds are believed to originate. However, the relationship between the characteristics of lung sounds and the geometry of the airway has yet to be explored.

Aim

The aim of this on-going study is to explore potential relationships between lung sound characteristics measured by CALSA and airway dimensions measured by HRCT.

Appendix 1

Methods

Data have been collected and analysed for five subjects: one moderate stage COPD, 2 healthy non-smokers and 2 healthy smokers. Lung sound data were recorded using a digital stethoscope connected to a laptop computer equipped with customised MATLAB software (version 7.0). HRCT was conducted using a Siemens Sensation 64 CT scanner with a high resolution reconstruction algorithm.

Results

Preliminary analysis showed a trend for negative correlation between the two cycle duration (2CD) recorded at left lower lobe (LLL) and airway diameter and perimeter at generation 3 (G3) of LLL and a trend of positive correlation between the number of crackles per respiratory cycle at LLL and branching angle (BA) at G3 of LLL. There was also a positive correlation between the 2CD at right upper lobe (RUL) and BA of G3 at RUL. However when the airways become smaller, there was a negative correlation between the 2CD at right lower lobe (RLL) and BA of G5 at RLL.

Conclusion

Current results are unlikely to reject the alternative hypothesis that there is a relationship between added lung sound characteristics and the geometry of airways, but more evidence is required before this hypothesis can be accepted.

European Respiratory Society Annual Congress, 1-5 September 2012, Vienna Austria (poster presentation)

The prediction of airway wall thickening by computer aided lung sound analysis

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Computer Aided Lung Sound Analysis (CALSA) has been used to detect and analyse added lung sounds to aid diagnosis of many respiratory diseases. The wall area of the main bronchi, expressed as a percentage of the cross-sectional area of each branch, is a biomarker of chronic airway inflammation which is raised in COPD. The objective of this study was to explore the possible relationship between characteristics of crackles measured by CALSA and percentage of wall area of the main bronchi measured by High Resolution Computed Tomography (HRCT), and hence the possibility of using crackles as a biomarker of COPD. 26 participants (9 healthy non-smokers, 9 healthy smokers and 8 COPD) were recruited. Lung sound data were recorded using a digital stethoscope. HRCT scans were conducted using a Siemens Sensation 64 CT scanner and the resulting data were analysed using the Pulmonary Workstation 2 (Vida Diagnostics, Iowa, US) software to give measurements of airway geometry. The results showed that the percentage of wall area at the right upper bronchus correlated with the two cycle duration of crackles ($r=-0.39$, $p=0.025$) recorded at the right upper lobe (RUL), the number of crackles per breathing cycle (NCpB) at RUL ($r=-0.49$, $p=0.005$) and NCpB at right lower lobe ($r=-0.49$, $p=0.006$). Additionally, the NCpB at RUL was found to significantly predict the percentage of wall area at the right upper bronchus (adjusted $R^2=0.20$, $R^2=0.24$, $p=0.010$). These initial results suggest NCpB

might be useable to predict changes in percentage of wall area caused by the chronic inflammation of the main bronchi, though a larger sample is needed to confirm it. This suggests that crackles could possibly be used as a biomarker of COPD.

Appendix 2: Terms applied in lung sound analysis

Standard references were used to define these concepts (McClellan et al. 1998; Sovijarvi et al. 2000a; Huckvale 2003; Bores 2006).

Aliasing – Aliasing occurs when the sampling rate used to record a signal is too low to adequately capture all of the frequency components, leading to the introduction of false frequencies.

Amplitude – is the strength of sound waves or an electrical signal used to indicate the size of the variation in a signal, or the amount of a sinewave component present in a signal. When describing the amplitude of a sinewave component of a sound, typically a decibel scale is used, with respect to some reference amplitude

Amplification – a proportional increase of the magnitude of a physical quantity in order to better measure or observe it

Amplifier– an electronic device that increases the amplitude of a signal

Attenuate– to make weaker. An attenuator uses resistance to reduce output voltage, as with a volume control

Decibel (dB) – the decibel scale is a logarithmic amplitude scale, in which the size of a vibration is expressed in terms of its relative size to some reference vibration. To convert relative amplitude to decibels, it is satisfactory to take the logarithm of the ratio (to base 10) and to multiply by 20. An increase of 6 dB equals twice the sound pressure. It is used to indicate loudness.

Cut-off frequency – is the frequency at which the frequency response of a filter (or other circuit) is 3 dB below the maximal value of the frequency response.

Filter – filtering is a process of selecting, or suppressing, certain frequency components or remove unwanted frequency or noise from a signal. This allows a filter to shape the frequency spectrum of the signal for a particular purpose. Usually, the transformation aims to remove unwanted frequency components, e.g., noise. Filters can be classified as analogue filters (e.g., implemented by

operational amplifiers, resistors and capacitors) and digital filters (e.g., implemented by programmable digital hardware or software). High/Low-pass filters allow components above/below specific frequencies to pass, attenuating or stepping, all the other components.

Fourier Transform – is a mathematical operation for decomposing a time function into its frequency components (amplitude and phase). The process is reversible and the signal can be reconstructed from its Fourier components. The Fourier Transform (FT) is a mathematical tool using integrals or sums. The Discrete Fourier Transform (DFT) is its numerical equivalent using sums instead of integrals. The Fast Fourier Transform (FFT) is a popular, computationally fast algorithm to calculate DFTs.

Frequency – the number of sound waves or complete cycle that pass a given point in one second. The frequency of sinewave vibrations is measured in Hertz.

Frequency domain – the space of the variable 'frequency' associated to the space of the variable 'time' by a Fourier transform (or any other frequency transformation). In the frequency domain, a signal is described by its spectrum. A signal can be studied in the time and/or frequency domain. The latter is advantageous for signals with periodic content. For example, a pure sinewave can be described by only its frequency, amplitude and phase.

Frequency resolution – is a measure of the ability to extract the frequency content of a given signal. It depends on the duration of the signal and on the sampling rate.

Frequency response – is a measure of the systems response to sinewaves of different frequencies. A frequency response graph plots the ratio of the input/output amplitudes of sinewave signals as a function of their frequencies.

Frequency spectrum – is the collection of the frequency components of a given signal.

High-pass filter – a filter that allows components above specific frequency to pass attenuating or stepping all lower-frequency components.

Initial Deflection Width (IDW) – is the duration of the first deflection in a crackle waveform (Murphy et al. 1977; Hoevers & Loudon 1990).

Largest Deflection Width (LDW) – is the duration of the deflection of the largest amplitude in a crackle waveform (Murphy et al. 1977; Hoevers & Loudon 1990).

Loudness – is related to the quantity of sound, and it is affected by the logarithm of the amplitude. That is why decibel is commonly used for sound analysis. Loudness is the perceptual correlate of amplitude.

Low pass filter– A filter that allows components below a specific frequency or stopping all higher frequency components

Octave band analysis– To identify frequency components of a sound, there is octave band analysis in which frequencies are segmented into proportionate widths (octave bands) and analyzed. The sound pressure level of a single octave band is called the "octave band level", while that analyzed for 1/3 of the octave band is called a "1/3 octave band level". The frequency band in the octave band and 1/3 octave band is expressed as the center frequency of that band. Using f_1 and f_2 as the upper and lower end frequencies of the band, the center frequency f_c is as follows.

$$f_c = \sqrt{f_1 \cdot f_2} \qquad f_2 = 2f_1 \quad \text{(For 1 octave band)}$$

$$f_2 = \sqrt[3]{2} f_1 \quad \text{(For 1/3 octave band)}$$

(http://www.menlh.go.id/apec_vc/osaka/eastjava/noise_en/1/page5.html)

Piezoelectric material– Material which, when strained (distorted) by the action of external forces, becomes electrically polarized and produces voltages linearly related to the mechanical strain. Electrically, it behaves as it capacitance of which the charge varies with the imposed strain. Piezoelectric transducers are used to measure many variables, including acceleration, force and sound (International Electrotechnical Vocabulary, 1960, cited in Sovijärvi et al 2000a)

Piezoelectric microphone- A microphone in which incident sound acts on a piezoelectric material to generate an output voltage (International Electrotechnical Vocabulary, 1960, cited in Sovijärvi et al 2000a)

Pitch – relates to the frequency of the sound as perceived by human beings. Pitch is the perceptual correlate of the fundamental frequency of an acoustical signal.

Power spectrum – is the frequency domain data representing the power distribution of a sound with respect to frequency. A power spectrum graph plots the amplitude (usually expressed in decibels) of each sinewave component against the frequency of the component.

Sampling frequency – the repetition frequency (number of times per second) at which an analogue signal is measured and converted to a digital format.

Spectrogram – graphical representation of the change of a spectrum with time. The horizontal axis is time, the vertical axis is frequency and the amplitude of the sinewaves components of the signal at any given time and frequency is displayed on a grey scale.

Time domain – is the natural space in which the analogue signal is represented as instantaneous amplitude versus time, i.e., by its waveform.

Time-expanded waveform – the time-expanded waveform (TEW) is the display of a signal with a time scale of ≥ 800 mm/s. From a visual inspection of such a display, it is possible to study the waveforms of normal breath sounds, tracheal sounds and adventitious sounds (crackles, wheezes) and to distinguish them from each other (Murphy et al. 1977; Sovijarvi et al. 2000a).

Two Cycle Duration (2CD) – is the time from the beginning of the initial deflection of a crackle to the point where the waveform of the crackle has completed two cycles (Murphy et al. 1977; Hoevers & Loudon 1990).

Appendix 3: Technical terms used in HRCT

Standard reference was used to define these concepts (Romans, 2011)

Attenuation correction- is a method for reducing artifacts in the PET image. In PET, loss of counts can occur because of anomalies in attenuation, scatter and random image noise, image artifacts and image distortion. When PET/CT is used, data from the CT images can be used for attenuation correction of the PET emission data, eliminating the need for a separate, time-consuming transmission scan.

Collimator - refers to mechanical hardware that resembles small shutters and adjusts the opening based on the operator's selection.

Detector aperture- is the size of the detector opening.

Detector- element in a CT system that collects attenuation information and measures the intensity of the transmitted x-ray radiation along a beam projected from the x-ray source to that particular detector element.

Effective dose- a measurement, reported in Sv or rem, that attempts to account for the effects particular to the patient's tissue that has absorbed the radiation dose. Although the methods to calculate the effective dose have been established they depend on the ability to estimate the dose to radiosensitive organs from the CT procedure. It is also called effective dose equivalent.

Fan beam- the radiation emitted from the collimated x-ray source in single-detector row CT systems.

Gantry- ring shaped part of the CT scanner that houses many of the components necessary to produce and detect x-ray.

Gray (Gy)- SI unit of absorbed dose.

Gray scale-system that assigns a certain number of Hounsfield values to each shade of gray.

Helical scanning- scanning method that includes a continually rotating x-ray tube, constant x-ray output, and uninterrupted table movement. It is also called spiral, volumetric, or continuous acquisition canning.

Hounsfield units (HU)- measure of the beam attenuation capability of a specific structure. It is also called pixel values, density numbers or CT numbers.

kV- Kilovolt-peak., defines the quality (average energy) of the x-ray beam.

mA- miliampere- measure of the tube current used in the production of x-ray energy. In conjunction with the scan time, it is the quantitative measure of the x-ray beam.

mAs- miliampere-seconds- the product of miliampere setting and scan time.

Matrix- Grid formed from the rows and columns of pixels.

mSv- the mili- sievert. Once the quality factor has been applied to the radiation absorbed dose the new quantity is called the dose equivalent. The SI equivalent unit is the sievert.

Pitch- relation of table speed to slice thickness. It is most commonly defined as the travel distance of the CT scan table per 360 degree rotation of the x-ray tube, divided by the x-ray beam collimation width.

Pixel- picture element. Two-dimensional square of data. When arranged in rows and columns, they make up the image matrix.

Reconstruction algorithm- determined how the data are filtered in the reconstruction process. The appropriate reconstruction algorithm selection depends on which parts of the data should be enhanced or suppressed to optimize the image for diagnosis.

Spatial resolution- an ability of a system to resolve, as separate forms, small objects that are very close together. It is also called high-contrast resolution or detail resolution.

Spiral scanning- scanning method that includes a continually rotating x-ray tube, constant x-ray output, and uninterrupted table movement. It is also called helical, volumetric, or continuous acquisition canning.

Tube current- measured in thousandths of an ampere, or miliampere, it controls the quantity of electrons propelled from the cathode to anode.

Voxel- volume element. Three-dimensional cube of data acquired in CT.

Window level- mechanism that selects the centre CT value of the window width, also called window centre.

Window width- mechanism that determines the quantity of Hounsfield units represented as shades of gray on a specific clinical situation.

Appendix 4: Ethical approved and protocol of the large project

An Investigation into the Application of Imaging to the Characterisation of the Phenotypes of Chronic Obstructive Pulmonary Disease

(Biomedical Research Unit Programme 5.2)

SUHT R&D Number:

Study Protocol Version 1.0

11th March 2009

Chief Investigator: Dr Peter Howarth

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Background and Rationale

Chronic Obstructive Airways disease is defined on the basis of the single physiological measurement of incompletely reversible airflow limitation or obstruction as measured by spirometry. However it is well recognised even within the disease definition that there is significant variability within the natural history of the disease[1]. Previous drug development which treats COPD as a single entity leaves the clinician with little choice but to treat all COPD patients similarly despite their marked clinical heterogeneity[2]. Greater understanding of the underlying inflammatory processes has highlighted several new targets for novel drug therapies[3].

It is likely that the reason underlying the clinical heterogeneity and the prognostic variability is that COPD is not a single disease entity but a group of different inflammatory processes that often, but not always, co-exist in the same patient. The variations between these inflammatory processes are likely

to be the end result of a complex interplay between different underlying genetic factors and subsequent environmental exposures.

If novel therapies are to be successfully targeted at specific inflammatory or pathological mechanisms then the predominant disease processes will have to be identified in each COPD patient. Furthermore if the disease characterisation is to be clinically useful in routine clinical practice then the imaging and diagnostic tools should ideally be those that are already employed in day to day practice.

Techniques developed to categorise the disease processes in COPD could then go on and be refined as validated endpoints in the development of novel therapies.

Variations in the Pathophysiology of COPD

It is already established that COPD is made up of a combination of different disease processes[4]. The underlying theme is one of inflammation however many different aetiologies and mechanisms for this inflammation have been proposed and the resulting pathological processes vary between sites in the lung.

The small airways obstruction that characterises the disease is the result of several disease processes. Those that have been described so far are chronic obstructive bronchiolitis with fibrosis, increased formation of lymphoid follicles, mucosal thickening, intra-luminal exudate and loss of airway wall support due to parenchymal destruction (emphysema) [5]. The underlying mechanisms for each of these processes are not all fully understood.

There is tissue destruction within the lung parenchyma leading to the enlarged airspaces characteristic of emphysema. The distribution of the emphysema varies within the lung lobules and within the lobes of the lung. There is only a weak correlation between the severity of emphysema and various tests of lung function[6] and so these standard measures do not accurately reflect the severity of this aspect of COPD.

Within the central airways there is epithelial activation, neutrophil recruitment, an increase in CD8 +ve lymphocytes and mucous gland hyperplasia. It is recognised that the large airway pathology gives rise to the chronic sputum production that is characteristic of the clinical syndrome of chronic bronchitis. It has long been recognised that the symptoms of chronic bronchitis are not necessarily linked to airways obstruction[7] however more recently it has been recognised that if the symptoms do co-exist with airways obstruction the prognosis is worse[8;9].

The relationship between these different pathophysiological processes is an area of significant current interest but is still poorly understood. There is conflicting evidence, for example, as to whether the distribution and occurrence of small and large airways disease are linked or if they are independent disease processes with some aetiological commonality. The relationship between the presence of emphysema and small airway obstruction is not known. The parenchymal changes probably contribute to the small airways obstruction but the extent to which this occurs is not known and probably varies from patient to patient.

Emphysema and small airways fibrosis commonly co-exist and are both caused by cigarette smoke. How a destructive process and a fibrotic process occur in such close regional proximity and with such aetiological commonality has not been explained. We do not know if they are different manifestations of the same disease process or two linked but completely different pathologies.

Measures of inflammation, especially sputum neutrophil counts, have been correlated to COPD disease severity as measured by spirometry. O'Donnell et al.[10] from our group showed that these classical patterns of COPD inflammation are more associated with measures of small airway obstruction than parenchymal disease.

Accurate measures of these subsets or phenotypes of COPD will allow further research into the inflammatory processes involved in each and allow better targeting of therapy.

Regional variation of COPD within the lung

COPD is not only heterogeneous within the population but its distribution within the lung is very variable. Almost all of the commonly used measures of COPD treat the lung as a single unit with no ability to account for regional variation. This is unavoidable for most measures of lung function such as spirometry and gas transfer however we intend to use new imaging techniques to differentiate aspects of function between regions of lung that are differently affected by processes of COPD.

It is not known if the regional variations in morphological appearance that are seen in COPD are matched by accompanying variations in the inflammatory processes. The pathological and inflammatory processes associated with COPD are often measured by analysis of induced sputum. Induced sputum usually originates from the large airways and it is not known whether or not it is representative of small airway or parenchymal disease.

Quantifying the disease processes and their regional variation in COPD

Lung density measurements on inspiratory HRCT imaging has already been shown as an accurate correlate of the severity of emphysematous destruction[11]. Lung density measurements in expiration and particularly the ratio of expiratory to inspiratory mean lung density have been shown to be strongly correlated to measures of small airways disease and gas trapping[10]. All of these measures can be calculated for the whole lung or on a broad regional basis in the lung. Thus far it has not been possible to accurately calculate it with respect to anatomical regions within the lung, specifically lung segments. As far as we are aware lung segment mapping software developed within our group[12;13] makes this anatomical analysis possible for the first time.

In addition to the structural images as described above we can use isotope images to measure ventilation. The three dimensional images that are made possible by radionuclide scintigraphy with Single Photon Emission Computed Tomography (SPECT) allow us to map the ventilation images to low dose CT images. These accurate measures of lung ventilation can then be mapped onto anatomical regions by the use of referencing software that has been developed by our group.

Bronchial wall thickening has been associated with the inflammatory changes of COPD in both the large and small airways. CT has already been used to measure bronchial wall thickness in large airways. More recently, fully 3-D image analysis has allowed the bronchial tree to be reconstructed down to the sixth subdivision order. Bronchial wall measurements of the small airways beyond this have proved more challenging due to the limits of resolution of the scanners[14]. Some studies have used manual measurements of bronchial wall thickness but this is very time consuming and therefore not easily applicable to clinical practice. Several centres are working on algorithms to automatically calculate bronchial wall thickness, and recently the first studies have been published using these measurements as an end point in a therapeutic study[15].

Separate from these forms of imaging is endobronchial ultrasound (EBUS). This does not involve ionising radiation and provides the necessary resolution and penetration for imaging the airway wall. EBUS measurement of airway wall dimensions is, however, invasive as it necessitates the introduction of a fibre-optic bronchoscope into the airways to allow insertion of the radial ultrasound probe. To optimally obtain images it is necessary to inflate a latex balloon with liquid around the transducer to provide sonic coupling with the airway wall. We have used this technique successfully in asthma and have identified that the proximal airways are thicker in asthma than those in healthy controls[16]. Furthermore this form of imaging allows assessment of the epithelial and sub-mucosal layers of the airway walls as distinct from the entire airway wall thickness, which is the only measure available by CT approaches. To date the EBUS images have been taken from large central airways and the extent to which this approach may be used to image smaller airways has not been evaluated but would be limited by the radial ultrasound probe size

Identifying patterns of COPD

The detailed analysis of the lungs of a range of patients with and without COPD will allow us to accurately identify the various disease processes of COPD and map it to specific anatomical regions within their lungs. This will then enable us to compare measures of inflammation between areas of different disease activity within the same patient. We can also test the relationships, if any exist,

between the characteristic disease processes of small airways obstruction, emphysema and large airways inflammation.

Developing new methods of measuring COPD

This study plans to extremely accurately characterise the disease patterns within COPD as described above. This presents an ideal opportunity to test novel diagnostic methods for COPD. SPECT, as described above, can provide an accurate picture of regional ventilation. The lower radiation dose from the short lived Kr-81m labelled air, if validated, could be a useful measure of COPD regionality in longitudinal studies. The low dose may enable repeated imaging over time in a safer way than with other modalities which deliver a higher radiation dose such as HRCT.

Lung Sounds Analysis

Sounds generated in the lungs should relate directly to airway geometry, the movement of air and the presence of secretions. Computer aided lung sound analysis (CALSA) is designed to access some of the information that is not possible using standard auscultation techniques, by removing subjectivity and allowing lung sounds to be quantified and characterised. CALSA has previously been investigated as a potential diagnostic tool [17;18] but the validity of the technique has yet to be established in COPD populations. Guidelines for recording and analysing lung sounds have been published to standardise terminology and techniques [19].

CALSA has potential to be a simple to use and safe tool for diagnosing lung diseases and possibly grading and the location of airways obstruction. This study will provide accurate disease characterisation which will allow detailed referencing of the lung sounds.

Alveoscopy

Fibered confocal fluorescence microscopy (FCFM) is a new technique that produces microscopic imaging of a living tissue through a 1-mm fiberoptic probe that can be introduced into the working channel of a bronchoscope. By spectral analysis of the fluorescence signal disruption of the elastin component of the basement membrane in the bronchial walls can be measured[20]. The thin

probe can be advanced into the peripheral airways where the alveolar sacs and respiratory airways can be imaged[21] in both smokers and non-smokers.

Thus far this method of imaging has been mainly used for measuring dysplasia and metaplasia within the airways. It has been used safely in a smoking population but has not been compared to other measures of COPD changes. It has significant potential, especially as a research tool, to allow detailed and longitudinal assessment of the microscopic airway changes in COPD without the invasion, discomfort and risk of performing multiple biopsies. In addition it enables imaging of the most peripheral airways which is not possible by any other method.

Summary

This project will, for the first time, allow several individual disease processes of COPD to be measured in detail in a group of participants with varying stages of the disease. It will, by comparing several measurement modalities, be able to validate each of those modalities against each other. Also for the first time we will be able to map the disease processes to anatomical lung regions. The development and validation of these image analysis tools is a significant end point of the study as they will potentially be of significant clinical and research use for measuring disease progression and/or therapeutic effect.

The regional radiological mapping of the disease processes within the lung to anatomical segments allows these processes to be correlated to regional variations in disease activity, as measured by bronchoscopic sampling. This is a significant step forward in the investigation of the COPD disease processes which until now has tended to treat the COPD affected lung as a homogenously affected organ.

Aim

To explore the role of lung imaging and lung sound analysis in characterizing the phenotypes of COPD.

Hypothesis

The hypothesis to be tested is that lung imaging approaches will provide a more sensitive index of extent and severity of disease in COPD than spirometric lung function and that these approaches will detect disease when spirometry shows no evidence of airflow obstruction.

Study Objectives

In healthy controls, smokers without COPD and patients with mild and moderate COPD:

1. To compare CT and EBUS measures of airway wall dimensions.

2. To relate the structural measurements made on CT and US to measures of airway inflammation.

3. To develop an algorithm to enable accurate registration of CT images taken at different stages of respiration.

4. To assess by SPECT imaging whether the presence and extent of regional ventilation dysfunction is more sensitive than lung function or airway wall imaging in the early detection of COPD in cigarette smokers.

5. To relate ventilation measurement by SPECT CT to evidence of small airway gas trapping on CT.

6. To investigate relationships between: the functional measurements of the SPECT CT; the structural measurements of the EBUS and HRCT; markers of airway inflammation; and structural changes within the extracellular matrix.

7. To evaluate how these imaging methods relate to lung function measures and dyspnoea scores.

8. To evaluate EBUS in the measurement of both large and small airways.

9. To develop existing image analysis to improve the resolution and measurement of airway walls.

10. To develop software to enable automatic measurement of the bronchial walls.

11. To identify a relationship between airway wall measurements of large and small airways using EBUS and/or HRCT.

12. To investigate the potential value of alveoscopy imaging at bronchoscopy as an indicator of airway structural change.

13. To investigate the use of Computer Aided Lung Sound Analysis (CALSA) as a non-invasive tool in the evaluation of COPD by comparing lung sound data with imaging data

The Research Subjects

Subject recruitment

Some patients may be recruited.

Volunteers may be recruited by:-

- Contact via clinical appointments at the respiratory centre at Southampton General Hospital (SGH), out-patient appointments at SGH and possibly from clinical and outpatient settings at other hospitals in the region. The request for participation would be made at the end of their clinical attendance, would not interfere with their clinical visit and the researchers would make clear that participation or non-participation in the trial would have no bearing on their ongoing clinical care. Any participation request would be made with the full permission of the consultant supervising the care.
- Contact made via the existing III Division database. The database contains names of individuals with COPD as well as healthy controls who have expressed an interest in being willing to participate in clinical studies.
- Contact via hospital and community pulmonary rehabilitation classes. The request for participation would not interfere with their visit and the researchers would make clear that participation or non-participation in the trial would have no bearing on their ongoing care. Any participation

request would be made with the full permission of the supervising health professional

- Contact via primary care organisations, including General Practices.
- A participation request to participants who are nearing completion in existing studies ensuring that the twelve week 'wash-out' period is observed.
- Advertisements and information leaflets formally approved by the ethics committee and distributed or posted in the following places:
    - At stands or stalls at educational events.
    - In public areas with the permission of the owner or proprietor
    - In newspapers or other literature for circulation
    - On a website operated by our group or in conjunction with our group
    - On public information websites
    - At health events, rehabilitation and exercise classes.
    - By radio advertising
    - By e-mail distribution to a group or a list only with the network administrator or with equivalent authorisation

Approval for the study will be obtained from the Southampton and West Hampshire local research ethics committee and the SUHT Research and Development department. Subjects will be provided with an approved patient information sheet and after at least 24 hours to consider this they will be asked to give written informed consent. General practitioners will be informed of their patients' participation by letter.

Participants will be reimbursed for the time that they spend in the clinic and compensated for the inconvenience of the procedures. The total compensation is likely to be £300, given at the end of the study. If participants do not complete the study they can be partially compensated approximately based on the following calculation: £10 for visit 1, £20 for visit 2, £20 for visit 3, £250 for visit 4.

All participants will be entered into the national TOPS database to prevent over-volunteering in clinical trials.

Appendix 4

Subject groups

There will be four study arms. Initially 10 subjects per arm will be studied, and then further subjects may be added up to a maximum of 50 subjects per arm.

There are two COPD groups comprising of current or ex cigarette smokers with proven COPD as indicated by an FEV_1/FVC ratio of <75%. The two COPD groups will be divided into ten subjects with GOLD stage 1 disease (FEV_1/FVC ratio of <70% and an FEV_1 of ≥80%) and ten subjects with GOLD stage 2 disease (FEV_1/FVC ratio of <70% and an FEV_1 of 50-79%).

There are 2 control groups: One non-smoking healthy control group and one group of cigarette smokers who have no physiological evidence of obstructive airways disease.

To preserve resources and reduce the impact on the participants we will accept historical screening data that has been obtained less than a year prior to the date of inclusion into this study. This is also subject to the equipment used to obtain the historical results producing data that is directly comparable to the data relating to other subjects in the study i.e. on the same equipment using the same measurement protocol. The screening data that this statement refers to is baseline spirometry, bronchial reversibility testing, skin prick testing, bronchial responsiveness testing, and exhaled nitric oxide testing.

Inclusion criteria

- Age 40 – 70 years
- Either gender
- Willingness to participate in the study

GOLD Stage 1 COPD patients

- Current or ex smokers.
- Physiological evidence of obstructive airways disease (FEV_1/FVC ratio <70%)
- FEV_1 of ≥80%

- Incomplete disease reversibility (<10% improvement with 400ug inhaled salbutamol)
- Symptoms of breathlessness, cough or sputum production
- No evidence of exaggerated diurnal variation to suggest asthma
- Normal exhaled NO

GOLD Stage 2 COPD patients

- Current or ex smokers.
- Physiological evidence of obstructive airways disease (FEV_1/FVC ratio <70%)
- FEV_1 of 50-79%
- Incomplete disease reversibility (<10% improvement with 400ug inhaled salbutamol)
- Symptoms of breathlessness, cough or sputum production
- No evidence of exaggerated diurnal variation to suggest asthma
- Normal exhaled NO

Smoking controls

- Current smoker
- Normal spirometry
- Normal exhaled NO
- PC_{20} (Histamine) > 8 mg/ml (non-cumulative)

Healthy Controls

- Life long non-smoker (<5 pack years, last tobacco >2yrs ago)
- No history suggestive of airways disease.
- Normal spirometry
- Normal exhaled NO
- PC_{20} (Histamine) > 8 mg/ml (non-cumulative)

Exclusion criteria

- Pregnancy or breast feeding
- Significant co-morbidity (eg symptomatic ischaemic heart disease)

- Current acute lung infection or acute exacerbation of COPD, investigation will be deferred until the infection is treated.
- Inability to understand directions for dosing and study assessment
- Inability to be contacted in case of emergency
- Past or present tuberculosis, systemic lupus erythematosis or multiple sclerosis
- Any clinically significant neurological, renal, endocrine, gastrointestinal, hepatic or haematological abnormalities uncontrolled with standard treatment
- History of psychiatric, medical or surgical disorders which may interfere with study

Subject visits

This section describes the full study protocol. Depending on the physical well-being of the patient and their disease characteristics not all investigations may be performed in all participants.

There are four scheduled study visits as outlined below and the protocols for the investigations at each visit are subsequently described. As long as the validity of the investigations is not compromised the order of the visits or the order of the investigations within the visits may be changed. Circumstances where this may become necessary are among others, to coincide with equipment availability, volunteer availability and volunteer tolerance of procedures. In very unusual circumstances and if all the investigations cannot be completed within the proposed four visits then an additional visit may be considered.

The following are examples of when the safety or validity of the investigations could be compromised and infringement would constitute a protocol violation.

- Screening investigations that include inclusion or exclusion criteria (baseline spirometry, bronchial reversibility, bronchial responsiveness, skin prick testing and nitric oxide testing) should be performed prior to undertaking the HRCT and the bronchoscopy (unless either investigation is clinically necessary for non-research purposes).

- Participants should not receive bronchodilator medication less than 24 hours prior to the bronchial responsiveness challenge, bronchial reversibility testing, lung function testing, HRCT scanning, and the SPECT-CT scan.

- Bronchoscopy should be performed at least seven days after sputum induction and bronchial responsiveness challenge.

- Sputum induction, lung function testing and lung imaging should be performed at least six weeks after bronchoscopy

Summary of patients' visit

Visit 1

Skin-prick testing (5 min, 15 min observation and 1 min reading)

All subjects will undergo skin prick testing to assess atopic status. Six common aeroallergens (grass mix, cat, tree mix, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and aspergillus - Hollister-Stier Laboratories, Spokane, WA, USA) will be used. One drop of each solution will be placed on the volar aspect of the forearm and pricked through with a sterile lancet (Entaco Ltd, Studley, UK). Appropriate positive and negative controls will be included. The long axis wheal size will be measured at 15 minutes. Positive atopic status will be defined as a response of > 3 mm more than the control wheal, to one or more allergens.

Nitric Oxide testing (10 min)

Exhaled Nitric Oxide will be measured according to the ATS guidelines[22].

ECG testing (10 min)

ECG testing will be carried out using standard local guidelines.

Appendix 4

Lung function measurement (30 min)

Spirometry will be performed, computing FEV_1. FVC, PEF and FEF_{25-75} measures from the flow volume loop. The best of three measures will be taken for each separate recording. We will also measure carbon monoxide gas transfer and static lung volumes (TLC and FRC).

Assessment of bronchial responsiveness (30 min)

Airway responsiveness will be assessed using a modification of the method of Chai *et al.*[23] Subjects will be asked to abstain from using short acting β_2 agonists for at least eight hours prior to the histamine challenge and to discontinue long acting β_2 agonists for at least 24hrs . A Baseline FEV_1 will be obtained before five breaths from functional residual capacity to total lung capacity are made inhaling physiological saline then incremental increasing dilutions of histamine or methacholine from 0.03 mg/ml – 8 mg/ml administered by an Inspiron nebuliser (Bard Ltd, Sunderland, UK) with an output of 0.33ml/min. FEV_1 measurements will be made at one and three minutes after inhalation of each solution. A fall of >10% after the saline challenge will preclude continuation of the histamine/ methacholine challenge. The challenge will be stopped when a fall in FEV_1 of > 20% below the post saline baseline is achieved or the 8mg/ml concentration is reached. The non-cumulative concentration of histamine or methacholine required to produce a 20% fall in FEV_1 (PC_{20}) will be calculated by linear interpolation between the last two points of the log dose response curve administered.

Diary Card Recording (5min)

Volunteers will be supplied with a peak flow meter (Miniwright, Clement Clarke International Limited, Harlow, UK) to measure their PEF at home for two weeks after entry into the study. The best of three blows in the morning and evening, before taking any bronchodilators, will be recorded on a diary card with details of any chest symptoms or medication taken. The card will be collected on visit 4 before proceeding with the bronchoscopy.

Dyspnoea/ Quality of Life Score (10 min)

Symptoms of dyspnoea will be quantified using a validated measure such as the MRC[24] dyspnoea scale. We will also use a validated respiratory quality of life measure such as the St George's Respiratory Questionnaire[25].

Blood testing (5min)

Screening blood test (full blood count, U&E and clotting) will be taken to ensure the safety of the bronchoscopy. Serum will also be taken and stored in the -80°C for analysis for markers of inflammation.

Visit 2

SPECT imaging

Before the SPECT/CT scan the participants' spirometry, oxygen saturation measurement and a urine pregnancy test (for females of child bearing age) will be performed according to the Nuclear Imaging Department protocols. SPECT/CT ventilation and perfusion scans will be performed using a GE Infinia camera fitted with the Hawkeye 4 low dose CT system (GE Systems, Milwaukee, WI, USA). The ventilation scans will use Kr-81m labelled air and the perfusion scans will use Technetium-99m labelled human albumin in microsphere form. The low dose CT scan will enable attenuation correction of the SPECT scan providing a quantitative description of the three dimensional spatial variation of ventilation. The effective dose for the Kr-81m scan will be approximately 0.2 mSv and for the low dose CT, 0.8 mSv. The images will be transferred to an image processing workstation for analysis.

Reversibility Testing

Baseline spirometry and oscillometry measurements will be taken (see below for further information). If the subjects have an airways obstruction then reversibility will be tested. The bronchodilator will be 400 mcg of salbutamol inhaled from a multi-dose inhaler via a spacing device. The spirometry and oscillometry measures will be repeated 15 minutes after the salbutamol

administration using the same equipment as the baseline measurements, this is in accordance with published practice [26;27].

Spirometry for reversibility testing

This will be done using the same equipment as in visit 1.

Random Noise Oscillometry

The effort independent technique of random oscillometry will be used to provide information relating to small airway resistance (R0) and the findings will be compared to measures of small airways obtained by spirometric flow volume loop FEF25-75 measures. It will be done using on a Sensormedics pseudo random noise oscillatory spirometer.

Sputum Induction

Sputum will be collected using European Respiratory Society (ERS) recommendations, and protocols that consist of inhalation of hypertonic saline solution[28].

Visit 3

HRCT imaging

Prior to the HRCT all females of child bearing age will receive a urine pregnancy test. CT scans will be performed on a Siemens Sensation 64 CT scanner (Siemens Medical Solutions Erlangen Germany) using a high resolution algorithm, with detector thickness 0.6 mm, pitch 1, effective mAs 90, 120kV. The effective dose for this procedure will be approximately 3.8 mSv. This high resolution protocol is required to get the best visualisation of the airway tree[14]. Images will be captured with subjects in suspended full inspiration and on full expiration. The combined radiation dose of the inspiratory and expiratory scans will be approximately 7 mSv. The expiratory CT scan will require a lower radiation dose because the lung fields will be smaller.

The images will be initially reconstructed using a slice thickness of 0.75 mm, a reconstruction increment of 0.5 mm and a sharp reconstruction algorithm.

These parameters will be varied to determine the settings that provide the optimal visualisation of the airways. The DICOM images will be transferred from the CT scanner to a dedicated image-processing computer. This will be a high specification PC running Matlab and C++. It will also run the Pulmonary Workstation software (VIDA Diagnostics Inc, Iowa City IA, USA)

Computer Aided Lung Sounds Analysis

In this study, lung sounds will be captured using a digital stethoscope (WelchAllyn Meditron, 5079-402) recording for 25 seconds at 7 anatomical locations. The input from the stethoscope microphone will be connected via an integral amplifier to the sound card of a laptop with customised software, suitable for data acquisition and analysis, written in Matlab (version 7.1). A sampling frequency of 44.1 kHz will be adopted for all recordings.

Visit 4

Review of diary card

The Peak flow and symptom diary cards that were distributed in visit1 will be collected.

Fibreoptic bronchoscopy with EBUS and alveoscopy

Before the bronchoscopy the participants' spirometry, oxygen saturation measurement and a urine pregnancy test (for females of child bearing age) will be performed according to the departmental protocols. Bronchoscopic examination will be performed at least seven days after histamine challenge. All subjects will give their written consent and the procedure will be carried out according to the British Thoracic Society guidelines[29]. Spirometry and/or oscillometry at baseline and 15 minutes after nebulised salbutamol (2.5mg) and Ipratropium bromide (0.5mg) will be recorded prior to bronchoscopy in both the COPD and control groups. The post nebuliser value will be taken as the post bronchodilator value for calculation of reversibility. Intravenous access will be established and subjects will be premedicated and sedated in accordance with the operator preference, the participant's wishes and the clinical scenario. Sedation will be administered in accordance with the guidance

for conscious sedation issued by the Royal College of Anaesthetists in association with the Academy of Royal Colleges[30].

Topical 10% lignocaine spray (Astra pharmaceuticals, Kings Langley, UK) will be applied to the nose and pharynx. The bronchoscope (BFXT 40, Olympus, Keymed, Southend-on-Sea, UK) will be passed via the mouth or the nose with further 1-2% lignocaine instilled through the bronchoscope as necessary, up to a maximum of 5mg/Kg. Arterial oxygen saturation will be monitored throughout (Ohmeda, Louisville, USA) and supplemental oxygen given by nasal cannulae as required. ECG monitoring will be monitored throughout the procedure.

The EBUS probe (PL2220-20, Hitachi medical systems, Japan) will be introduced via the bronchoscope working channel. Ultrasound measurements will be taken from one or more airways. The balloon sheath will be inflated with saline until 360° contact with the airway wall is just achieved and an image recorded. The balloon inflation/deflation/image capture process will be performed up to three times in each airway to be measured.

If two lobes, segments or sub-segments are to be studied in the same patient they will be from the same lung. We will not biopsy or lavage both lungs in the same patient during one bronchoscopy.

For each lung segment under study a 120ml bronchoalveolar lavage and bronchial brushings may be undertaken.

For each bronchial area under study we will take three to five biopsies depending on the number of separate sites to be biopsied and the tolerance of the participant. Two endobronchial biopsy specimens will be taken from the subcarinae using 1.8mm disposable alligator cup forceps (BARD, Sunderland, UK order no. 100503) and immediately placed in ice cold acetone-containing protease inhibitors (2mM phenylmethylsulphonyl fluoride with 20 mM iodoacetamide, Sigma) for subsequent processing into glycol methacrylate resin. These biopsies will be used for immunohistochemical analysis. Another sample will be placed in Trizol for mRNA extraction and subsequent quantitative RT-PCR for gene expression analysis. Further biopsies that may be taken would be one snap frozen in liquid nitrogen for atomic force microscopy

and/or one processed for helium ion microscopy. No more than ten biopsies would be taken at a single bronchoscopy.

Samples will remain the property of the University of Southampton.

Alveoscopy

Alveoscopy may be undertaken depending on equipment availability and if the overall length of the procedure allows. Airway wall and alveolar images will be obtained at using the Cellvizio system and the images will be stored and analysed with respect to collagen and elastin fluorescence and the pattern compared between groups and in relationship to lung function, immunohistochemical staining of biopsy samples and the results of helium-ion microscopy.

Post procedure subjects will be observed for at least 2 hours after the procedure and will not be discharged until their FEV_1 had reached ≥90% of their baseline value.

Assessment of Safety

Safety will be assessed by the frequency, incidence and nature of adverse events and serious adverse events arising during the study.

Definitions

Adverse Events

An AE is any untoward medical occurrence in a volunteer which may occur during the study and does not necessarily have to have a causal relationship with the study. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study, whether or not considered related to the study.

Serious Adverse Events

An SAE is an AE that results in any of the following outcomes, whether or not considered related to the study.

• Death (i.e., results in death from any cause at any time)

• Life-threatening event (i.e., the volunteer was, in the view of the investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more serious form, might have caused death.

• Persistent or significant disability or incapacity (i.e. substantial disruption of one's ability to carry out normal life functions).

• Hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalisation (including inpatient or outpatient hospitalization for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.

• An important medical event (that may not cause death, be life threatening, or require hospitalization) that may, based upon appropriate medical judgment, jeopardize the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalization.

Reporting Procedures for all Adverse Events

All AEs occurring during the study observed by the investigator or reported by the volunteer, whether or not attributed to study medication, will be reported in the CRF (either on AE page or elsewhere on CRF for expected related AEs). All AEs that result in a patient's withdrawal from the study or that are present at the end of the study, will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned.

Reporting Procedures for Serious Adverse Events

SAEs will be reported on SAE forms to the local safety committee (see section 11.6), within 1 working day of the investigators being aware of their occurrence.

SAEs will not normally be reported to the ethics committee sponsor unless there is a clinically important increase in occurrence rate, an unexpected outcome, or a new event that is likely to affect safety of trial volunteers.

In addition to the expedited reporting above, the investigator shall submit once a year on the anniversary date of the CTA throughout the study, or on request, a safety report to the Competent Authority and Ethics Committee.

Procedures to be followed in the event of abnormal findings

Abnormal clinical findings from medical history, examination or blood tests, will be assessed as to their clinical significance. If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the volunteer will be informed and appropriate medical care arranged as appropriate and with the permission of the volunteer.

Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

Image and Data Processing and Analysis

Storage of Data

All identifiable electronic data will be stored in a secure password protected area on the SUHT network drive. All data for analysis will be anonymised by subject number.

Study documents (paper and electronic) will be retained in a secure location during & after the trial has finished. All essential documents and source data, including any medical records where entries related to the research have been made, will be retained for a minimum period of 15 years following the end of the study. A 'DO NOT DESTROY' label stating the time after which the documents can be destroyed will be placed on the outer cover of relevant medical records.

Capture and storage of EBUS images

The EBUS ultrasound machine has an attached laptop computer with image capture and Digital Imaging and Communications in Medicine (DICOM) archiving software (WinPax, Medical Diagnostic Systems, Meckesheim,

Germany). The subject files will then be anonymised by subject number. The image analysis software will be manually calibrated using the internal side scale on EBUS images before analysis.

Capture and storage of HRCT images

The GE systems CT console will be connected to a workstation with CD burning capabilities. The images will then be stored on a computer as DICOM files anonymised to a study number. Calibration information will be automatically included in the DICOM files generated by the CT scanner.

Reading of CT and Radio-isotope images

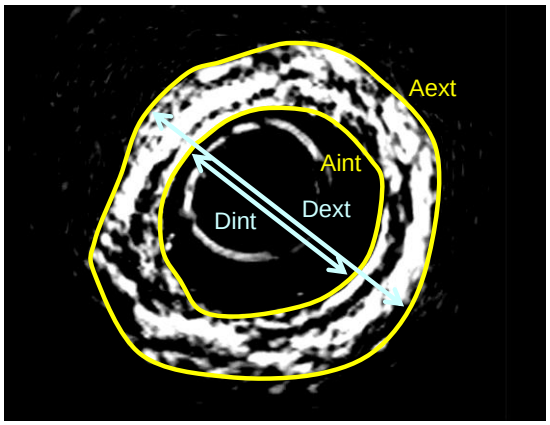
The HRCT and SPECT/CT images will be read by a radiologist blinded to the COPD disease category of the participant. This is to detect any unexpected abnormalities on the CT scans and to provide a subjective reference for the severity of any underlying lung disease.

Measurement of airway wall thickness

Manual measurement

The initial calculations of wall thickness from both the HRCT and EBUS images will be made using previously validated methods. These are calculations of short axis wall thickness to external diameter ratio (T/D) and percentage wall area (%WA). These calculations will be taken from a mean of measurements taken from the 3 EBUS images, or the 3 most appropriately orientated CT slices. All measurements will be by manual guidance of the image analysis software conducted by an observer blinded to the study group.

Image analysis calculations



T/D ratio= (Dext–Dint/2)

Dext

%WA = Aext–Aint x 100

Aext

We intend to develop algorithms, based on existing 3D image segmentation algorithms, to improve on these calculations.

Development of computer analysis

The images will also be transferred to the image-processing computer where they will be first analysed by the Vida software to segment the airway tree as far down the tree as is possible. Software will then be developed in Matlab to identify each visible airway and define lengths diameters and spatial orientation. From this data we intend to be able to develop automated analysis of airway wall thickness and other airway dimensions from the CT images. The automated analyses can then be validated against the manual measurements.

Lung density mapping

The small airways dysfunction of the lung and emphysematous change will be analysed first by lung density mapping as described in previous work. We will then use more sophisticated approaches[31] to refine the analysis. This enables lung tissue to be allocated to one of a number of different classes.

Further software will define the segmental structure of the lung from the airway description[12]. This will provide a means of describing changes in structure and function on a local basis.

Analysis of SPECT Ventilation Images

The SPECT/CT images will be able to accurately show regional variations in lung ventilation. We will then be able to build on existing work to refine algorithms that will allow mapping of the ventilation images to lung segments[13].

Image referencing between inspiratory and expiratory HRCT images and SPECT/CT images

We will build on already existing work[13] to develop an algorithm to reference the inspiratory and expiratory HRCT images and the SPECT/CT images (which are acquired at tidal ventilation) to each other. This will allow us to compare the lung structural measurements at different phases of respiration. We should then be able to compare these measurements with those taken by EBUS.

This image referencing analysis will then allow the SPECT image of ventilation to be aligned to the high resolution CT images via the low dose CT acquired with the SPECT. The relative ventilation will be correlated with structural changes observed on CT on a segmental basis.

Analysis of Lung Sounds

Lung sound data will be processed using customised algorithms written in Matlab based, in part, on an algorithm developed by Vannuccini et al[32].These algorithms permit automatic detection and analysis of adventitious sounds (crackles and wheezes) and automatic detection of breathing cycles. Lung sound data will be compared with image data, based on post-processing of the CT scans to extract 3-D reconstructions of the bronchial tree that can be analysed to locate sputum more precisely than in the raw image[33].Any relationship between lung sounds and airway geometry will be sought.

Data Analysis

All the parameters derived from imaging will then be correlated with the clinical findings. Results from the four groups studied will be compared with a view to finding parameters showing significant differences between groups. The parameters will also be studied together with other measurements to see if there are any typical patterns of combined values that would enable patients to be classified as being of a particular phenotype.

The results of the lung sound analysis will also be correlated with both clinical and imaging findings with a view to determining its potential as a non-invasive tool in the assessment of COPD.

Software development

It is anticipated that new software algorithms will be developed during the course of this study that will have general application in providing biomarkers of respiratory disorders from imaging. The prototype software developed in Matlab will then be written in more efficient C++ code to produce software, which is robust, user-friendly and generalisable and therefore suitable for distribution to other hospitals

Indemnity

This is an NHS-sponsored research study. For NHS sponsored research HSG(96)48 reference no. 2 refers. If there is negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed, NHS Indemnity covers NHS staff, medical academic staff with honorary contracts, and those conducting the trial. NHS Indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm. Ex-gratia payments may be considered in the case of a claim.

Appendix 4

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Principal Investigator:	Dr Tom Havelock
Participant number:	
Subject initials:	

VOLUNTEER CONSENT FORM (Staged)

An Investigation into the Application of Imaging to the Characterisation of the Phenotypes of Chronic Obstructive Pulmonary Disease

PART A: This section relates to your consent for the current study
(samples to be destroyed on study completion unless parts B/C completed)

Initials

1. I confirm that I have read the information sheet version ____ dated _____ for the above study and have been given a copy to keep. I have been able to ask questions about the study and I understand why the research is being done and any risks involved. 1. ____
2. I have received enough information about the study. 2.____
3. I agree to take participate in the study as detailed in the information sheet. 3.____
4. I understand that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected 4.____
5. I agree to my GP being contacted and being asked to share information about my medical history and give access to any other medical records as required. 5.____
6. I agree to my details being registered with and checked against a confidential national database (TOPS) to prevent me taking part in more than one trial at a time. 6.____
7. I agree that the relevant sections of my medical notes and data collected from the study may be looked at by responsible individuals from Southampton University Hospitals Trust and Southampton University and individuals who required to review the data as part of the regulation of the study (study monitors and the regulatory authorities). 7.____
8. I agree to give blood and sputum, lavage fluid, biopsies and brush samples to be taken at bronchoscopy for research as part of this study project. I understand how the sample will be collected, that giving a sample for this research is voluntary and that I am free to withdraw my approval for use of the sample at any time without my medical treatment or legal rights being affected. 8.____

Initials

9. I understand that my Doctor and I may be informed if any of the results of tests done as part of the research are important for my health. However, I also understand that the research may not directly benefit my health. 9.____

10. I understand that by signing this consent form I am releasing the samples, the by-products and derivatives of the sample to my physician and to any third party. 10.____

11. I understand that I will not benefit financially if this research leads to the development of a new treatment or test. 11.____

12. I know how to contact the research team if I need to. 12.____

_____	_____	_____
Name of patient	Date	Signature
_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Name of researcher	Date	Signature

Participant number:		Participant initials:	

Part B & C: These sections concern samples gifted for storage and their use in possible future studies

PART B ***This section refers to linked or linked-anonymised samples:***

13. The statements in this section give permission for my sample and any derived cells to be stored (potentially for many years) for the specific types of studies. Some of these projects may be carried out by other researchers, including researchers working for commercial companies. These **future studies will be reviewed and approved by a Research Ethics Committee prior to your sample being used, and that you can alter these decisions at any stage by letting the research team know.** These studies may not be for the purpose of direct benefit for my health.

		Initials
a)	I give permission for the sample to be used for research about medical conditions relating to asthma and chronic obstructive pulmonary disease and potential treatments thereof.	13a.____
b)	I understand that future research using the samples I give may include genetic research aimed at understanding the genetic influences of airways disease, but that the results of these studies are unlikely to have any implications for me personally.	13b.____
c)	In the case of linked anonymised samples, I give permission for a member of the research team to look at my medical records to obtain information on my medical history. I understand that the information will be kept confidential.	13c.____

PART C: ***This section refers to unlinked anonymised samples:***

14 The statements in this section give permission for my sample and any derived cells to be stored (potentially for many years) for the specific types of studies. Some of these projects may be carried out by other researchers, including researchers working for commercial companies. These **future studies will be reviewed and approved by a Research Ethics Committee prior to your sample being used. Once the samples become unlinked anonymised samples they can not be traced back to you and so it may not be possible to withdraw them if you change your mind.** For as long as the samples in our possession can be traced back to you (as linked anonymised samples) you can alter your decision about them becoming unlinked anonymised samples. These studies may not be for the purpose of directly benefiting my health.

		Initials
a)	I give permission for the sample to be used for research about medical conditions relating to asthma and chronic obstructive pulmonary disease and potential treatments thereof.	14a.____
b)	I understand that future research using the samples I give may include genetic research aimed at understanding the genetic influences of airways disease, but that the results of these studies are unlikely to have any implications for me personally.	14b.____

Name of patient	Date	Signature
Name of person taking consent (if different from researcher)	Date	Signature
Name of researcher	Date	Signature

Appendix 4

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PARTICIPANT INFORMATION SHEET

REC Number: 09/H0502/91

R&D/ SUHT sponsorship number: RHM MED0873

An Investigation into the Application of Imaging to the Characterisation of the Phenotypes of Chronic Obstructive Pulmonary Disease

Name of Principal Investigator: Dr Tom Havelock

Sponsor: Southampton University Hospitals Trust, Tremona Road, Southampton, SO16 6YD.

Funder: The Respiratory Biomedical Research Unit from a grant supplied by the National Institute of Health Research

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it would involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish.

Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Consumers for Ethics in Research (CERES) publish a leaflet entitled 'Medical Research and You'. This leaflet gives more information about medical research and looks at some questions you may want to ask. A copy may be obtained from CERES, PO Box 1365, London N16 0BW.

Thank you for reading this information sheet, there is a lot of information to consider so we have divided it into three parts:

- Part 1 is a shorter summary of the study and gives an outline of what is involved.
- Part 2 gives more detail about the study, why we are doing it and what will happen to you if you take part.
- Part 3 gives you more detailed information about the conduct and the official processes of the study.

If you are planning to take part in the study you need to read all three parts.

Part 1
What is the study about?

We are investigating a lung disease called Chronic Obstructive Pulmonary Disease (COPD). COPD is a very common disease which may affect up to about a quarter of adults over the age of forty and it is still getting more common. COPD causes narrowing of the airways of the lungs and usually is caused by smoking.

COPD affects different people in different ways and affects different parts of the lung in different ways. Sometimes it causes destruction of the lung itself (which is called emphysema). It also causes narrowing of the airways of the lung (which makes people wheeze). It can also cause inflammation in the main airways of the lung which causes people to cough up sputum (which we call chronic bronchitis). We call these different ways that COPD can affect the lungs the 'phenotypes' of COPD.

At the moment there is no good way to measure these different phenotypes in the lung and that is what this study is setting out to do. We are using different types of scans to look at the lungs in very fine detail. All of the scans are normally used in hospital practice. We are then using new computer programs that we have developed to analyse the results of the scans in new way.

The system that we are creating to do this would then have many uses. It could be used to accurately plan lung surgery, it would help us to investigate each of these 'phenotypes' of COPD in more detail and then help us to develop treatments that are specifically one of the 'phenotypes'. It may even be useful in measuring other lung diseases than COPD as well.

Who do we need to take part?

We need to compare the structure of the lungs of people with different amounts of COPD and people who don't have COPD. We are therefore looking for different groups of people:

- People who don't smoke and who do not have any lung disease
- People who smoke but do not have lung disease and
- People who have COPD.

We are looking for two groups of people who have COPD, people with 'mild' disease and people who have 'moderate' disease. Don't worry if you do not know if you have 'mild' or 'moderate' COPD, we can measure this by doing a breathing test.

We need people who do not have asthma and who do not have any serious illnesses other than COPD. We will also not be able to enter pregnant women into the study.

What does the study involve?

If you chose to take part in the study then we will ask you to come for four visits.

The first visit will last about 2½ hrs. During this time we will check that you understand what the study involves and you will have plenty of opportunity to ask questions. We will ask you to sign a form saying that you agree to take part in the study. We will ask you about any medical problems you may have, examine you, take a heart tracing and take some blood tests. We will also perform a number of breathing tests to check your lung function and a skin test for allergies. After this visit we will ask you to do a breathing test at home twice a day for two weeks and keep a diary of the result.

The second visit will last about 2 hrs. During this visit you will have a special type of lung scan called a SPECT-CT scan. This scan measures your breathing function and the blood flow through your lungs in a lot of detail. After the scan there will be two more breathing tests.

The third visit will last 1-1½ hrs. During this visit you will have a CT scan of your lungs, which is like a very detailed three dimensional X-Ray. Because the hospital CT scanner is very busy we might have to do this scan in the evening time. After this scan we will record your breathing sounds using an electronic stethoscope. This is like when a doctor listens to your breathing with a stethoscope except that the stethoscope is attached to a computer instead of the doctor's ears.

The fourth visit will be for the bronchoscopy and will last about three hours. At this visit we will want to look at the diary card you will have kept for your breathing tests. We will also do some more breathing tests to recheck you lung function. During the bronchoscopy we use a thin instrument to look down into your lungs. You will be awake while this is happening but we will give you medicine so that you are a bit drowsy and so that you don't really feel it. When we are looking into your lungs during the bronchoscopy we will take some samples from the lining of your airways. Because of the medication we give you, you will not be able to drive or work for the rest of the day after this visit.

Will I be paid for taking part in the study?

We do not pay people to take part in research studies but we do reimburse them for the inconvenience and discomfort of partaking in the research, the time spent taking part in the research and the travel costs. The reimbursement is broken down by visit: £10 for visit 1, £20 for visit 2, £20 for visit 3, £250 for visit 4 giving a total of £300 for the completion of the study.

What are the risks involved with taking part?

We have designed the study to be very safe and to minimize any potential harm to people who are participating in the study. The risks of the study are described in much more detail in Part 2. In summary there is a small risk associated with having the CT scans and SPECT scan because they involve exposure to radiation. The dose that we are using gives a lifetime risk of cancer of 1 in 2000which is equivalent to having a normal CT scan of your abdomen and pelvis.
The bronchoscopy is a very safe procedure to undergo but it can be uncomfortable for some people. It is described in more detail in part 2.

Do I have to take part?
It is completely up to you to decide whether or not to take part. After reading this information sheet you can have as much time as you need to make the decision. You are free to talk it over with family members, friends and other relatives if you wish. Some people even make an appointment with their GP to get their advice.

If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. Even after you have signed the consent form and agreed to take part in the study you can change your mind at any time. You are do not have to give any reason for not continuing and any decision to leave the study would not have any effect on any medical care that you are receiving. You would receive reimbursement for time that you spent partaking in the study prior to your decision to discontinue.

Part 2

What does the study involve?

The study involves four visits and at each visit involves a number of different activities. A summary of what is done in each visit is outlined in table 1. A description of what each of the activities involves is then after the table. Please note that all guidance about the time required for the test is an estimate and vary a lot from person to person.

Table 1. Summary of study visits

Visit 1 (about 3 hours)	Visit 2 (about 2 hours)	Visit 3 (about 1½ hour)	Visit 4 (about 3 hours)
Informed consent Medical history and examination Skin prick testing Exhaled NO ECG testing Lung function tests Provision of diary card and PEF meter Dyspnoea/ Quality of life score Blood tests	SPECT lung imaging Reversibility testing by Spirometry and Oscillometry Induced sputum	HRCT chest CALSA measurement	Review of diary card Fibre-optic bronchoscopy EBUS assessment of airway wall Endobronchial biopsies, bronchoalveolar lavage and bronchial brushings. Alveoscopy

Visit 1

A lot of the tests that we will do in visit one are to make sure that you are put into the correct study group and that you do not have any other medical problems that would

mean that the study was dangerous for you to take part. We also need to make sure that you do not have any illnesses that would make our study inaccurate, the most common example of this is asthma.

Some people may find out on this visit that they are not eligible to take part in the rest of the study.

Before this visit you may need to hold off taking some of your medication for a while, these instructions are written in more detail in the 'What do I need to do section?' which is later on in this sheet.

Informed consent

The first thing that we do when you choose to enter into our study is to thoroughly check that you understand what the study involves. We check that you have read all the information in this sheet and that you understand it. You will be given plenty of opportunity to ask any questions.

We then ask you to sign two consent forms which say that you agree to take part in the study and that you understand what the study involves. Even after you have signed this form if you choose that you no longer want to be in the study you are free to stop at any time.

Medical history and examination

A doctor will then see you and ask you questions about your health, any illnesses or health problems that you may have had in the past, your medications or any medications that you may have taken in the past and about any family medical problems. They will then examine you.

We do this to make sure that you do not have any medical problems that would mean that taking part in the study is dangerous for you.

Skin prick testing *(5 min, 15 min observation and 1 min reading)*

This is a test to detect any common allergies that you may have. Things that cause allergy (like dog fur, grass pollen, and house dust mite) are called allergens. Small drops of a purified liquid form of some common allergens are put onto the skin of the underside of your forearm. We will then scratch the skin once through the drop to introduce the substance under the skin. We then leave it for fifteen minutes to see if your skin reacts. If you are allergic to the substance the surrounding skin will redden, itch and come up in a small bump that rapidly resolves.

Nitric Oxide (NO) measurement *(10 min)*

You will be asked to breath into a tube where the nitric oxide (NO) in your exhaled breath will be measured using a computer. The nitric oxide levels in the air that you breathe out can be used to measure some types of inflammation in your lungs.

ECG testing *(10 min)*

This is a test that looks at the electrical activity of your heart and it can identify some heart problems. You will be asked to lie on a couch and stickers will be placed on your chest. A computer will then measure the electrical activity of your heart through wires connected to the stickers.

Lung function testing/ breathing tests *(90 min)*
You will be asked to breathe into a number of machines that will take different measurements of your lungs. There will expert nurses or doctors helping you to make sure that you get the tests right.

Spirometry involves you taking a big breath in and then breathing out as much air as possible and this tests how much air you can breathe in and out and if there is any narrowing of the tubes of your lungs.

To measure *gas transfer* you will need to breath in a special measure of gases and then hold your breath for a short while before breathing out through the machine. This gives us a measurement of how well gases are transferred from the air in your lungs into the blood, which is one of the jobs of the lungs.

To measure *static lung volumes* you will be asked to get inside a large clear Perspex box and breath through a mouthpiece. This enables us to take measurements of the size of your lungs.

The *random noise oscillometry* machine is not nearly as scarey as the name sounds. You will be asked to put your mouth around a mouthpiece and to breathe normally while the machine gently vibrates the air in your lungs. It is then able to measure the resistance to air flow in your lungs and which gives us an idea about the air flow through the small airways in the lung.

We will then what to test how 'twitchy' your airways are. We do this with a *provocation test* which is also sometimes called an *airway responsiveness test*. If your airways are very twitchy this would suggest that you probably have asthma and you would then not be eligible to take part in the trial. You will be asked to inhale a solution in increasing strengths at set intervals to see if the airways narrow. This will be evaluated by repeatedly doing breathing tests. The test is stopped either when the top concentration is reached (16mg/ml) or the airways narrow by 20%. If the airways narrow this will cause a feeling of chest tightness and sometimes make you cough. These effects are transient and you will be given a rescue medication, salbutamol (200 micrograms via a metered dose inhaler) to return the airways to normal. Trained staff will always be present.

Diary Card Recording *(5min)*

You will be given a peak flow meter which is a small hand held device that is used for measuring how quickly you can breath out. This gives us an idea of any narrowing in the tubes of your lungs. We will teach you how to use the peak flow meter.

Then we will ask you to measure your peak flow twice a day for two weeks and keep a diary of the results. We will also ask to make a note in the diary of any breathing symptoms that you may have over the two weeks. We will collect the diary from on visit 4.

Dyspnoea/ Quality of Life Score *(10 min)*

We will ask you a number of questions from a questionnaire about your breathing and any breathing symptoms that you may have. These questionnaires give us a reliable way of comparing different peoples breathing symptoms to each other.

Blood Tests *(5min)*

We will take about 50mls of blood. This is 5 or 6 little blood bottles worth and less than an eighth of the amount of blood that would be taken if you were to give some blood for blood donation. Some of the sample will be used to check that you have no underlying medical problems that would make the bronchoscopy more risky. The others will be analysed for chemicals in the blood that might give us information about the amount of inflammation in your lungs.

Visit 2

Before this visit you may need to hold off taking some of your medication for a while, these instructions are written in more detail in the 'What do I need to do section?' which is later on in this sheet.

SPECT-CT scan *(30 min)*

Before and after the visit:
We would ask you not to have any caffeine containing drinks, not to undertake any strenuous exercise, and not to drink excessive amounts of alcohol in the 24 hours preceding and following this visit. If you smoke, you should continue to smoke at your normal level. As respiratory infections affect the imaging, this visit cannot be done if you are currently suffering from a chest infection, or have had a chest infection in the previous 8 weeks. If this is the case, it may be possible to reschedule this visit once this period has passed.

At the visit:
Firstly we will recheck your lung capacity using the breathing tests (spirometry) mentioned above. We will also check your oxygen levels using a small probe attached to your finger. If you are female and of child bearing age we will ask you to take a urine pregnancy test.

We will ask you to lie on your back on a table and two cameras will be positioned in front of and behind you (you are not enclosed). Initially the cameras will move around you to take a modified CT picture of the lungs, and then the cameras will be stationary.

We will then ask to breath in a special gas (called Krypton 81-m) mixed with air. The Krypton is very mildly radioactive which means that the scanner can measure the air you are breathing into and out of your lungs. This is called a ventilation scan and takes about five minutes.

We will then give you an injection which is also very mildly radioactive. This is so that we can measure the blood flow around your lungs. This is called a perfusion scan and takes about ten minutes.

We will then repeat the breathing tests to make sure the inhaled molecules have not made your airways 'twitchy' (they are not known to in the past, and even if they do cause

some twitchiness this can be reversed with a dose of standard asthma reliever medication, salbutamol).

Bronchial reversibility test *(20 min)*

This test is very similar to the spirometry test that we measured during visit one. We will first do a spirometry test. We will then give you four puffs of a salbutamol (Ventolin) inhaler. After a pause of a few minutes we will take your spirometry measurements again to see if the inhaler has made any difference.

Sputum Induction *(30 min)*

You will also be asked to provide a sputum (phlegm) sample. You may not be able to provide a sputum (phlegm) sample automatically so you will be asked to inhale a mist of a salty solution. This will help you cough up the sputum, they may be a little discomfort as you cough. To prevent the coughing narrowing your airways you will have been given extra salbutamol (ventolin) beforehand. Trained personnel will be there with you all of the time. We will then be able to analyse the sputum for signs of lung inflammation.

High Resolution Computed Tomography (HRCT) chest scan *(45 min)*

Prior to having this scan all females of child bearing age will be asked to give a urine sample for pregnancy testing. Because the scanner is also used in the NHS and is very busy many of these scans will probably have to take place in the evenings.

For this scan you will be asked to lie on a couch that runs through the scanner. The scanner itself is a big ring that runs all the way round the bed. You will not be enclosed by the scanner and your head will not be inside it. The bed will move through the scanner until it is correctly positioned. Everything is controlled by a specialist technician called a radiographer. When the scan is being done all the staff members will have to be outside the room but they will still be able to hear you and talk to you by a microphone. They will also be able to see you through a big window and a small camera in the scanner.

We will then perform two scans, one with holding you breath after you have taken a big breath in and the second with you holding your breathe after you have breathed out as much as possible. Each scan takes less than a minute.

Computer Aided Lung Sounds Analysis *(30 min)*

You will be asked to sit down, with either a bare chest or with you wearing minimal undergarments to allow access to your chest area. The researcher will then mark your skin, with a pen, on six different locations around your chest. These marks will then be used to position the end of a digital stethoscope (this looks exactly like the stethoscope used by doctors to listen to your heart and lungs, but is attached to a computer). You will then be asked to lie on your back with your head slightly up and your knees slightly bent (we will use pillows to make you comfortable). We will then record your lung sounds while you take deep breaths through your mouth, for around 25 seconds at each location we marked on your skin. You can rest in between recordings if you feel breathless or tired. The whole lung sounds recording session should take around 10 minutes. After we finish recording, the pen marks on your skin will be cleaned.

Visit 4 *(about 3 hours but can be longer)*

This visit will take place at least seven days after visit 2 (when you have the sputum induction test). The visits usually take place in the mornings and we ask that you do not eat anything for at least six hours before hand. You can drink clear fluids (water, black tea or coffee) up to four hours before the test.

During the bronchoscopy you will receive some medications (called sedatives) that will probably make you feel drowsy. It is very important that you do not drive or operate any dangerous machinery for at least 24 hours after having these medications. We also strongly advise that you are not at home alone for the 24 hours after receiving the medication. We will therefore nned to make sure that you do not drive to or from the hospital for this visit, if necessary we can arrange a taxi. You may alternatively like to arrange transport with a family member or friend if you prefer.

All females of child bearing age will be asked to take a urine pregnancy test.

Diary card

We will collect the diary card that we gave to you at the first visit. You will hopefully have had the opportunity to collect two weeks of information.

Bronchoscopy *(the test itself takes 30-45 min but the preparation takes 30-40 minutes and then we will keep you under observation for an hour or two afterwards)*

Before the bronchoscopy you will have another breathing test to make sure that there has been no worsening of your lung function and to make sure that it is within the sfare limits for the bronchoscopy. We will insert a small cannula (which is like a plastic needle) into a vein in your arm or on the back of your hand. We will also give you a nebulizer which is a machine that makes a fine mist of medication that you breathe in. This medication, called salbutamol, makes sure all the tubes of your lung are fully opened out and relaxed. The doctor who will do the bronchoscopy will come to see you to make sure everything is OK, that you haven't had any recent medical problems and to make sure that you do not have any further questions about the procedure.

The bronchoscope looks like a thin black pipe which is about the thickness of a pencil but much more flexible. It has a bright light in one end with a tiny camera. We use it to look down into your lungs and to take samples from the lining of the lung. Usually the easiest way to get down to the lungs is through the nose because this means that we avoid the structures at the back of the throat that can make some people gag. Sometimes, if the volunteer prefers it or because the bronchoscope will not fit through the nose we go through the mouth. You will be given sedation which might make you feel a bit sleepy but you will be awake through the procedure. You will be able to breathe normally throughout.

When you have the bronchoscopy you will be sitting up on a couch. You will be given some medication (sedation) into the cannula that was inserted into your arm, this may make you feel a little bit sleepy. We then give some local anaesthetic into your nose and into the back of your throat, this can be a jelly or a spray. The local anaesthetic numbs the inside of your nose and the back of your throat so that the bronchoscope doesn't hurt when it goes through. The local anaesthetic stings a bit when it is first given but then this quickly goes away. In the back of your throat you can get a sensation of swelling, a bit like the feeling you get in your mouth when you have had the injections at the dentist. This is the anaesthetic working and there is not actually any swelling it just feels like there is.

When the anaesthetic is working we will pass the bronchoscope through your nose or mouth into the back of your throat here we will spray some more local anesthetic which may make you cough a little.

We use the bronchoscope to look down into your lungs.

The first thing that we will do is to take some measurements with an endobronchial ultrasound probe which looks like a thick wire and goes down through the bronchoscope to take ultrasound picture of the walls of the tubes of your lungs. You probably will not feel anything in you lungs when this is being done.

We will then take three types of samples from your lungs.

1. We plan to take 3 types of sample: Firstly a lavage sample (a wash of a small part of the airway lining) will be taken to see what cells are present in the airways of your lungs rather than in the tissue itself. This is done by introducing a small amount of salt solution (about 4 teaspoons at a time) into one corner of the lung, and then sucking out the fluid. In this way, any free cells in the airspace of your lung can be removed and studied.
2. Secondly, we will take brushings which obtains a sample of the lining of the airway (epithelium) by gently rubbing against it with a small brush.
3. Lastly biopsies will be taken, where a small pair of forceps is used to pinch off a small piece of airway (since there are no nerves to sense pain this is not uncomfortable although sometimes you might feel a bit of a tugging senstation). The size of the biopsies is about the same size as a tip of a ball point pen.

For some participants we may also use a new instrument called an *alveoscope* which also passes down the bronchoscope. This looks like a long wire but it is able to look at the microscopic detail in the walls of the tubes of the lung. When we are using this instrument your lungs you should not feel anything other than what you normally would from the bronchoscopy.

The bronchoscopy will be done by a doctor assisted by at least two nurses who are all experienced and trained in bronchoscopy. If at any point you do not want to continue, the bronchoscopy would be stopped. It may also be stopped if the staff think that it is in your best interests. Our first priority is your comfort and safety.

After the bronchoscopy you will be observed for at least one hour. This will allow some of the effects of the sedation to wear off. More importantly it will allow the effects of the local anaesthetic that has been given to the back of your throat to wear off. This local anaesthetic can affect your swallowing and temporarily make eating and drinking dangerous so you will have to wait for an hour before you can do this. At the end of the hour you will be given something to eat and drink so that we can check that it is safe. We will also check your breathing tests (spirometry) again to make sure that the bronchoscopy has not affected your breathing (it is very rare that this happens).

Why is the study being done?

This study is bringing together several bits of research that have been done in Southampton. Some of these previous studies have created computer programmes that can automatically identify specific regions in the lungs, others can analyse levels of COPD and others that can analyse lung function. With this study we hope to be able to combine all of these computer programmes to make a programme that can automatically make a

three dimensional 'map' of the COPD in a persons lungs. We will then use this map firstly to guide us so that we can take samples and measurements from areas of the lung that have got bad COPD or areas that have got less COPD. We then hope to be able to identify why some areas of a persons lung are more affected by COPD than other areas and maybe also why some people get worse COPD than others.

If the three-dimensional COPD 'map' is successful it could then possibly be used for other reasons as well. It may be used to guide surgery on the lungs or to be able to predict the effects of removing a particular part of a person's lung before surgery is performed. It may also be used to assess the effects of future COPD treatments.

What will happen to the images and samples I have provided during this study and will I know the results?

All of the images and samples that we take from you will be labeled only with a study number and not your name. We do this so that scientists can analyse the samples and the images without knowing who you are. This is important for confidentiality so that only a few doctors and nurses involved with the study will know your personal details. It is also important scientifically so that the scientists and people analyzing the samples and images cannot be influenced by any knowledge of your medical history such as if have COPD or not.

For some of the analysis that we do we may need to trace your samples back to you. This is done by keeping a paper file in a locked office that links your study number to your personal details. No-one except for the study team can access this file. Samples that are numbered in this way are called *linked anonymised samples*. For other studies the samples are labeled with a number that cannot be traced back to you at all these are called *unlinked anonymised samples*.

The samples will be analysed using a variety of scientific techniques in the laboratory. Most of the analysis will be done here in Southampton but for some tests we may need to send some of the sample elsewhere. We do not usually tell the volunteers the results of these tests because they are not usually relevant to medical care. If we do discover any result that is medically relevant then we will tell you and make sure that you are put into contact with the appropriate medical specialists.

Some of the blood samples that we take are for safety to make sure that you do not have any unknown medical problems that might affect the tests that wwe are doing. These samples will be analysed through the hospital in the same way that would happen for any other blood test and the results would be stored in the hospital system. If there are any abnormal results that might be medically relevant we would inform you and advise you what to do.

The images that we take from the HRCT will be stored in the secure image storage system that Southampton University Hospitals Trust uses in exactly the same way as if you had had a scan done in the NHS for a medical problem. It will also be read by a CT expert doctor (a consultant radiologist) and an official report made in exactly the same way as what would happen with a normal NHS scan. This means that we stand the best chance possible of spotting any medical problems on your CT scan that you or we do not know about. We cannot of course guarantee to spot any medical problems but we will make every effort to make sure that we do so. We will let you have access to the report of your scan and will be happy to discuss it with you if need be.

In addition to the normal reporting of the scan we will analyse the images with a number of different computer programmes. This analysis is important for our research but for this the images will only be labeled with numbers.

The SPECT-CT scans will not be reported in the same way as the HRCT scans and will only be analysed by the computer programs. We will therefore not be able to give you a report from this scan.

What do I have to do?

a) Medication changes:

For some of the visits you made need to hold off your medication for a while, please see the table below:

Medication	Which visits?	Instructions
Salbutamol (Ventolin) or Terbutaline (Brincanyl) inhalers	1 and 2	Please try to avoid taking these inhalers for six hours before the visit. If you have bad breathing difficulties and have to take them please tell the person who is doing your breathing tests.
Medications that contain Salmeterol (Serevent or Seretide) or Eformoterol (Oxis or Symbicort) inhalers.	1 and 2	Please try to avoid taking these inhalers for twelve hours before the visits. If you have bad breathing difficulties and have to take them please tell the person who is doing your breathing tests.
Tablets that contain amminophylline or theophylline (Slo-phylin, Phyllocontin,	1 and 2	If you have bad breathing difficulties and have to take them please tell the person who is doing your breathing tests.
Anti-histamine tablets	1	Please try to avoid taking these tablets for four days before the visit.
Anti-coagulant medication (warfarin, clopidogrel (Plavix),	4	It is very important for you to tell doctor who sees you about these medications. If you take warfarin it will have to be stopped for a few days until your blood clotting comes back to normal. Clopidogrel (Plavix) will need to be stopped for seven days before visit 4. If it is not safe for you to stop taking these medications then you will not be able to take part in the study. The study doctor or your GP will be able to advise you about this.

Most other medications you should continue to take as normal. You will be able to discuss this when the doctor takes your medical history during visit 1.

b) Lifestyle changes:

In the 24 hours preceding the SPECT-CT visit (visit 2), we would ask you not to drink any caffeine containing drinks, and to refrain from strenuous exercise.

As we said before you must not have anything to eat for six hours before visit 4 but can drink clear fluids only up to four hours before visit 4.

c) Private medical Insurance

If you have private insurance you should check with the company before agreeing to take part in the trial. You will need to do this to ensure that your participation will not affect your medical insurance.

d) Income tax

If you pay income tax you must declare the reimbursement payments that you receive to the Inland Revenue.

What are the possible disadvantages, side effects or risk of taking part?

These can be divided into those associated with the screening tests, those related to the imaging visits and those associated with the bronchoscopy:

a) Related to screening:

Blood taking: Occasionally when donating blood some people feel faint because their blood pressure falls. For this reason blood will always be drawn in a special chair which can be reclined fully which normally results in rapid recovery.

Skin prick tests: The skin prick test for allergy can cause itching and swelling of the skin if you have a positive response. This only lasts a short time (<30 minutes).

Provocation airway tests: The inhalation challenges to test the irritability of the airways can cause a sensation of chest tightness, but this is only slight and the challenge is done incrementally with careful measurements. You will be given reliever medication (salbutamol) afterwards to reverse this sensation.

Lung volume tests: Some people can find the box where you sit to have the volumes of your lungs measured claustrophobic. Someone will be with you at all times, and you can get out of the box quickly and easily at any time (it is not locked).

b) Related to the SPECT-CT and the HRCT (radiation exposure):

The main risk from both the SPECT-CT scan and the HRCT scan is from the radiation exposure. All X-rays, CT scans and nuclear medicine scans (like the SPECT-CT scan) involve some radiation exposure. We are also exposed to low levels of radiation exposure every day from the sun and from some radioactive rocks in the ground.

Radiation is measured in units called millisieverts which is abbreviated to mSv. Day to day radiation exposure varies where you live but on average it is about 2.2mSV per year, if you live in Devon where background radiation is higher it is about 7mSv per year. The combination of the SPECT-CT scan and the HRCT scan will expose you to a total radiation dose of about 10 mSv. This is equivalent to the radiation that you would be normally exposed to in 4½ years or if you lived for 1½ years in Devon. It is the same radiation exposure that you would receive if you had a CT scan of your abdomen and pelvis.

The main problem with radiation exposure is that it can cause a slightly increased chance of causing cancer. The background radiation exposure of 2.2mSv gives about a 1 in 10000 life time chance of getting cancer, the radiation from our study (10mSv) gives a 1 in 2000 life time risk of getting cancer.

We will not enroll anyone who is pregnant, breastfeeding or intending to get pregnant as there is a risk of radiation exposure harming the unborn child. High levels of radiation can increase risks of miscarriage, malformation, childhood cancer and still birth. To this end,

we will require all women of childbearing age to take a urinary pregnancy test, and will also ask about the possibility of pregnancy immediately prior to each imaging session.

c) Related to the bronchoscopy

Bronchoscopy is a very safe procedure and we take a lot of care to make sure that any risks are kept to an absolute minimum. Bronchoscopy can be uncomfortable to have done which is why we use the local anaesthetic in your nose, throat and mouth and the sedation medication to make you a little sleepy during the procedure.

The biggest risk from the bronchoscopy is from the sedation because it can cause problems with some peoples breathing. This is why we carefully monitor your breathing during the procedure and we have medications on hand which reverse the sedation. The doctors and nurse who are undertaking the bronchoscopy are very experienced at using the sedation medications. The overall risk of death from a bronchoscopy is between 1 and 2 per 10,000 bronchoscopies this is about the same as the risk of being struck by lightning in the next year.

After the bronchoscopy it is common to cough up small amounts of blood. This is coming from the lining of the lungs where we have taken the samples and will stop within about a day after the procedure. You should not cough up a lot of blood or clots of blood and you should not cough up blood for more than two days. If any of these things happen or you are concerned then you should contact a member of the research team or seek the advice of a doctor.

It is also quite common to get a fever for a day or so after the bronchoscopy, this is because the lavage sample can sometimes cause a bit of inflammation in the lungs. The fever is usually mild and does not last longer than two days. Very rarely the inflammation can progress to a lung infection. So if the fever lasts longer than two days, or you feel unwell or you are concerned about your symptoms then again please seek advice from the research team or a doctor.

What are the possible benefits of taking part?

The study itself will not benefit you directly. The information we get from this study may allow us to do further studies looking at COPD, and thus help us with future patient care.

Part 3

What happens when the research study stops?

You would continue any normal treatment you are receiving after the study has completed. There are no lasting effects from the study.

What if something goes wrong?

This study is covered by indemnity of Southampton University Hospitals Trust and the University of Southampton. In the unlikely event that you come to any harm as a result of taking part in this study, the usual NHS and University complaints mechanisms are open to you.

Would my taking part in this study be kept confidential?

Any information that is collected about you during the course of the research would be kept strictly confidential.

As described before all the samples and images that we take for scientific analysis are not labeled with any personal details. However for safety we do have to have a way of tracking any of your samples or images back to you. This is done by keeping a paper file which links your personal details to the study number that is used to label all of your images and samples. This file is kept secure in a locked office and only registered people on the research team have access to it.

Your GP will be notified of your participation in this study. Before doing this, we need your approval for us to write to your GP. If you do not want us to contact your GP about the trial then you would not be able to continue in the trial.

The data collected in the study including the personal details of the participants have to be inspected by independent monitors to ensure that the study is being managed correctly and in accordance with international regulations. These monitors our bound by the same rules of confidentiality as all of the research staff.

What if relevant new information becomes available?

Sometimes we get new information about the treatments or imaging techniques being studied. If this happens, your research doctor will tell you and discuss whether you should continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. If you decide to continue in the study he/she may ask you to sign an updated consent form.

In addition if this happens, your research doctor might consider that you should withdraw from the study. He/she will explain the reasons and arrange for your care to continue.

If the study is stopped for any other reason, we will tell you and arrange your continuing care.

What if I want to withdraw from the study?

You can withdraw from the study at any time, without giving a reason. Information collected may still be used, unless you ask for your images to be deleted from the records.

What will happen to the results of the research?

The results of the research will be evaluated and may be published in scientific papers. You will not be identified from these results. If you wish we can notify you if an article based on the results of this study is published. The results of this research may indicate that there is potential for new treatments for asthma that may be further researched and developed.

What do I do if I want to make a complaint?

If you are unhappy about something in the study and wish to make a complaint then you can contact the doctor whose details are written below. If for some reason this is not possible or you do not feel able to do this then you can contact the Patient Advice and Liason Service (PALS) on:

Will any genetic tests be done?

No.

Who is organising and funding the research?

This study is sponsored by the University of Southampton, and funded by the Biomedical Research Unit, a department of the University of Southampton.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the Southampton and South West Hampshire Local Research Ethics Committee.

Contact for further information:

Further information is available from: Dr Tom Havelock
Wellcome Trust Clinical Research Fellow
Wellcome Trust Clinical Research Facility
Mailpoint 218, Level C, West Wing
Southampton General Hospital
SO16 6YD
Tel no 02380794989
Mobile 07725364661
Email t.havelock@soton.ac.uk

If you decide to take part in the study, you will be given a copy of the information sheet and a signed consent form to keep.

Appendix 5: Demographic data and pulmonary function test for the sample population

Demographic data

Subject No.	Gender	Group	Age (year)	Weight (kg)	Height (m)	BMI
1	F	2	51	65.80	1.73	21.99
2	F	2	45	85.20	1.75	27.82
3	M	1	47	69.80	1.63	26.27
4	M	1	65	102.80	1.82	31.03
5	F	1	46	60.80	1.63	22.88
6	M	1	66	84.70	1.84	25.02
7	F	2	62	50.20	1.59	19.86
8	F	2	55	84.40	1.58	33.81
9	F	1	47	77.00	1.72	26.03
10	M	1	45	78.50	1.68	27.81
11	M	1	46	76.20	1.73	25.46
12	M	1	40	80.60	1.81	24.60
13	F	2	45	67.00	1.61	25.85
14	M	2	47	70.80	1.75	23.12
15	F	2	64	69.90	1.64	25.99
16	M	2	62	94.20	1.71	32.22
17	F	2	51	68.40	1.62	26.06
18	F	1	46	70.55	1.64	26.23

Appendix 5

Subject No.	Gender	Group	Age (year)	Weight (kg)	Height (m)	BMI
19	F	3	60	57.00	1.57	23.12
20	M	3	60	89.20	1.76	28.80
21	M	3	60	107.00	1.74	35.34
22	M	3	56	74.60	1.82	22.52
23	F	3	60	75.60	1.60	29.53
24	M	3	60	89.00	1.68	31.53
25	M	3	54	73.30	1.73	24.49
26	M	3	65	91.60	1.66	33.24

F= Female, M= Male, BMI= Body mass index

Pulmonary function test

Subject No.	FVC (l)	FEV1 (l)	FVC (%PP)	FEV1 (%PP)	FEV1/FVC	PEF (%)
1	3.61	2.61	104.70	88.26	72.28	103.80
2	4.57	3.60	123.00	111.90	78.58	97.00
3	3.57	2.80	113.30	103.50	78.07	57.10
4	5.05	4.29	97.50	100.50	84.84	92.10
5	4.29	3.33	136.80	123.80	77.67	109.00
6	4.43	3.49	115.60	104.40	78.61	96.00
7	3.36	2.46	132.10	115.40	73.21	123.10
8	3.04	2.32	111.70	100.50	76.19	139.90
9	3.53	3.04	125.60	108.30	74.25	115.30
10	4.90	4.11	117.60	119.90	83.87	85.60
11	4.28	3.57	96.60	97.70	82.60	96.10
12	4.75	3.88	93.10	92.50	81.69	109.70
13	4.49	3.08	146.30	116.90	68.54	103.00
14	5.34	4.43	111.70	111.90	83.02	115.40
15	2.99	2.00	110.20	87.80	66.94	84.00
16	4.50	2.98	115.60	97.10	66.10	113.70
17	3.14	2.33	106.10	92.20	74.03	84.70

Appendix 5

Subject No.	FVC (l)	FEV1 (l)	FVC (%PP)	FEV1 (%PP)	FEV1/FVC	PEF (%)
18	4.14	3.34	128.50	120.50	80.56	131.60
19	2.66	1.36	106.00	64.90	61.00	73.40
20	3.97	2.61	93.80	78.30	65.79	76.20
21	3.72	2.43	90.20	74.70	65.28	59.70
22	5.31	3.54	112.70	94.60	66.67	96.10
23	2.10	1.33	77.50	58.40	63.58	52.80
24	3.28	1.90	86.20	62.90	58.04	66.20
25	4.24	2.23	125.80	77.40	52.66	62.40
26	1.60	3.46	57.80	98.1	46.09	59.10

Appendix 6: Characteristics of added lung sounds

Subject No.	Crackle 2CD (ms)						Number of crackle per breathing cycle					
	AL	LL	PL	AR	LR	PR	AL	LL	PL	AR	L R	PR
1	16.15	16.15	13.07	15.10	14.32	12.80	3.11	4.75	3.00	2.40	6.00	7.40
2	16.55	17.31	16.08	16.50	13.70	15.40	1.80	2.40	3.67	1.00	7.20	3.20
3	14.65	16.02	8.92	16.49	17.43	12.67	1.11	1.85	3.14	2.88	1.75	2.00
4	17.11	16.24	11.36	18.02	17.92	12.94	2.33	11.10	7.00	7.66	3.00	8.66
5	15.51	14.27	10.64	15.73	13.88	11.69	3.00	8.00	6.40	2.40	10.00	6.50
6	17.19	16.64	12.19	17.26	16.36	12.71	1.75	7.00	4.66	5.00	4.66	5.50
7	14.64	13.78	17.00	16.35	14.36	15.44	4.75	4.20	9.00	5.80	3.60	6.40

Appendix 6

Subject No.	Crackle 2CD (ms)						Number of crackle per breathing cycle					
8	15.46	14.91	12.36	16.92	14.16	15.76	4.80	5.14	7.75	4.40	6.33	5.20
9	16.54	18.45	16.70	14.95	15.78	15.03	2.40	2.00	2.00	2.57	2.60	1.50
10	15.52	15.41	16.28	17.04	13.80	10.65	2.20	4.60	3.50	2.26	3.40	6.80
11	13.91	14.98	15.87	13.53	15.73	16.61	6.16	3.28	3.00	1.66	2.14	4.00
12	15.18	15.99	16.18	16.92	15.28	16.66	2.50	6.66	5.00	4.80	1.33	3.33
13	15.15	13.85	14.94	15.47	13.75	15.62	3.00	4.33	3.75	3.50	6.50	3.25
14	16.69	16.83	14.76	15.55	14.66	15.36	5.75	5.75	2.50	6.25	5.00	2.25
15	14.48	15.75	14.65	16.11	16.81	14.05	2.60	5.80	5.20	4.20	3.60	5.60
16	16.39	17.01	14.92	17.53	15.20	14.53	3.00	3.40	4.80	2.28	3.16	4.00

Subject No.	Crackle 2CD (ms)						Number of crackle per breathing cycle					
17	15.20	15.19	16.25	17.94	13.61	15.98	2.86	2.50	3.33	3.60	4.40	3.60
18	18.44	14.25	11.33	17.62	12.57	12.37	3.33	4.40	4.50	7.00	2.33	3.71
19	14.33	13.03	13.41	13.27	13.97	11.69	1.00	2.00	2.20	1.54	2.63	2.00
20	15.09	15.98	12.24	17.67	17.45	11.91	7.00	5.75	3.33	1.65	5.66	4.28
21	15.70	13.03	14.43	14.34	14.52	15.49	3.33	4.80	4.75	2.20	4.50	0.50
22	16.13	13.90	15.78	15.60	16.08	16.36	3.15	4.25	1.20	3.40	4.00	1.75
23	15.80	10.54	10.69	16.23	15.2	12.44	4.80	6.50	5.33	4.16	3.82	3.33
24	15.25	15.50	13.11	16.92	15.67	11.62	3.00	7.66	4.00	5.33	3.66	11.00
25	17.86	14.93	14.43	16.66	16.03	15.59	3.00	2.42	2.00	2.66	2.14	2.85

Appendix 6

Subject No.	Crackle 2CD (ms)						Number of crackle per breathing cycle					
26	15.79	14.81	14.83	15.35	15.83	15.88	4.75	3.60	4.00	2.75	2.50	5.00

AL = anterior left region of chest wall, AR = anterior right region of chest wall, LL =lateral left region of chest wall, LR= lateral right region of chest wall, PL= posterior left region of chest wall, PR= posterior right region of chest wall

Subject No.	Wheeze number per breathing cycle						Frequency of wheezes (Hz)					
	AL	LL	PL	AR	LR	PR	AL	LL	PL	AR	LR	PR
1	0.00	0.00	0.00	0.00	0.75	1.20	0.00	0.00	0.00	0.00	634.77	539.50
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.77	0.57	0.85	0.00	0.00	0.25	672.74	549.93	393.42	0.00	0.00	644.53
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.33	0.00	0.00	0.66	0.00	0.00	292.97	0.00	0.00	434.57	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 6

Subject No.	Wheeze number per breathing cycle						Frequency of wheezes (Hz)					
	AL	LL	PL	AR	LR	PR	AL	LL	PL	AR	LR	PR
10	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	566.41
11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	1.00	0.00	0.00	0.00	0.00	0.00	323.80	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Subject No.	Wheeze number per breathing cycle						Frequency of wheezes (Hz)					
	AL	LL	PL	AR	LR	PR	AL	LL	PL	AR	LR	PR
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	0.00	0.30	0.10	0.00	0.13	0.08	0.00	377.60	556.64	0.00	419.92	517.58
20	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21	0.60	0.00	0.00	1.50	1.50	0.25	343.42	0.00	0.00	209.42	143.00	126.95
22	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	234.38
23	0.00	0.00	0.16	0.00	0.16	1.16	0.00	0.00	190.43	0.00	190.43	347.38
24	0.00	0.33	2.00	0.00	0.00	0.00	0.00	312.50	545.25	0.00	0.00	0.00
25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 6

Subject No.	Wheeze number per breathing cycle						Frequency of wheezes (Hz)					
	AL	LL	PL	AR	LR	PR	AL	LL	PL	AR	LR	PR
26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

AL = anterior left region of chest wall, AR = anterior right region of chest wall, LL =lateral left region of chest wall, LR= lateral right region of chest wall, PL= posterior left region of chest wall, PR= posterior right region of chest wall

Appendix 7: Examples of Analysis of variance for pulmonary function tests among three groups

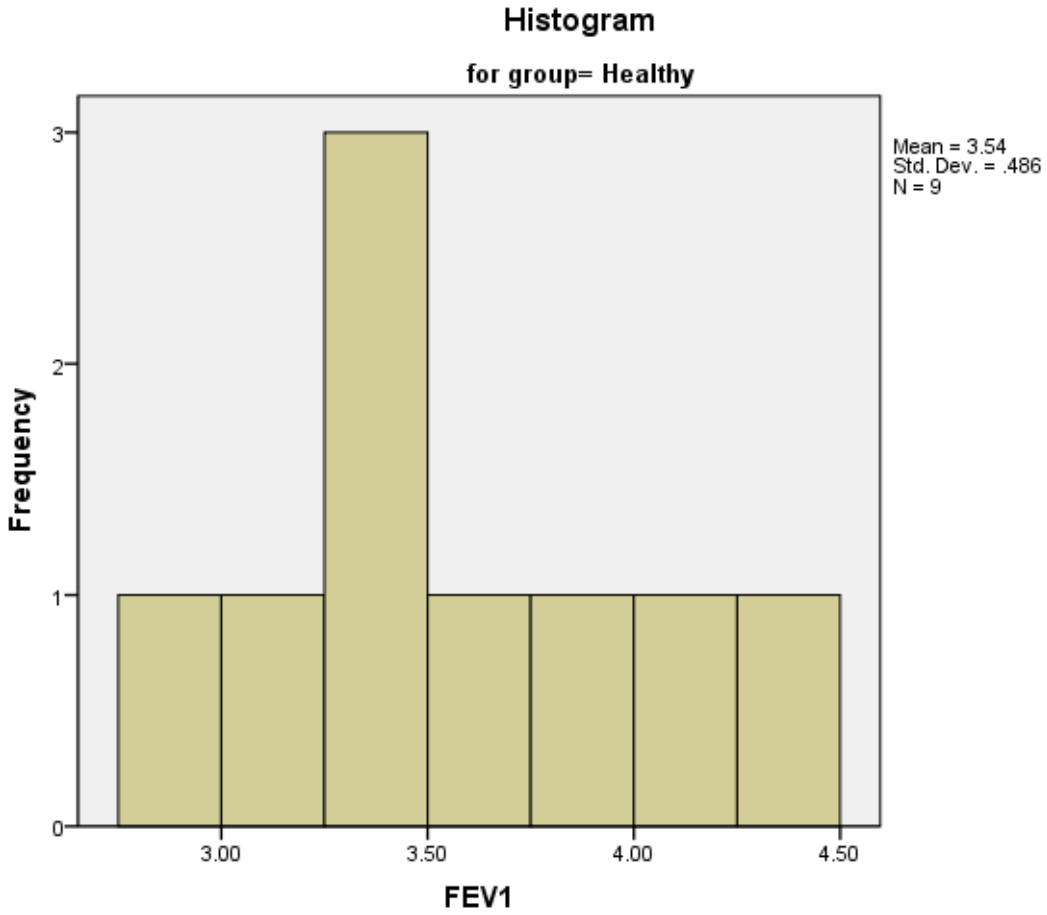
Tests of Normality

	1= healthy, 2=smoker, 3=COPD	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
FEV1	Healthy	.141	9	.200*	.972	9	.914
	Smoker	.188	9	.200*	.906	9	.289
	COPD	.143	8	.200*	.926	8	.479
FVCPC	Healthy	.189	9	.200*	.934	9	.523
	Smoker	.235	9	.166	.862	9	.101
	COPD	.143	8	.200*	.978	8	.955
FEV1PC	Healthy	.194	9	.200*	.923	9	.414
	Smoker	.234	9	.170	.881	9	.159
	COPD	.190	8	.200*	.910	8	.357

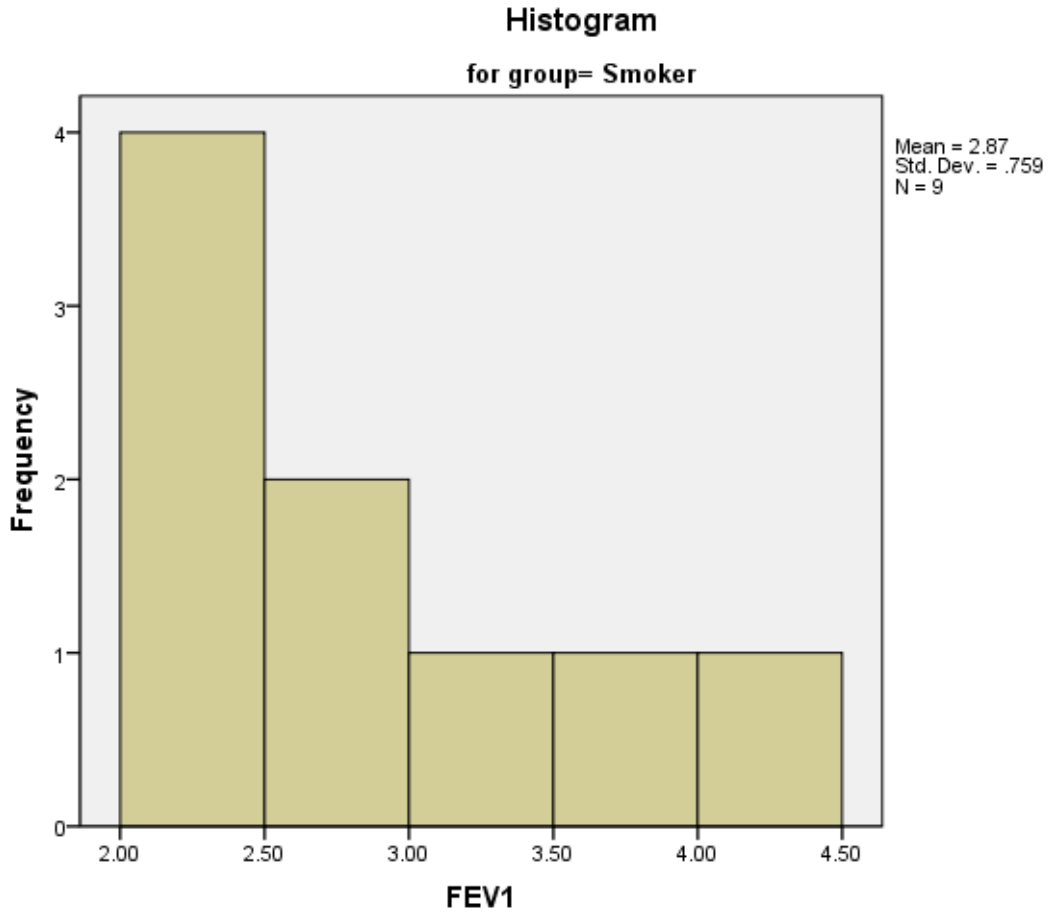
FEV1FVC	Healthy	.129	9	.200*	.968	9	.877
	Smoker	.133	9	.200*	.962	9	.816
	COPD	.194	8	.200*	.876	8	.171
PEF	Healthy	.148	9	.200*	.956	9	.760
	Smoker	.130	9	.200*	.959	9	.788
	COPD	.184	8	.200*	.901	8	.297

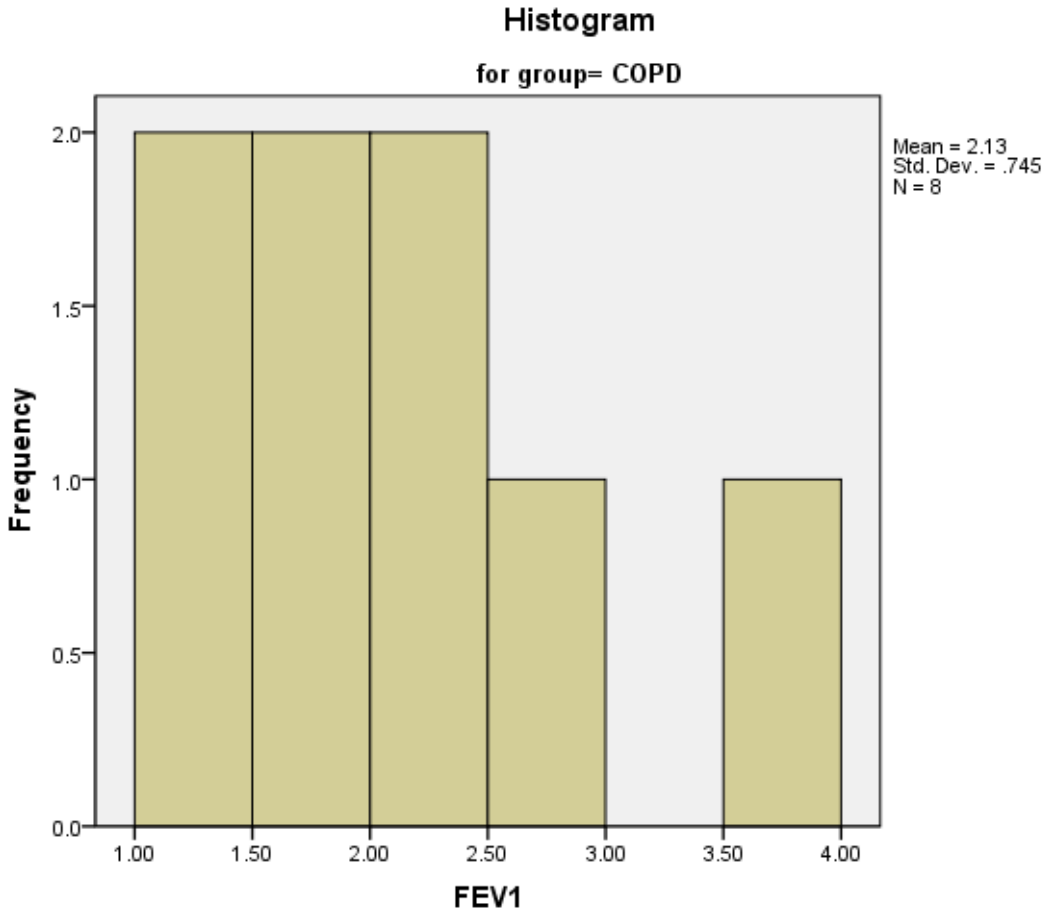
a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.


Histogram
for group= Healthy
Mean = 3.54
Std. Dev. = .486
N = 9
Frequency
0
1
2
3
3.00
3.50
4.00
4.50
FEV1

Appendix 7


Histogram
for group= Smoker
Mean = 2.87
Std. Dev. = .759
N = 9
Frequency
0
1
2
3
4
2.00
2.50
3.00
3.50
4.00
4.50
FEV1

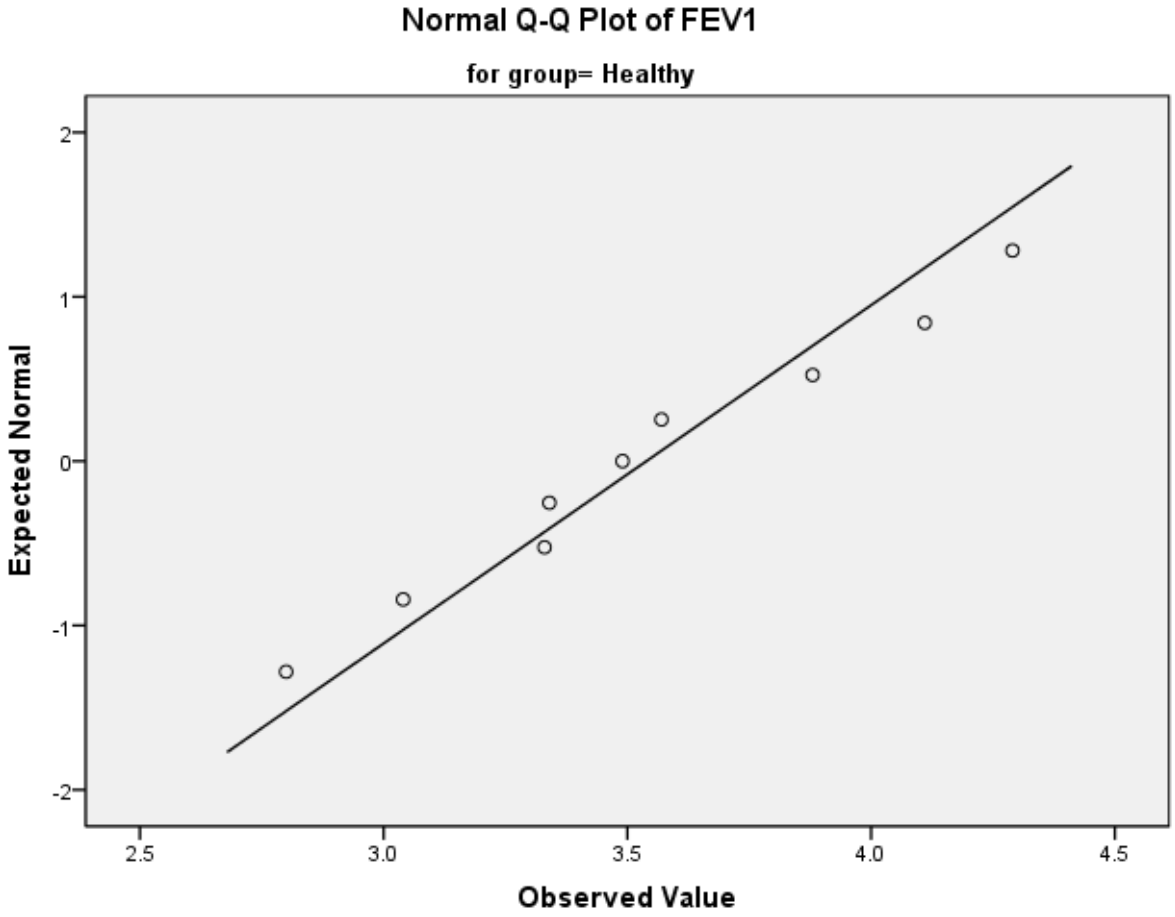

Histogram
for group= COPD
Mean = 2.13
Std. Dev. = .745
N = 8
Frequency
2.0
1.5
1.0
0.5
0.0
1.00
1.50
2.00
2.50
3.00
3.50
4.00
FEV1

Stem and leaf plots

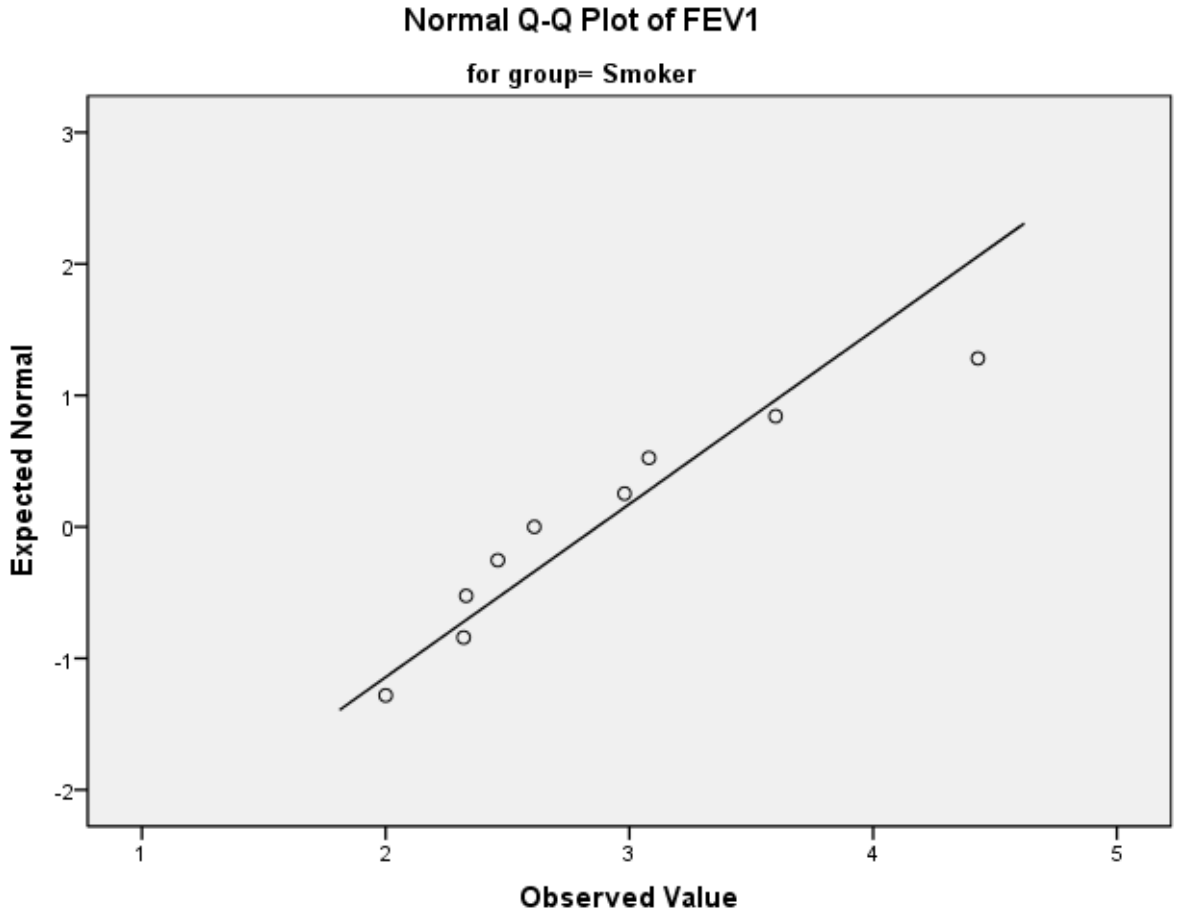
```
FEV1 Stem-and-Leaf Plot for
group= Healthy

Frequency    Stem &  Leaf

1.00         2 .  8
4.00         3 .  0334
2.00         3 .  58
2.00         4 .  12

Stem width:      1.00
Each leaf:       1 case(s)

FEV1 Stem-and-Leaf Plot for
```

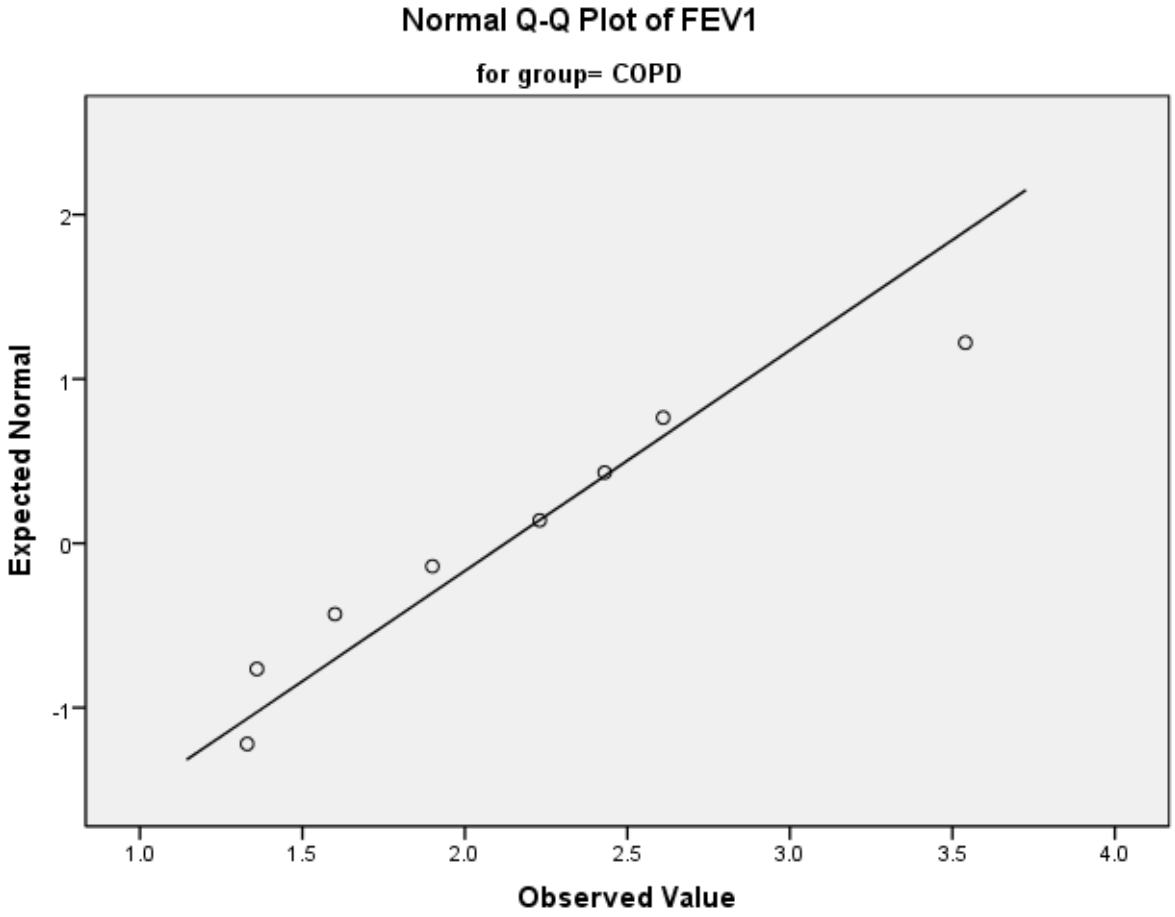
```
group= Smoker

Frequency    Stem &  Leaf

4.00        2 .  0334
2.00        2 .  69
1.00        3 .  0
1.00        3 .  6
1.00 Extremes    (>=4.4)

Stem width:      1.00
Each leaf:       1 case(s)


FEV1 Stem-and-Leaf Plot for
group= COPD
Frequency    Stem &  Leaf
```

```
4.00        1 .  3369

3.00        2 .  246

1.00        3 .  5


Stem width:      1.00

Each leaf:       1 case(s)
```

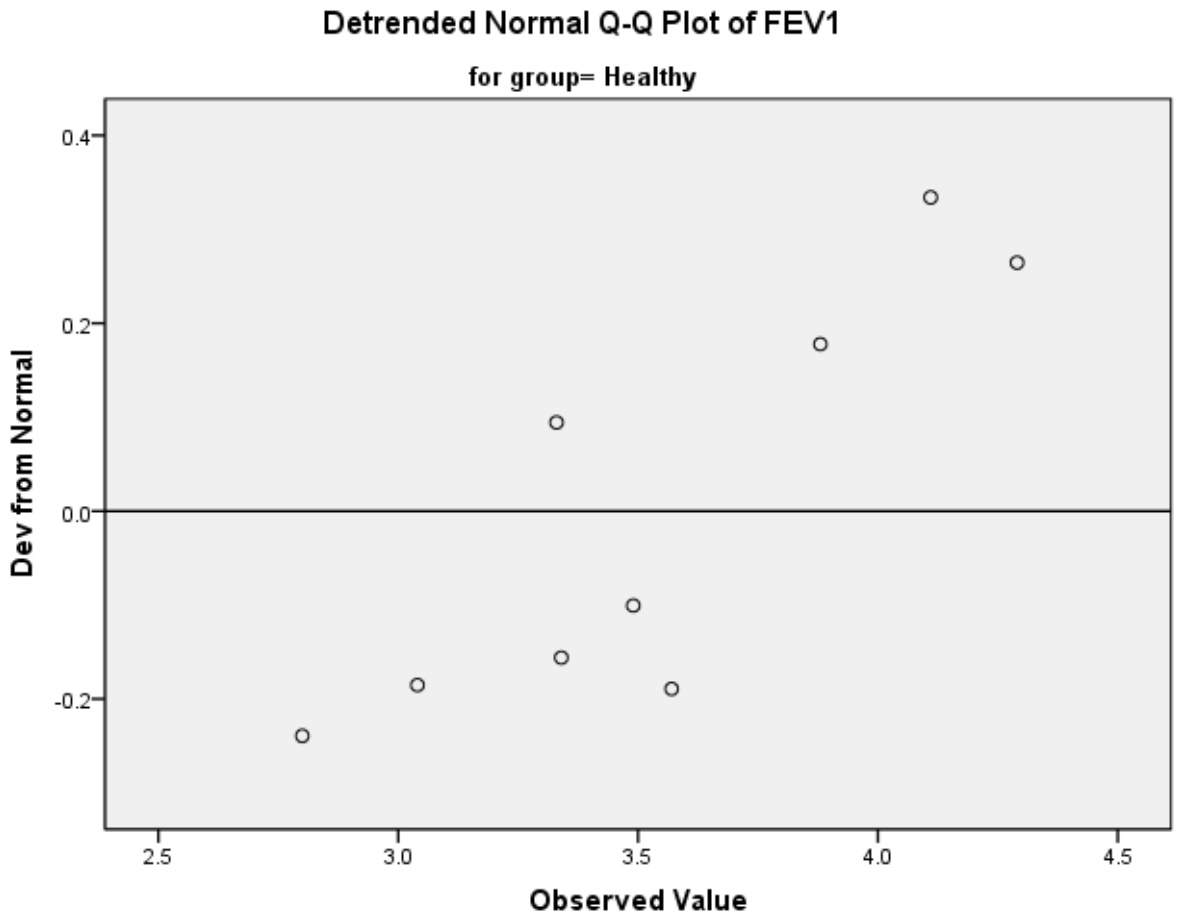

Normal Q-Q Plot of FEV1
for group= Healthy
Expected Normal
2
1
0
-1
-2
2.5
3.0
3.5
4.0
4.5
Observed Value

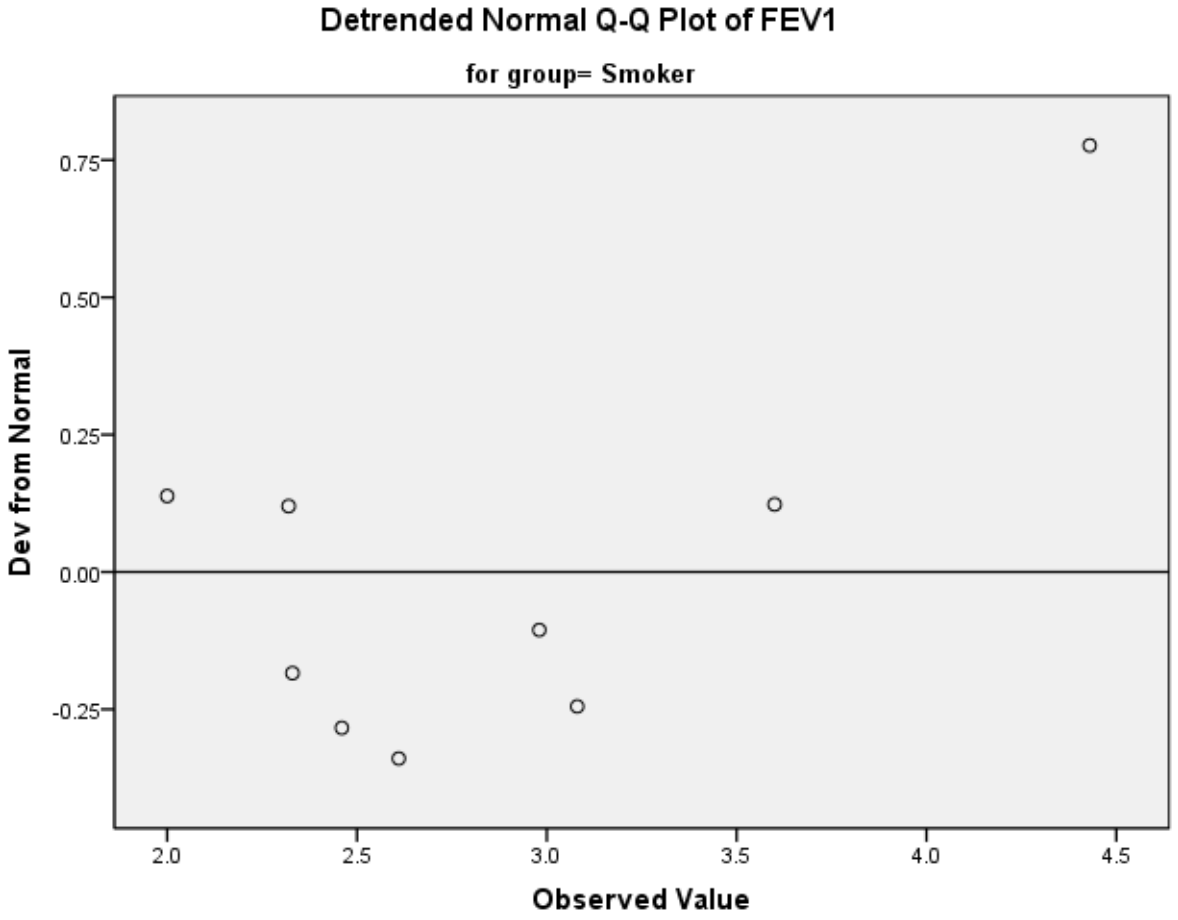
Appendix 7


Normal Q-Q Plot of FEV1
for group= Smoker
Expected Normal
Observed Value
3
2
1
0
-1
-2
1
2
3
4
5

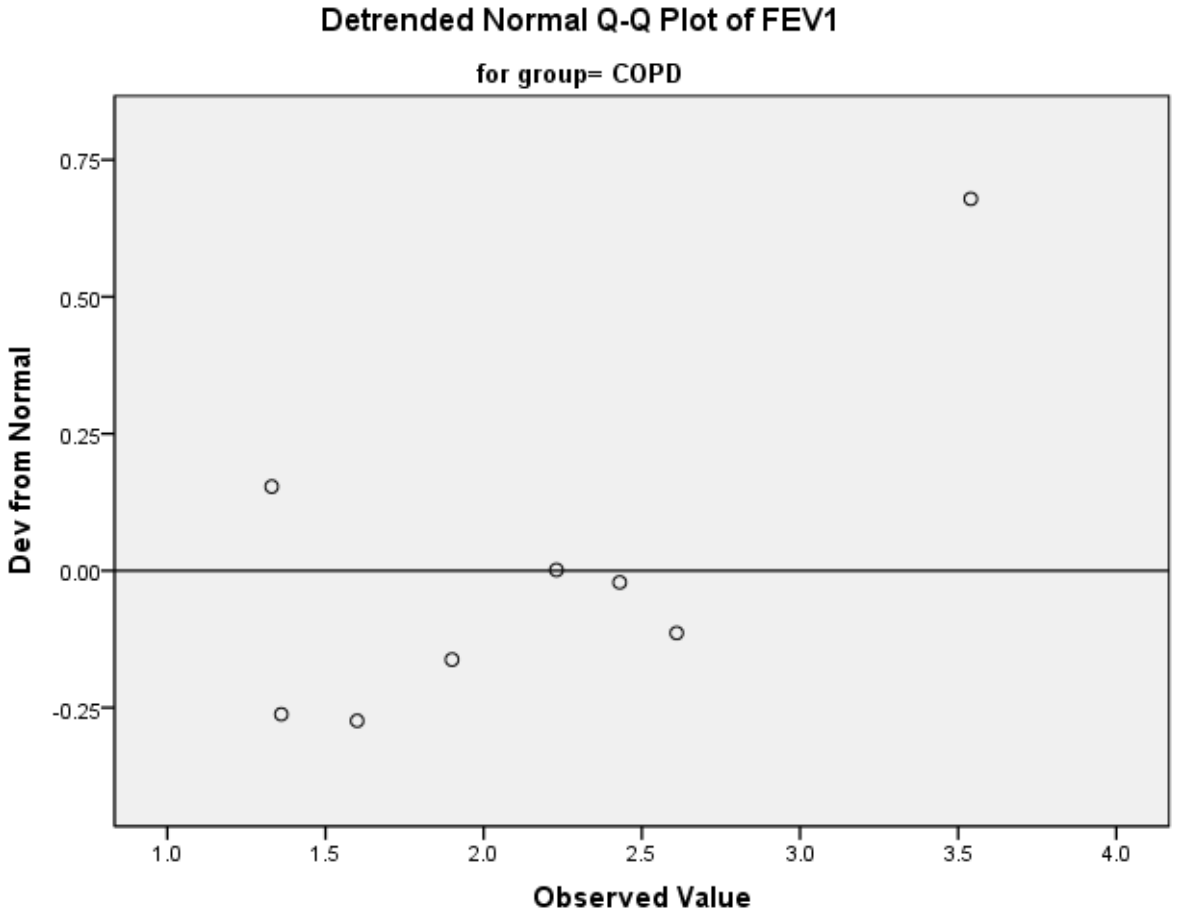

Normal Q-Q Plot of FEV1
for group= COPD
Expected Normal
2
1
0
-1
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Observed Value

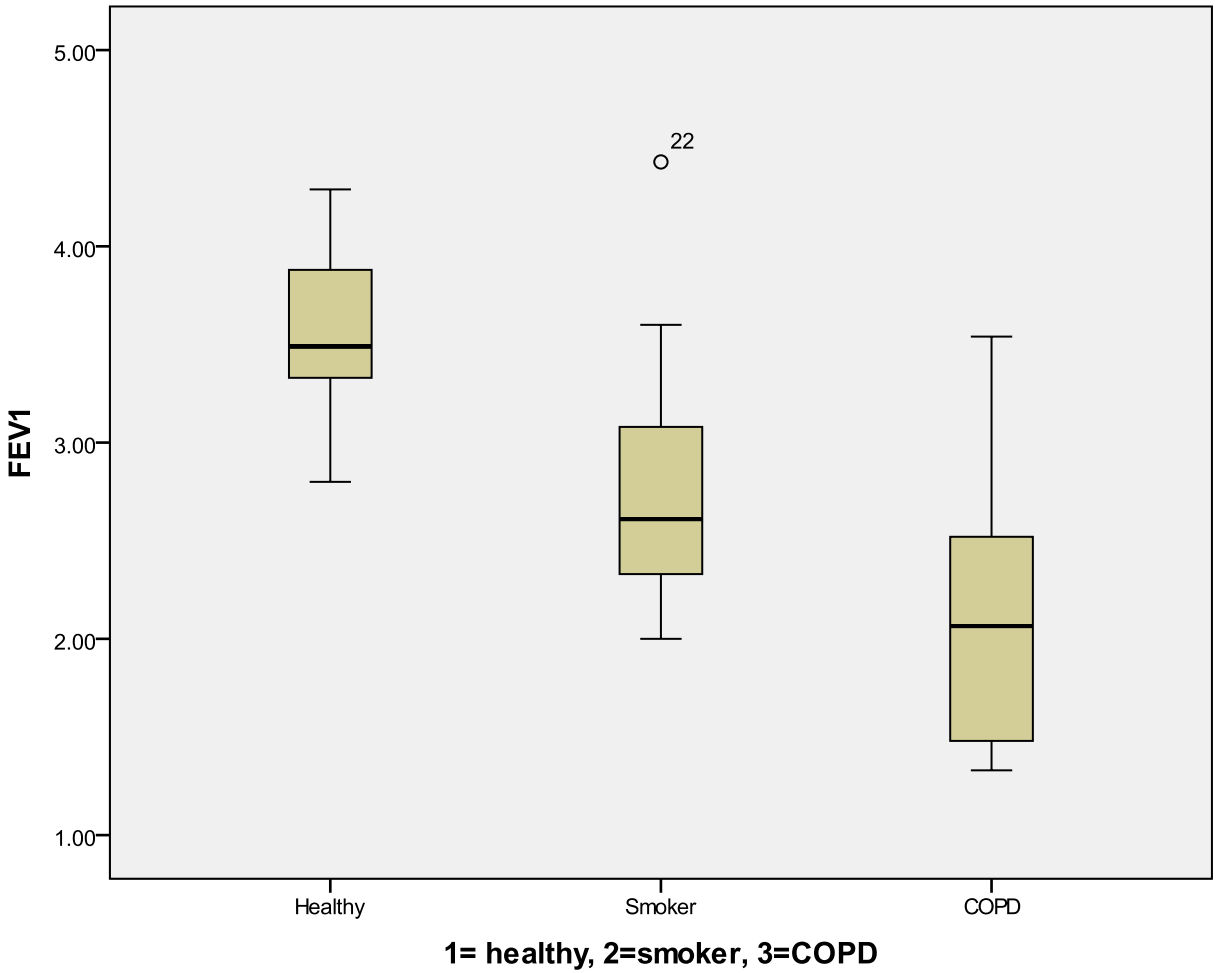
Appendix 7


Detrended Normal Q-Q Plot of FEV1
for group= Healthy
Dev from Normal
0.4
0.2
0.0
-0.2
2.5
3.0
3.5
4.0
4.5
Observed Value


Detrended Normal Q-Q Plot of FEV1
for group= Smoker
Dev from Normal
0.75
0.50
0.25
0.00
-0.25
2.0
2.5
3.0
3.5
4.0
4.5
Observed Value

Appendix 7


Detrended Normal Q-Q Plot of FEV1
for group= COPD
Dev from Normal
0.75
0.50
0.25
0.00
-0.25
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Observed Value


FEV1
5.00
4.00
3.00
2.00
1.00
22
Healthy
Smoker
COPD
1= healthy, 2=smoker, 3=COPD

Appendix 7

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
FEV1	Healthy	9	3.5389	.48581	.16194	3.1655	3.9123	2.80	4.29
	Smoker	9	2.8678	.75906	.25302	2.2843	3.4512	2.00	4.43
	COPD	8	2.1250	.74498	.26339	1.5022	2.7478	1.33	3.54
	Total	26	2.8715	.86832	.17029	2.5208	3.2223	1.33	4.43
FVCPC	Healthy	9	113.8444	15.37962	5.12654	102.0226	125.6663	93.10	136.80
	Smoker	9	117.9333	13.64341	4.54780	107.4461	128.4206	104.70	146.30
	COPD	8	98.7875	15.51934	5.48691	85.8130	111.7620	77.50	125.80
	Total	26	110.6269	16.44670	3.22546	103.9840	117.2699	77.50	146.30

FEV1PC	Healthy	9	107.9000	11.08659	3.69553	99.3781	116.4219	92.50	123.80
	Smoker	9	102.4400	11.77166	3.92389	93.3915	111.4885	87.80	116.90
	COPD	8	71.1250	12.53905	4.43322	60.6421	81.6079	57.80	94.60
	Total	26	94.6946	19.74441	3.87220	86.7197	102.6696	57.80	123.80
FEV1FVC	Healthy	9	80.2400	3.39047	1.13016	77.6339	82.8461	74.25	84.84
	Smoker	9	73.2100	5.55838	1.85279	68.9375	77.4825	66.10	83.02
	COPD	8	59.8888	7.27829	2.57326	53.8040	65.9735	46.09	66.67
	Total	26	71.5446	10.00453	1.96205	67.5037	75.5855	46.09	84.84
PEF	Healthy	9	99.1667	21.04424	7.01475	82.9906	115.3427	57.10	131.60
	Smoker	9	107.1778	18.06002	6.02001	93.2956	121.0599	84.00	139.90
	COPD	8	68.2375	13.62403	4.81682	56.8475	79.6275	52.80	96.10
	Total	26	92.4231	24.08288	4.72304	82.6958	102.1504	52.80	139.90

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
FEV1	.826	2	23	.451
FVCPC	.104	2	23	.902
FEV1PC	.130	2	23	.879
FEV1FVC	1.955	2	23	.164
PEF	.495	2	23	.616

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
FEV1	Between Groups	8.467	2	4.233	9.378	.001
	Within Groups	10.382	23	.451		
	Total	18.849	25			
FVCPC	Between Groups	1695.000	2	847.500	3.847	.036
	Within Groups	5067.351	23	220.320		
	Total	6762.351	25			
FEV1PC	Between Groups	6553.573	2	3276.786	23.607	.000
	Within Groups	3192.470	23	138.803		
	Total	9746.043	25			
FEV1FVC	Between Groups	1792.323	2	896.161	29.033	.000
	Within Groups	709.941	23	30.867		
	Total	2502.263	25			

Appendix 7

PEF	Between Groups	7048.132	2	3524.066	10.877	.000
	Within Groups	7451.494	23	323.978		
	Total	14499.626	25			

Multiple comparisons

Dependent Variable		(I) 1= healthy, 2=smoker, 3=COPD	(J) 1= healthy, 2=smoker, 3=COPD	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
FEV1	Bonferroni	Healthy	Smoker	.67111	.31672	.135	-.1467	1.4889
			COPD	1.41389*	.32647	.001	.5709	2.2568
		Smoker	Healthy	-.67111	.31672	.135	-1.4889	.1467
			COPD	.74278	.32647	.098	-.1002	1.5857
		COPD	Healthy	-1.41389*	.32647	.001	-2.2568	-.5709
			Smoker	-.74278	.32647	.098	-1.5857	.1002
	Games-Howell	Healthy	Smoker	.67111	.30040	.101	-.1177	1.4599
			COPD	1.41389*	.30919	.002	.5873	2.2405
		Smoker	Healthy	-.67111	.30040	.101	-1.4599	.1177
			COPD	.74278	.36523	.139	-.2071	1.6926

COPD	Healthy	-1.41389*	.30919	.002	-2.2405	-.5873
	Smoker	-.74278	.36523	.139	-1.6926	.2071

Multiple comparisons

FVCPC	Bonferroni	Healthy	Smoker	-4.08889	6.99714	1.000	-22.1556	13.9778
			COPD	15.05694	7.21248	.144	-3.5658	33.6797
		Smoker	Healthy	4.08889	6.99714	1.000	-13.9778	22.1556
			COPD	19.14583*	7.21248	.042	.5231	37.7686
		COPD	Healthy	-15.05694	7.21248	.144	-33.6797	3.5658
			Smoker	-19.14583*	7.21248	.042	-37.7686	-.5231
	Games-Howell	Healthy	Smoker	-4.08889	6.85302	.824	-21.7969	13.6191
			COPD	15.05694	7.50917	.146	-4.4856	34.5995
		Smoker	Healthy	4.08889	6.85302	.824	-13.6191	21.7969
			COPD	19.14583*	7.12662	.044	.5084	37.7832
		COPD	Healthy	-15.05694	7.50917	.146	-34.5995	4.4856
			Smoker	-19.14583*	7.12662	.044	-37.7832	-.5084

Multiple comparisons

FEV1PC	Bonferroni	Healthy	Smoker	5.46000	5.55384	1.000	-8.8801	19.8001
			COPD	36.77500*	5.72477	.000	21.9936	51.5564
		Smoker	Healthy	-5.46000	5.55384	1.000	-19.8001	8.8801
			COPD	31.31500*	5.72477	.000	16.5336	46.0964
		COPD	Healthy	-36.77500*	5.72477	.000	-51.5564	-21.9936
			Smoker	-31.31500*	5.72477	.000	-46.0964	-16.5336
	Games-Howell	Healthy	Smoker	5.46000	5.39016	.580	-8.4533	19.3733
			COPD	36.77500*	5.77152	.000	21.6859	51.8641
		Smoker	Healthy	-5.46000	5.39016	.580	-19.3733	8.4533
			COPD	31.31500*	5.92033	.000	15.8789	46.7511
		COPD	Healthy	-36.77500*	5.77152	.000	-51.8641	-21.6859
			Smoker	-31.31500*	5.92033	.000	-46.7511	-15.8789

Multiple comparisons

FEV1FVC	Bonferroni	Healthy	Smoker	7.03000*	2.61903	.040	.2676	13.7924
			COPD	20.35125*	2.69964	.000	13.3807	27.3218
		Smoker	Healthy	-7.03000*	2.61903	.040	-13.7924	-.2676
			COPD	13.32125*	2.69964	.000	6.3507	20.2918
		COPD	Healthy	-20.35125*	2.69964	.000	-27.3218	-13.3807
			Smoker	-13.32125*	2.69964	.000	-20.2918	-6.3507
	Games-Howell	Healthy	Smoker	7.03000*	2.17028	.016	1.3118	12.7482
			COPD	20.35125*	2.81050	.000	12.6003	28.1022
		Smoker	Healthy	-7.03000*	2.17028	.016	-12.7482	-1.3118
			COPD	13.32125*	3.17089	.003	4.9540	21.6885
		COPD	Healthy	-20.35125*	2.81050	.000	-28.1022	-12.6003
			Smoker	-13.32125*	3.17089	.003	-21.6885	-4.9540

Multiple comparisons

PEF	Bonferroni	Healthy	Smoker	-8.01111	8.48499	1.000	-29.9195	13.8973
			COPD	30.92917*	8.74613	.005	8.3465	53.5118
		Smoker	Healthy	8.01111	8.48499	1.000	-13.8973	29.9195
			COPD	38.94028*	8.74613	.001	16.3576	61.5229
		COPD	Healthy	-30.92917*	8.74613	.005	-53.5118	-8.3465
			Smoker	-38.94028*	8.74613	.001	-61.5229	-16.3576

Games-Howell	Healthy	Smoker	-8.01111	9.24376	.668	-31.9176	15.8954
		COPD	30.92917*	8.50931	.007	8.6234	53.2349
	Smoker	Healthy	8.01111	9.24376	.668	-15.8954	31.9176
		COPD	38.94028*	7.70988	.000	18.8642	59.0163
	COPD	Healthy	-30.92917*	8.50931	.007	-53.2349	-8.6234
		Smoker	-38.94028*	7.70988	.000	-59.0163	-18.8642

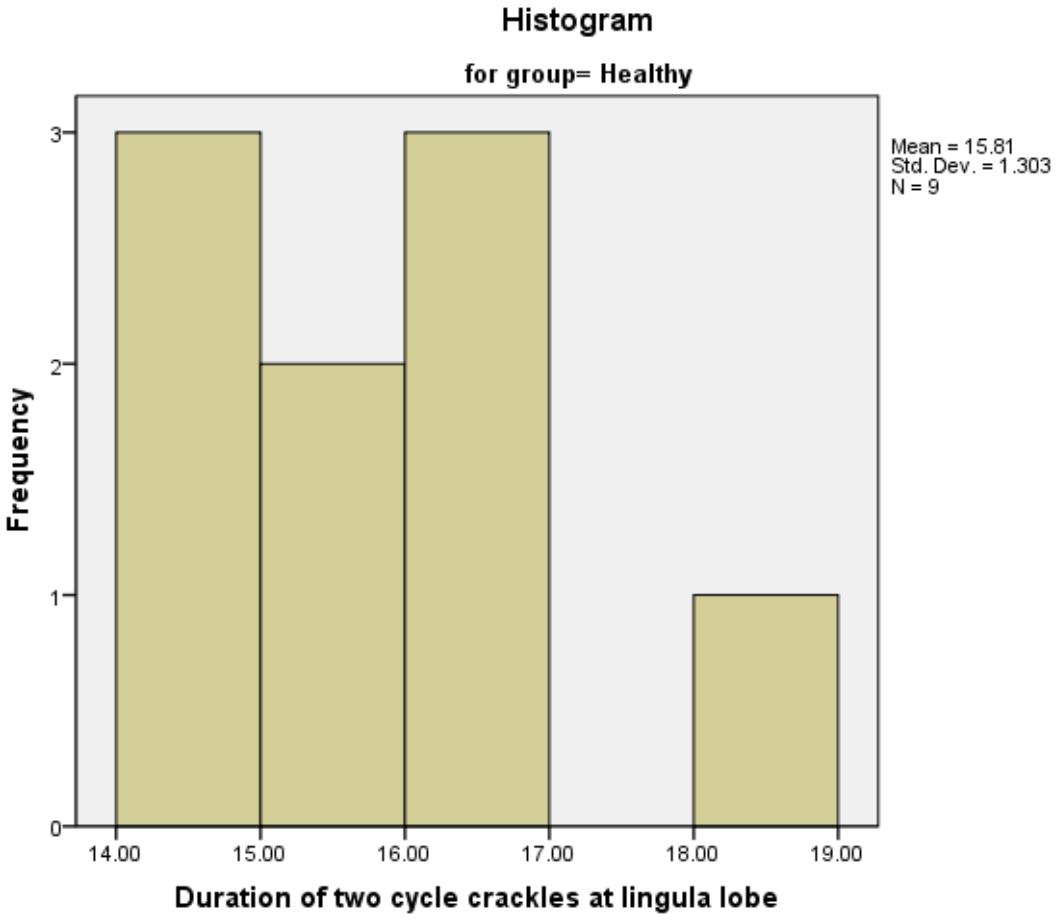
Appendix 8: An example of Analysis of variance for crackles 2 CD at lateral left region of the chest wall or lingula lobe among three groups

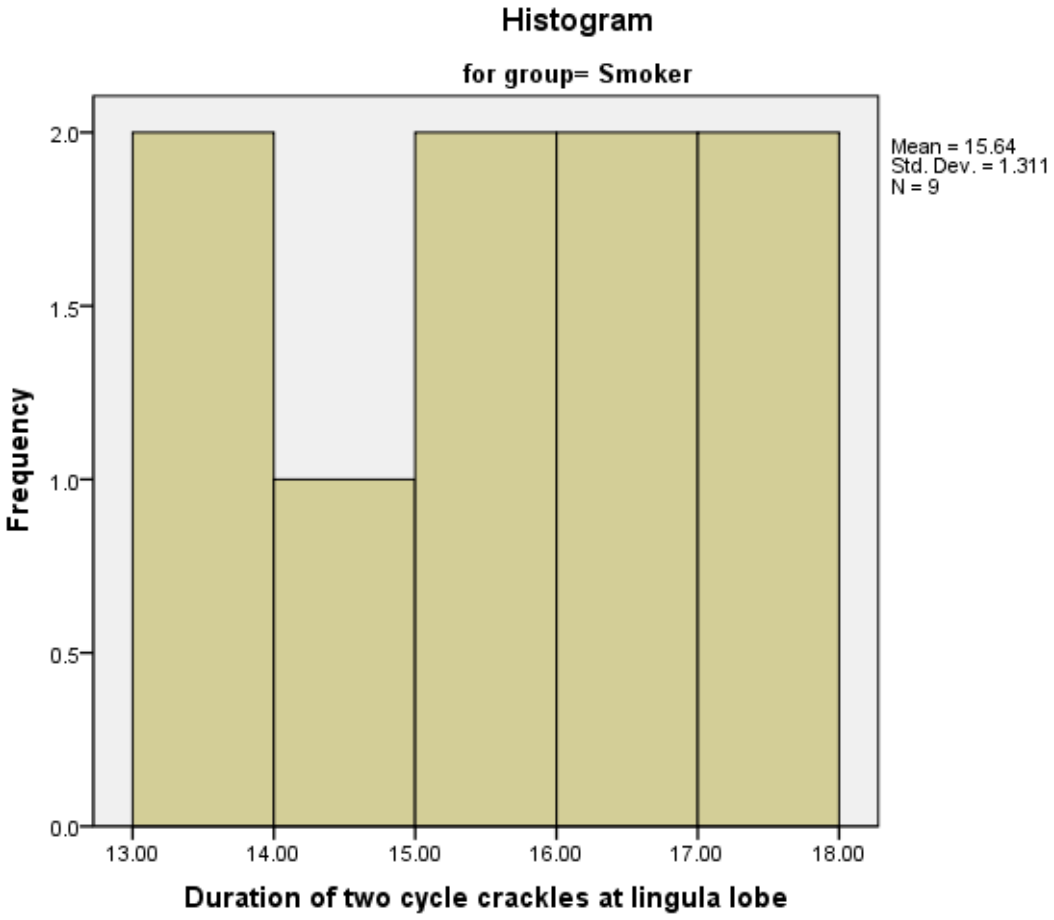
Tests of Normality

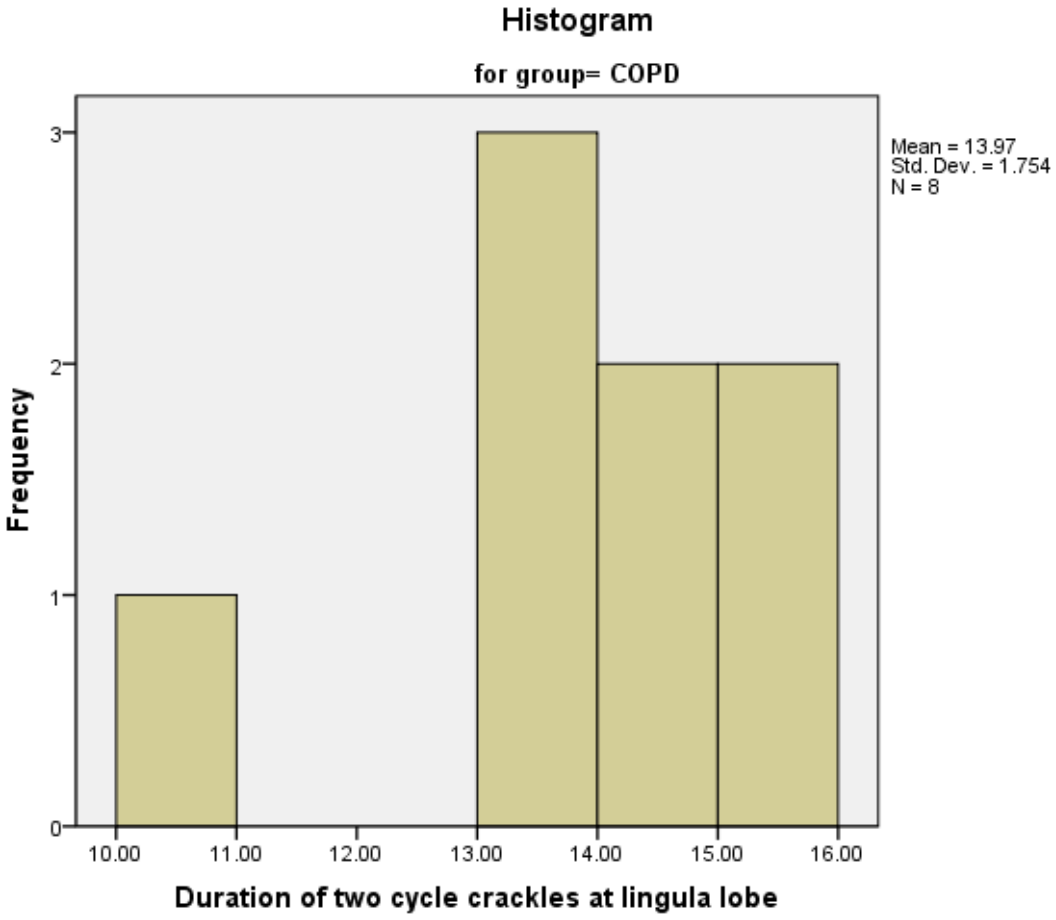
	1= healthy, 2=smoker, 3=COPD	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Duration of two cycle crackles at lingula lobe	Healthy	.150	9	.200*	.928	9	.460
	Smoker	.151	9	.200*	.929	9	.472
	COPD	.185	8	.200*	.922	8	.446

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.


Histogram
for group= Healthy
Mean = 15.81
Std. Dev. = 1.303
N = 9
Frequency
0
1
2
3
14.00
15.00
16.00
17.00
18.00
19.00
Duration of two cycle crackles at lingula lobe


Histogram
for group= Smoker
Mean = 15.64
Std. Dev. = 1.311
N = 9
Frequency
2.0
1.5
1.0
0.5
0.0
13.00
14.00
15.00
16.00
17.00
18.00
Duration of two cycle crackles at lingula lobe


Histogram
for group= COPD
Mean = 13.97
Std. Dev. = 1.754
N = 8
Frequency
0
1
2
3
10.00
11.00
12.00
13.00
14.00
15.00
16.00
Duration of two cycle crackles at lingula lobe

Duration of two cycle crackles at lingula lobe Stem-and-Leaf Plot for group= Healthy

Frequency	Stem &	Leaf
2.00	14 .	22
1.00	14 .	9
1.00	15 .	4
1.00	15 .	9
2.00	16 .	02
1.00	16 .	6
1.00	Extremes	(>=18.5)

Stem width: 1.00

Each leaf: 1 case(s)

Duration of two cycle crackles at lingula lobe Stem-and-Leaf Plot for group= Smoker

Frequency	Stem &	Leaf
2.00	13 .	78
1.00	14 .	9
2.00	15 .	17
2.00	16 .	18
2.00	17 .	03

Stem width: 1.00

Each leaf: 1 case(s)

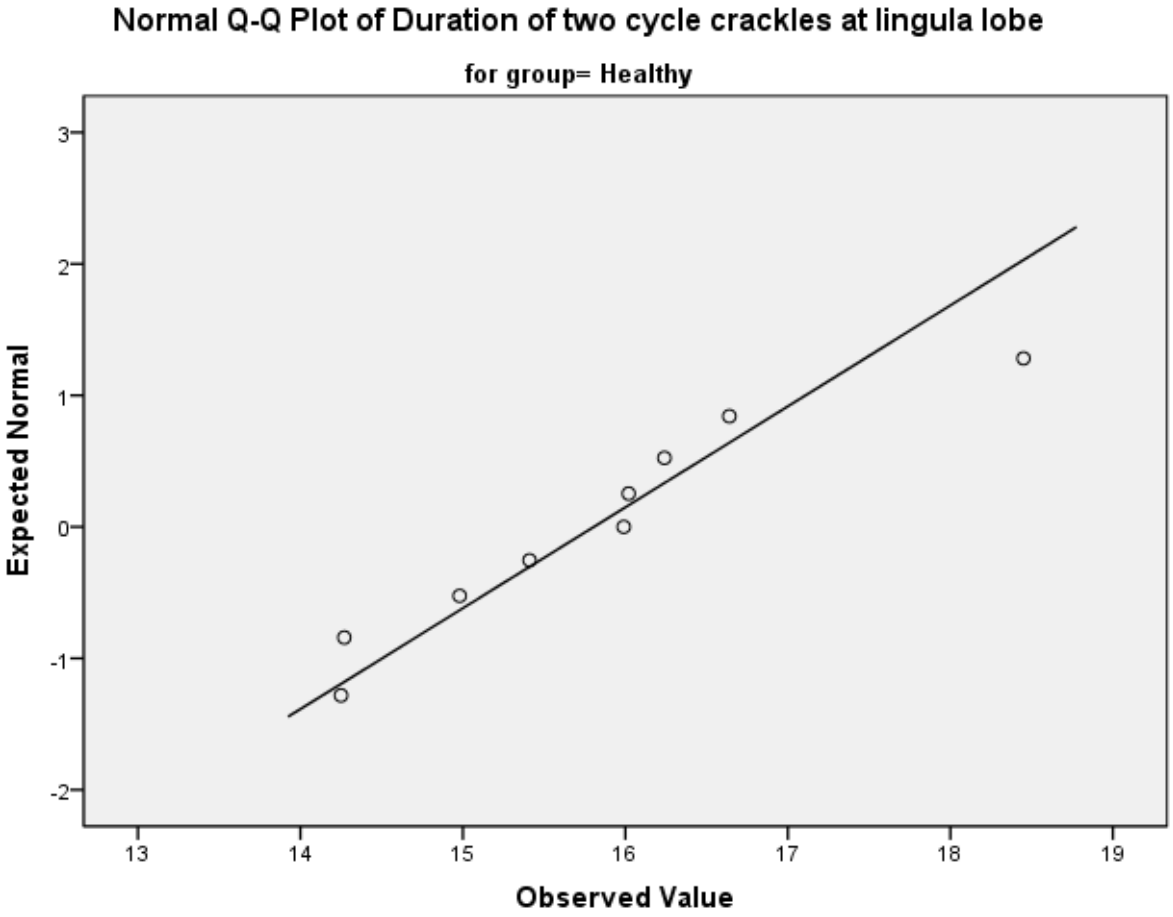
Duration of two cycle crackles at lingula lobe Stem-and-Leaf Plot for

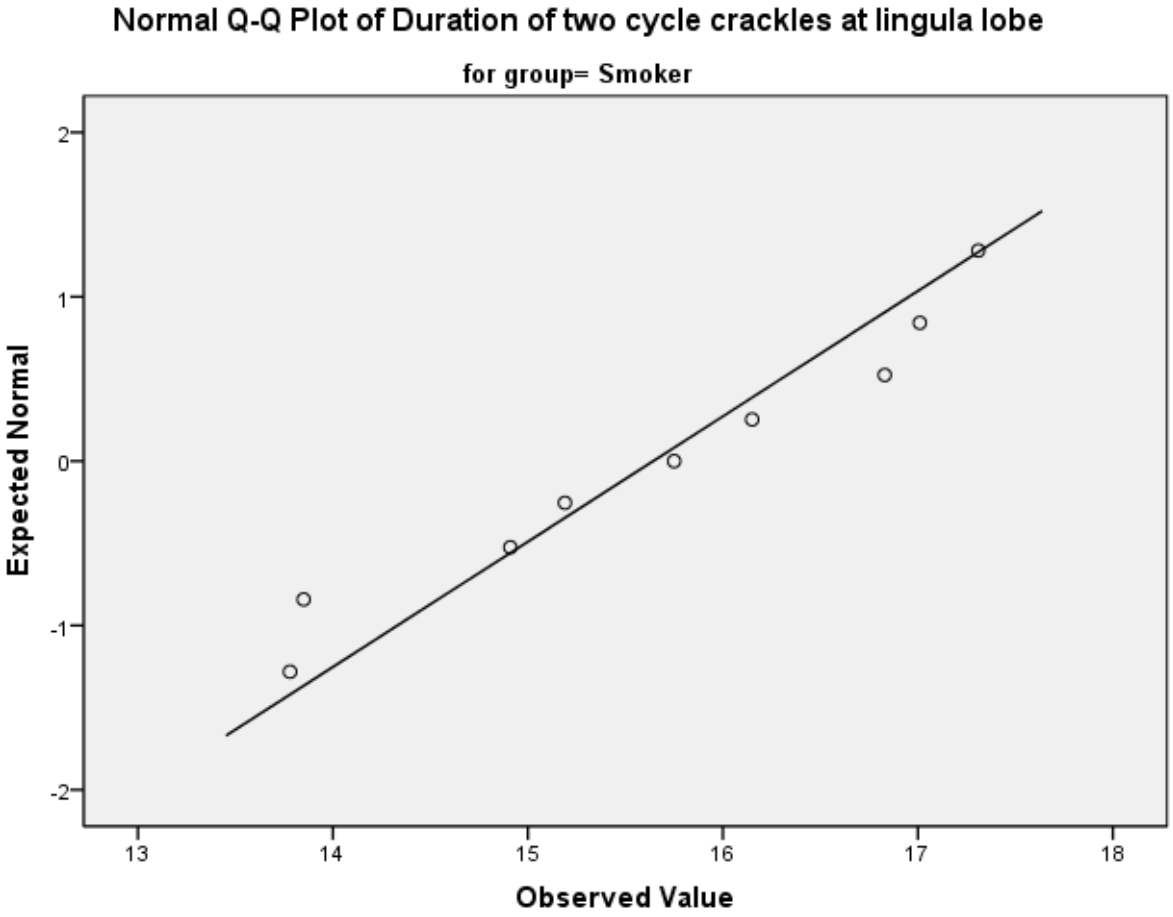
group= COPD

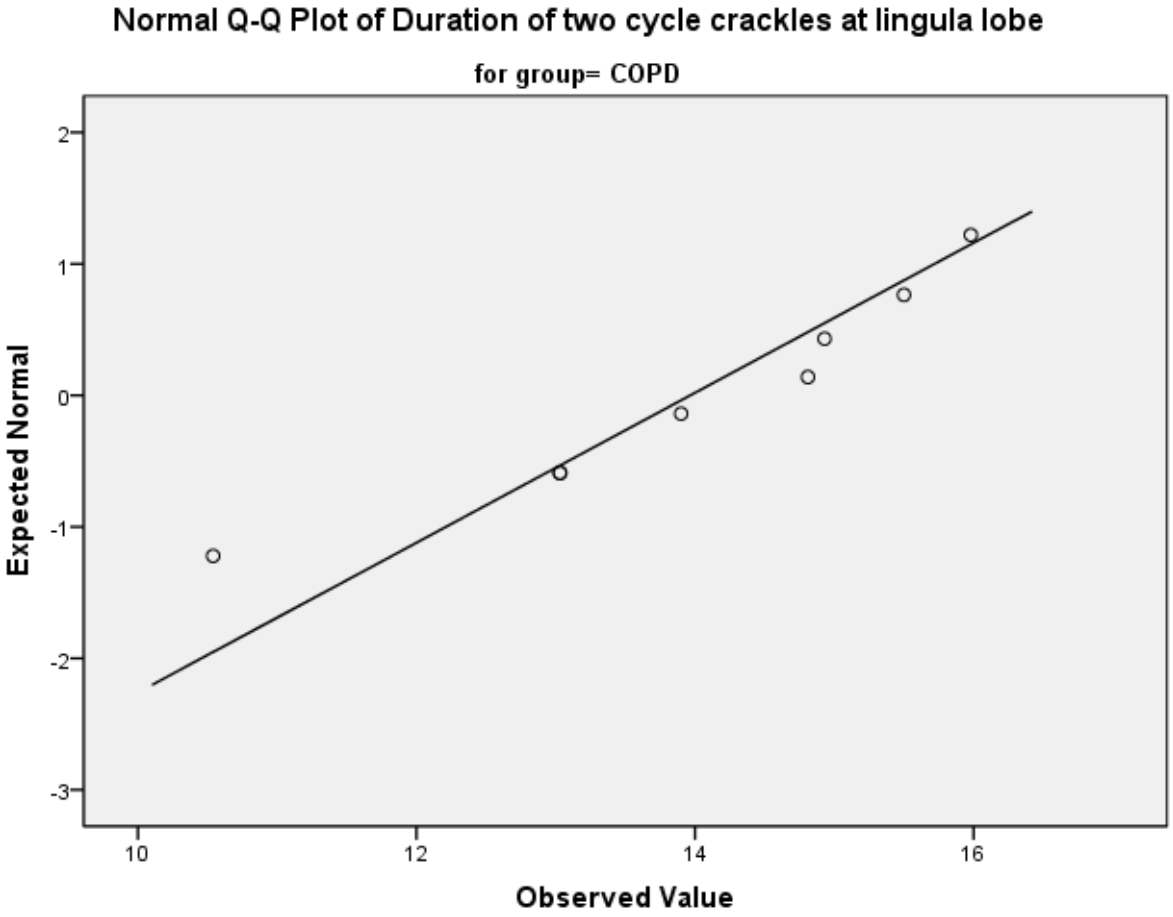
Frequency	Stem &	Leaf
6.00	1 .	033344
2.00	1 .	55

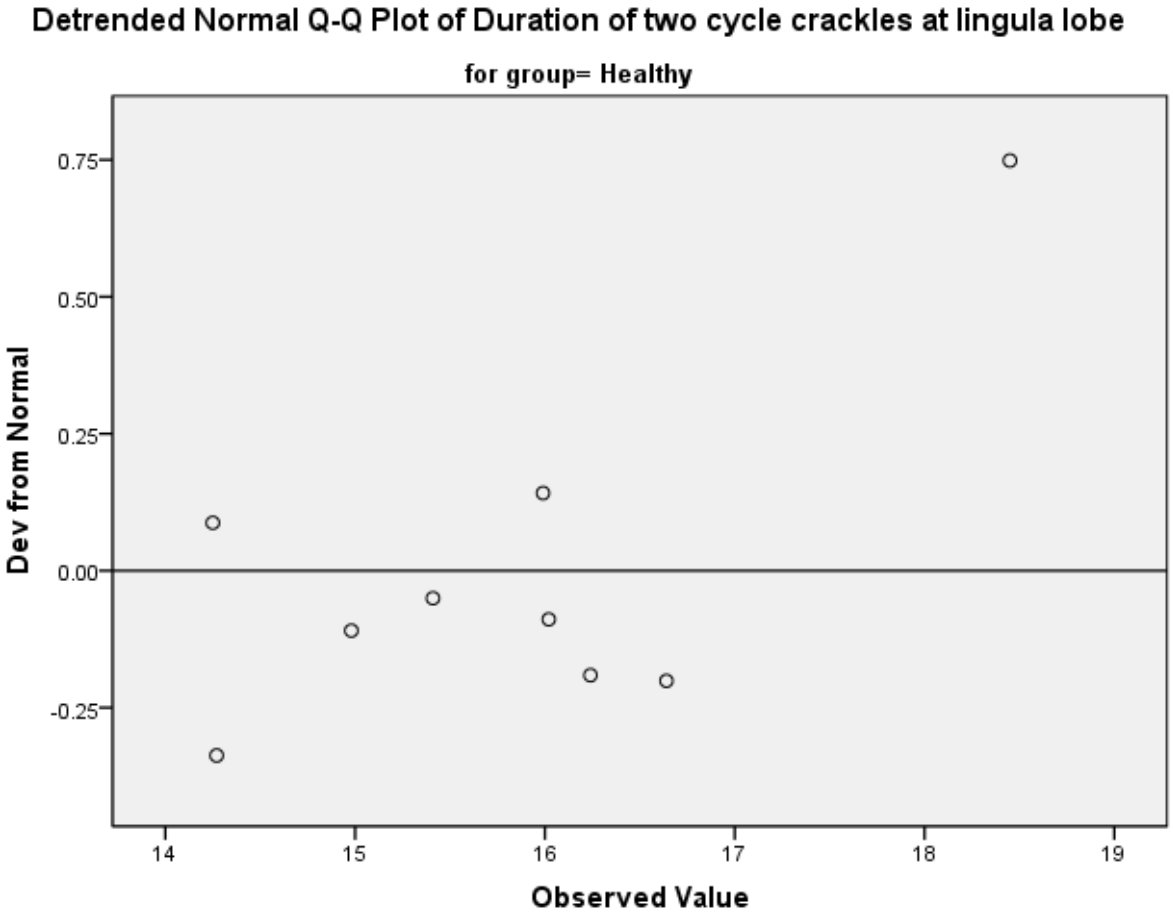
Stem width: 10.00

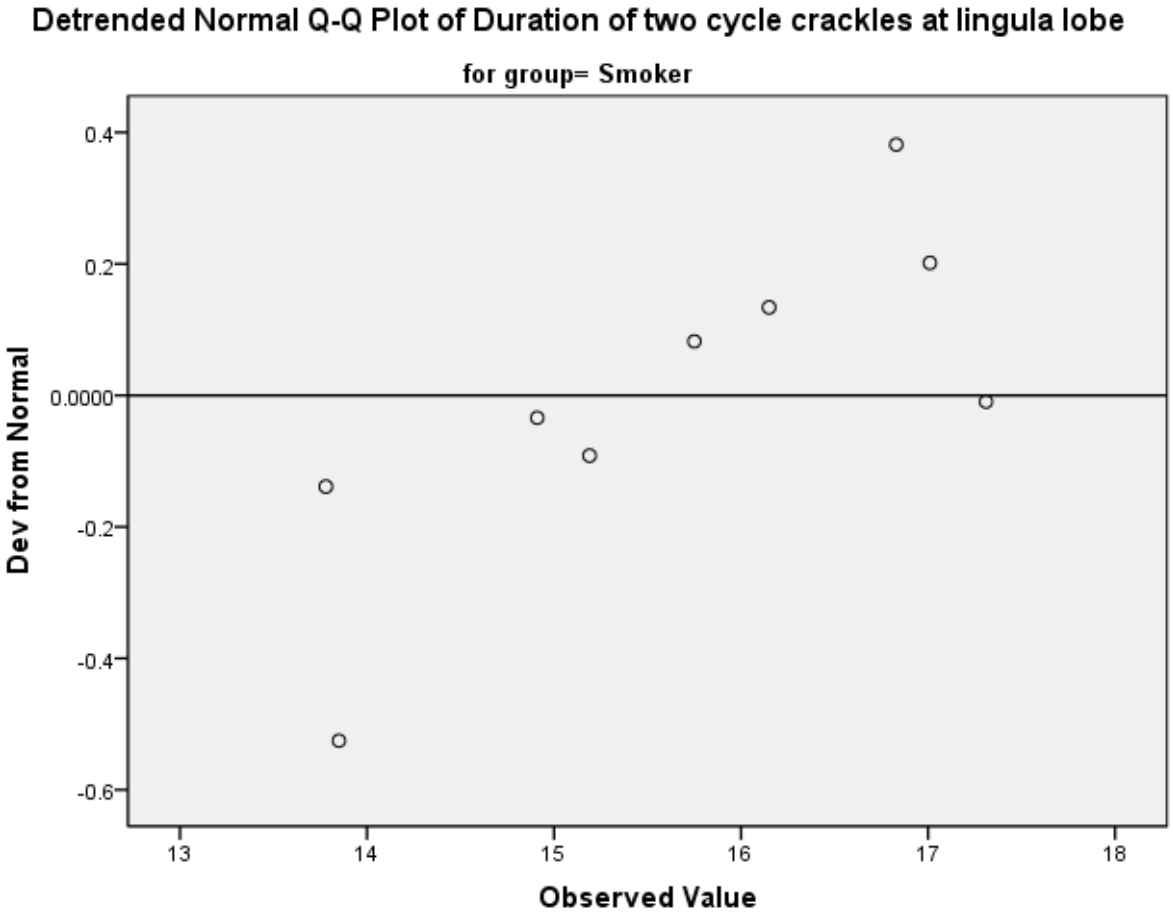
Each leaf: 1 case(s)

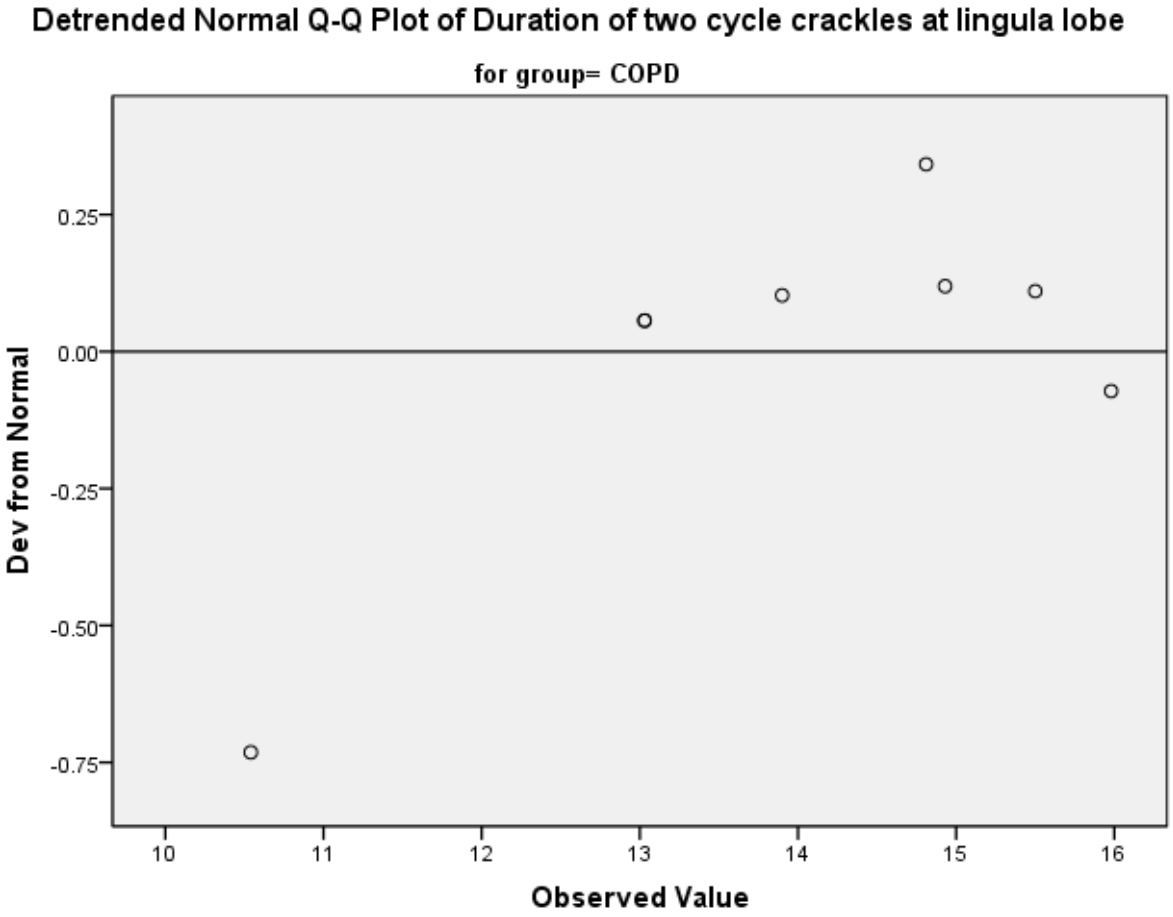

Normal Q-Q Plot of Duration of two cycle crackles at lingula lobe
for group= Healthy
Expected Normal
Observed Value
3
2
1
0
-1
-2
13
14
15
16
17
18
19

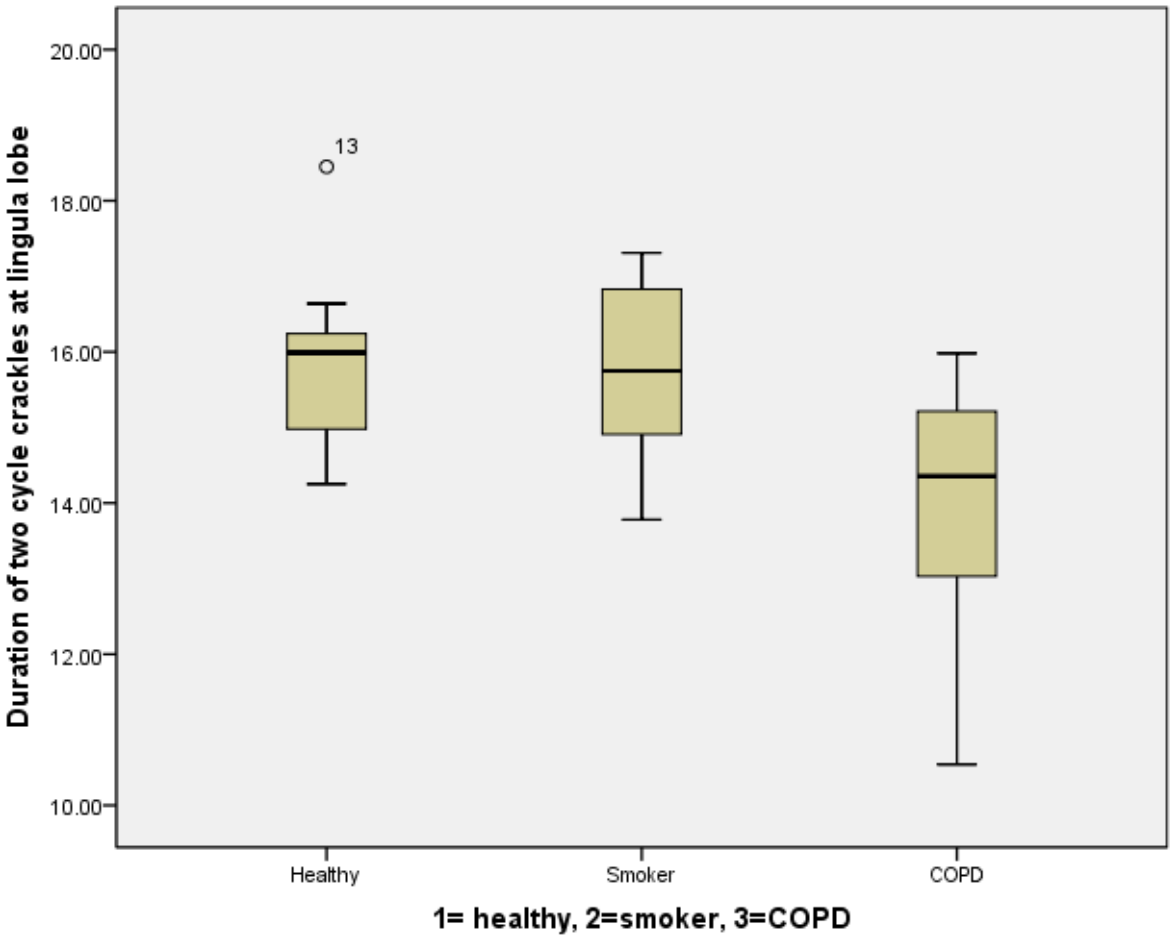

Normal Q-Q Plot of Duration of two cycle crackles at lingula lobe
for group= Smoker
Expected Normal
2
1
0
-1
-2
13
14
15
16
17
18
Observed Value

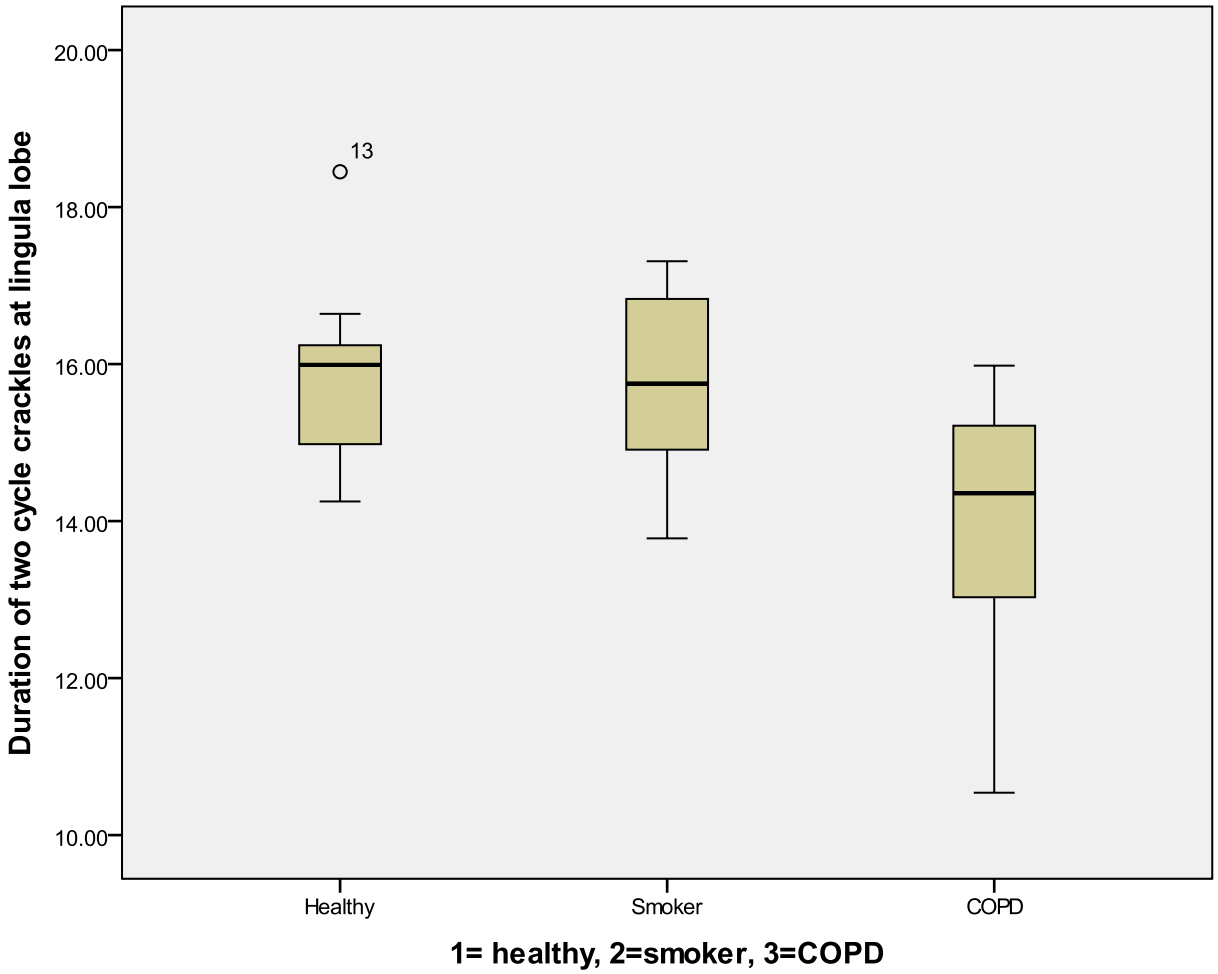

Normal Q-Q Plot of Duration of two cycle crackles at lingula lobe
for group= COPD
Expected Normal
Observed Value
2
1
0
-1
-2
-3
10
12
14
16


Detrended Normal Q-Q Plot of Duration of two cycle crackles at lingula lobe
for group= Healthy
Dev from Normal
0.75
0.50
0.25
0.00
-0.25
14
15
16
17
18
19
Observed Value


Detrended Normal Q-Q Plot of Duration of two cycle crackles at lingula lobe
for group= Smoker
Dev from Normal
0.4
0.2
0.0000
-0.2
-0.4
-0.6
13
14
15
16
17
18
Observed Value


Detrended Normal Q-Q Plot of Duration of two cycle crackles at lingula lobe
for group= COPD
Dev from Normal
0.25
0.00
-0.25
-0.50
-0.75
10
11
12
13
14
15
16
Observed Value


Duration of two cycle crackles at lingula lobe
20.00
18.00
16.00
14.00
12.00
10.00
13
Healthy
Smoker
COPD
1= healthy, 2=smoker, 3=COPD


Duration of two cycle crackles at lingula lobe
20.00
18.00
16.00
14.00
12.00
10.00
13
Healthy
Smoker
COPD
1= healthy, 2=smoker, 3=COPD

Descriptives

Duration of two cycle crackles at lingula lobe

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Healthy	9	15.8056	1.30259	.43420	14.8043	16.8068	14.25	18.45
Smoker	9	15.6422	1.31116	.43705	14.6344	16.6501	13.78	17.31
COPD	8	13.9650	1.75438	.62027	12.4983	15.4317	10.54	15.98
Total	26	15.1827	1.62636	.31896	14.5258	15.8396	10.54	18.45

Test of Homogeneity of Variances

Duration of two cycle crackles at lingula lobe

Levene Statistic	df1	df2	Sig.
.465	2	23	.634

ANOVA

Duration of two cycle crackles at lingula lobe

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.254	2	8.627	4.060	.031
Within Groups	48.872	23	2.125		
Total	66.126	25			

Multiple Comparisons

Dependent Variable:Duration of two cycle crackles at lingula lobe

	(I) 1= healthy, 2=smoker, 3=COPD	(J) 1= healthy, 2=smoker, 3=COPD	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bonferroni	Healthy	Smoker	.16333	.68716	1.000	-1.6109	1.9376
		COPD	1.84056*	.70831	.048	.0117	3.6694
	Smoker	Healthy	-.16333	.68716	1.000	-1.9376	1.6109
		COPD	1.67722	.70831	.080	-.1517	3.5061
	COPD	Healthy	-1.84056*	.70831	.048	-3.6694	-.0117
		Smoker	-1.67722	.70831	.080	-3.5061	.1517

Games-Howell	Healthy	Smoker	.16333	.61607	.962	-1.4263	1.7530
		COPD	1.84056	.75714	.073	-.1616	3.8428
	Smoker	Healthy	-.16333	.61607	.962	-1.7530	1.4263
		COPD	1.67722	.75878	.107	-.3283	3.6828
	COPD	Healthy	-1.84056	.75714	.073	-3.8428	.1616
		Smoker	-1.67722	.75878	.107	-3.6828	.3283

*. The mean difference is significant at the 0.05 level

Appendix 9: Examples of Analysis of variance for airway geometry among three groups

Tests of Normality

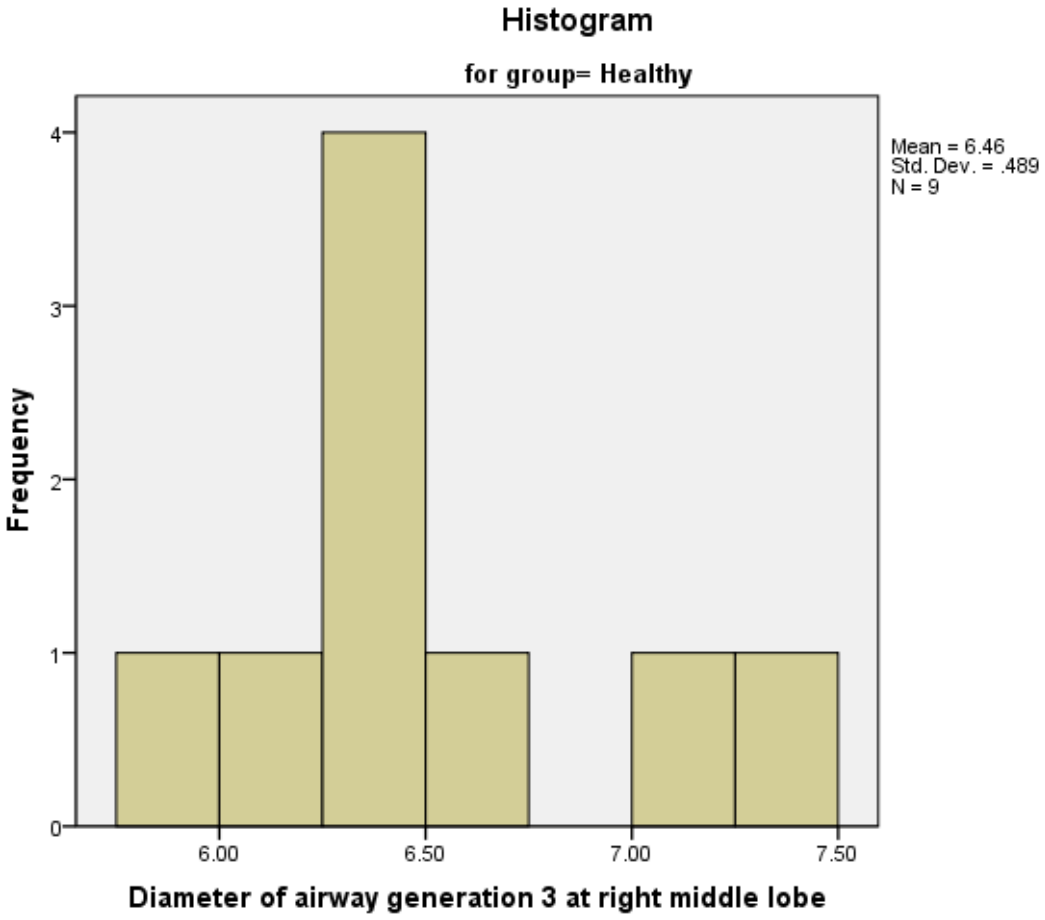
	1= healthy, 2=smoker, 3=COPD	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Diameter of airway generation 3 at right middle lobe	Healthy	.224	9	.200*	.910	9	.314
	Smoker	.219	9	.200*	.890	9	.201
	COPD	.201	8	.200*	.940	8	.614
Branching angle of airway generation 4 at lingula lobe	Healthy	.149	9	.200*	.951	9	.701
	Smoker	.170	9	.200*	.934	9	.524
	COPD	.272	8	.082	.911	8	.363
Internal perimeter of airway generation 3 at right middle lobe	Healthy	.220	9	.200*	.911	9	.320
	Smoker	.217	9	.200*	.891	9	.202
	COPD	.208	8	.200*	.938	8	.595

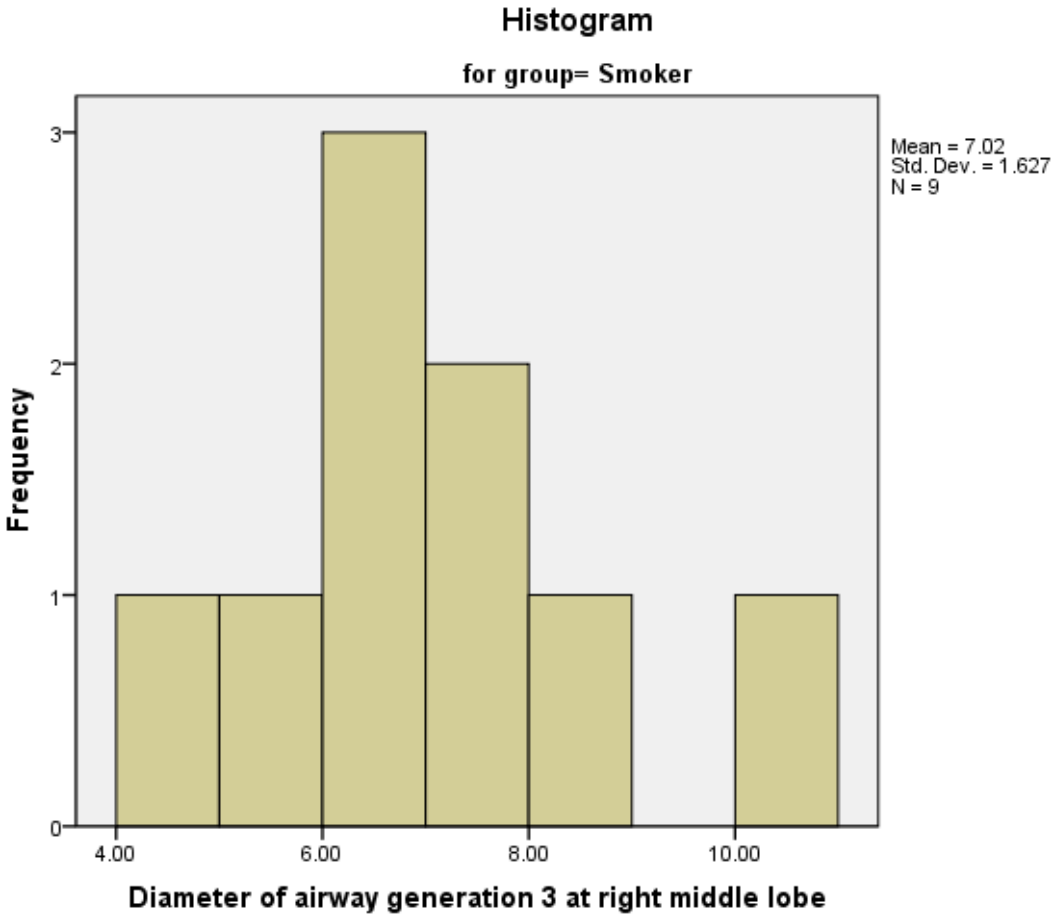
Tests of Normality

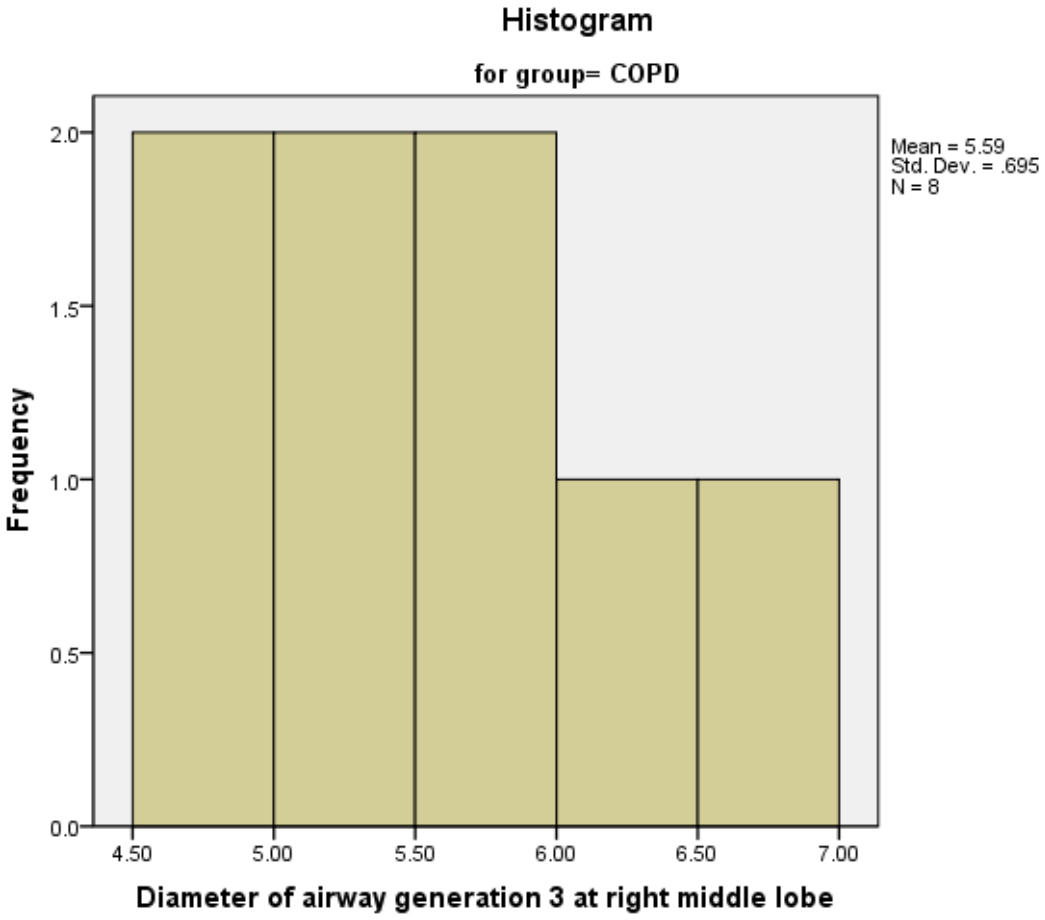
	1= healthy, 2=smoker, 3=COPD	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Diameter of airway generation 3 at right middle lobe	Healthy	.224	9	.200*	.910	9	.314
	Smoker	.219	9	.200*	.890	9	.201
	COPD	.201	8	.200*	.940	8	.614
Branching angle of airway generation 4 at lingula lobe	Healthy	.149	9	.200*	.951	9	.701
	Smoker	.170	9	.200*	.934	9	.524
	COPD	.272	8	.082	.911	8	.363
Internal perimeter of airway generation 3 at right middle lobe	Healthy	.220	9	.200*	.911	9	.320
	Smoker	.217	9	.200*	.891	9	.202
	COPD	.208	8	.200*	.938	8	.595

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.


Histogram
for group= Healthy
Mean = 6.46
Std. Dev. = .489
N = 9
Frequency
0
1
2
3
4
6.00
6.50
7.00
7.50
Diameter of airway generation 3 at right middle lobe


Histogram
for group= Smoker
Mean = 7.02
Std. Dev. = 1.627
N = 9
Frequency
0
1
2
3
4.00
6.00
8.00
10.00
Diameter of airway generation 3 at right middle lobe


Histogram
for group= COPD
Mean = 5.59
Std. Dev. = .695
N = 8
Frequency
2.0
1.5
1.0
0.5
0.0
4.50
5.00
5.50
6.00
6.50
7.00
Diameter of airway generation 3 at right middle lobe

Diameter of airway generation 3 at right middle lobe Stem-and-Leaf Plot for group= Healthy

Frequency	Stem &	Leaf
1.00	Extremes	(=<5.76)
1.00	61 .	6
2.00	62 .	57
1.00	63 .	0
1.00	64 .	4
1.00	65 .	3
2.00	Extremes	(>=7.10)

Stem width: .10

Each leaf: 1 case(s)

Diameter of airway generation 3 at right middle lobe Stem-and-Leaf Plot for group= Smoker

Frequency	Stem &	Leaf
1.00	4 .	9
1.00	5 .	8
3.00	6 .	229
2.00	7 .	02
1.00	8 .	0
1.00	Extremes	(>=10.7)

Stem width: 1.00

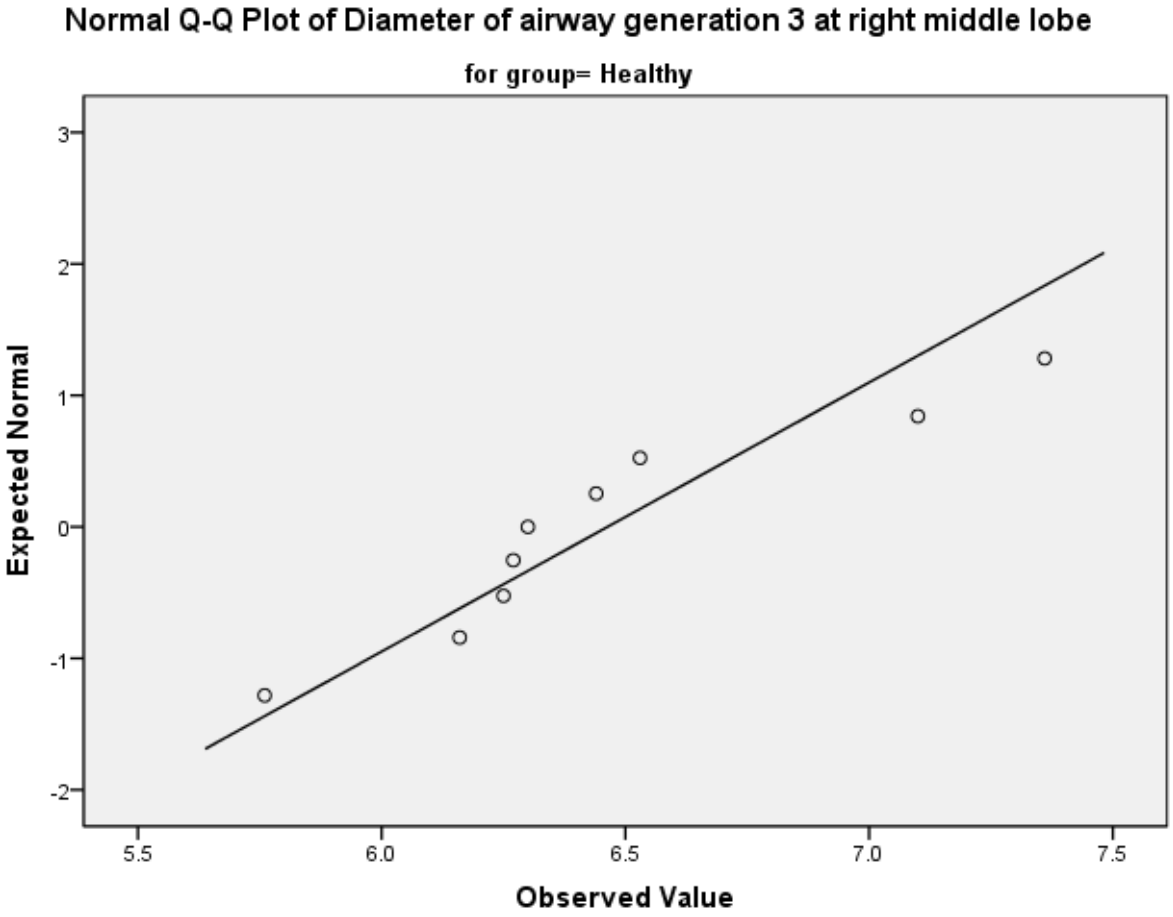
Each leaf: 1 case(s)

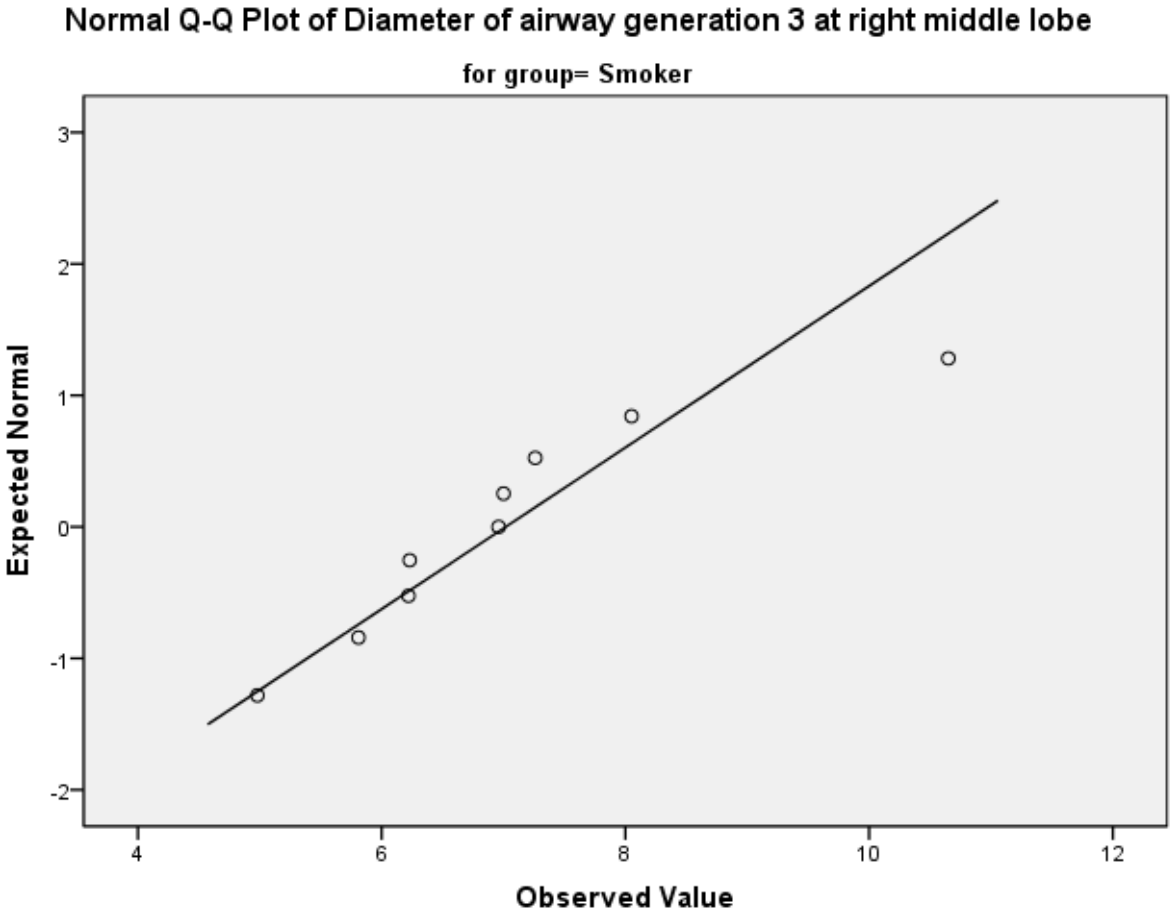
Diameter of airway generation 3 at right middle lobe Stem-and-Leaf Plot for group= COPD

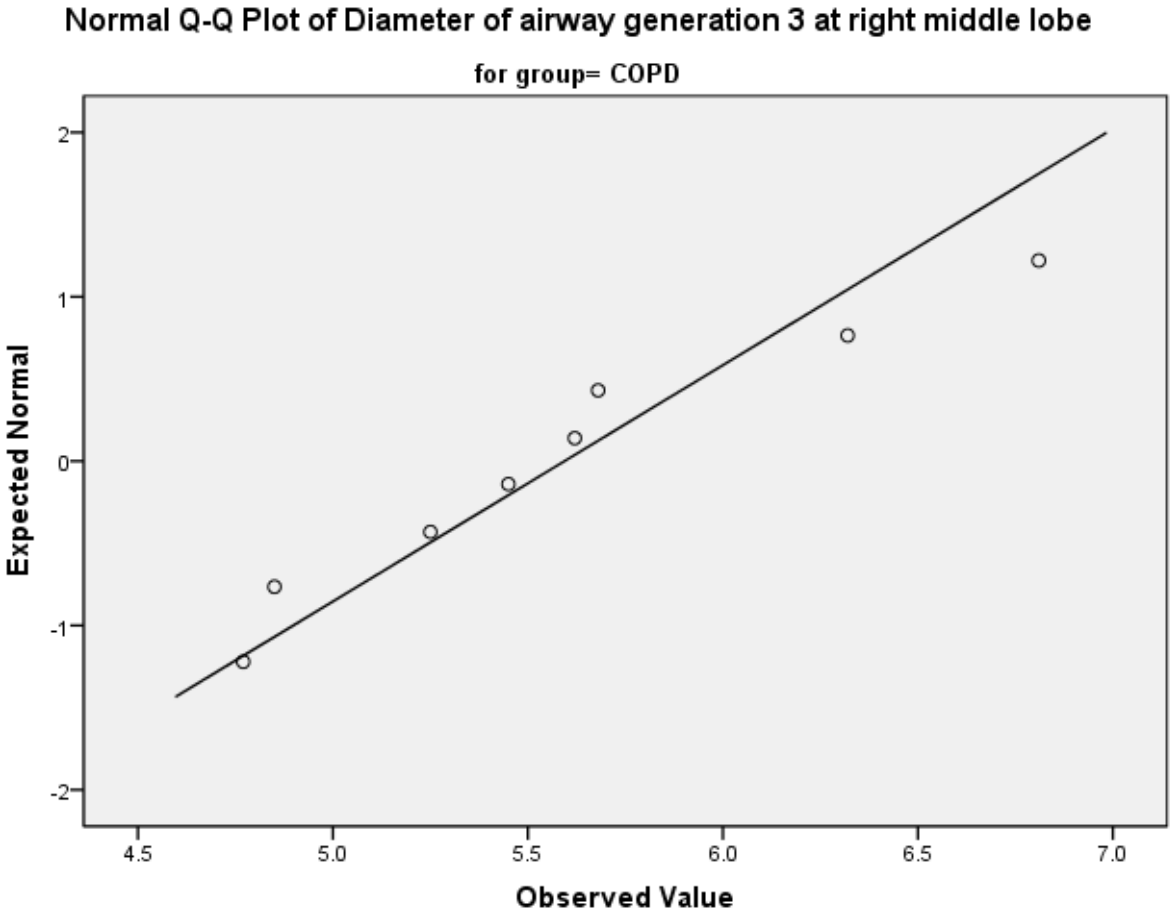
Frequency	Stem &	Leaf
2.00	4 .	78
4.00	5 .	2466
2.00	6 .	38

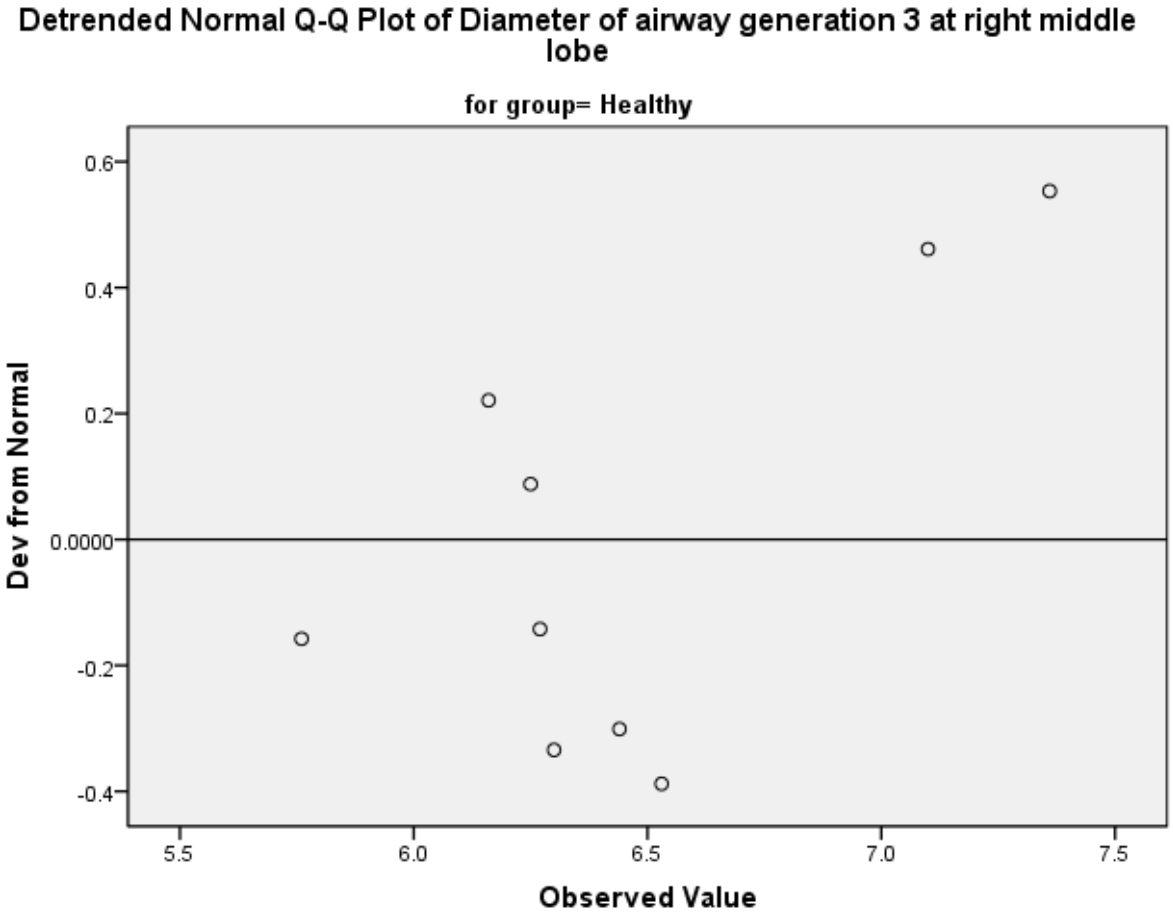
Stem width: 1.00

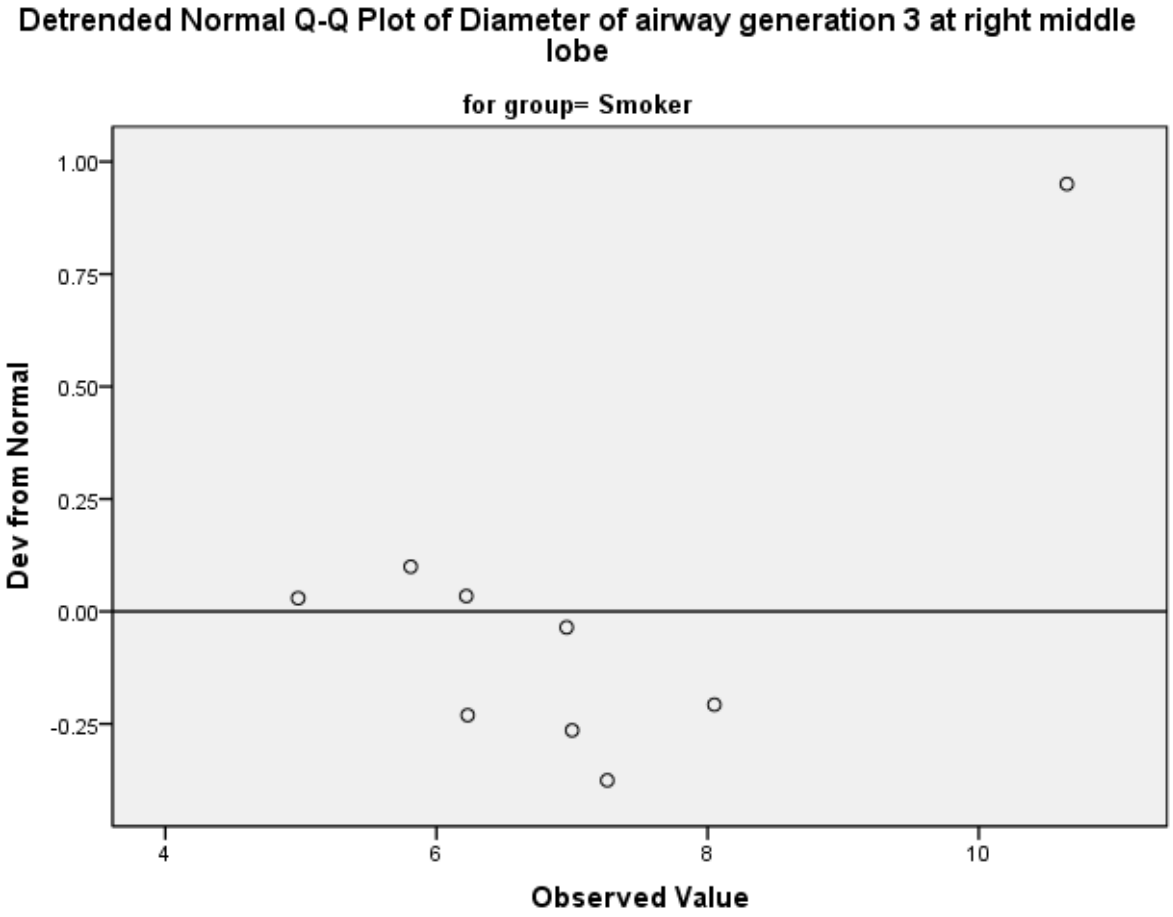
Each leaf: 1 case(s)

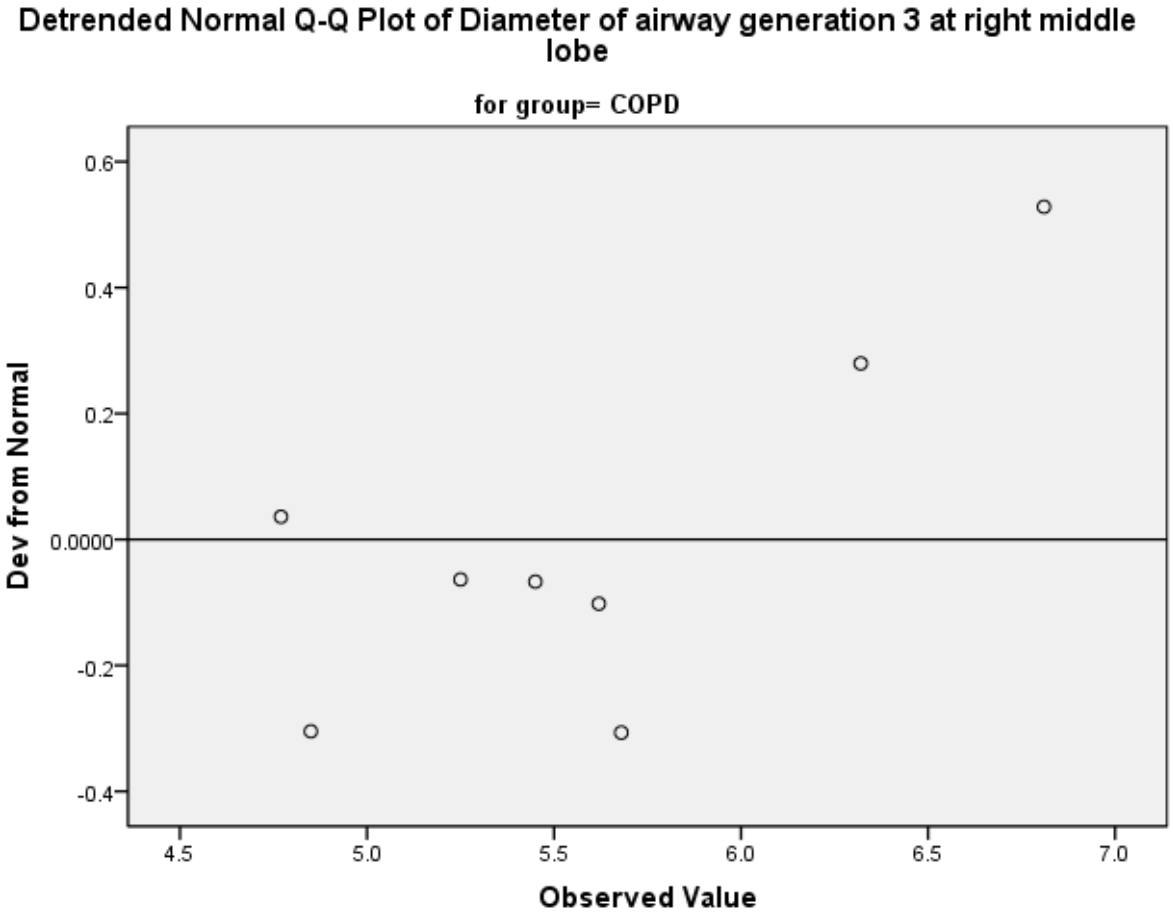

Normal Q-Q Plot of Diameter of airway generation 3 at right middle lobe
for group= Healthy
Expected Normal
3
2
1
0
-1
-2
5.5
6.0
6.5
7.0
7.5
Observed Value

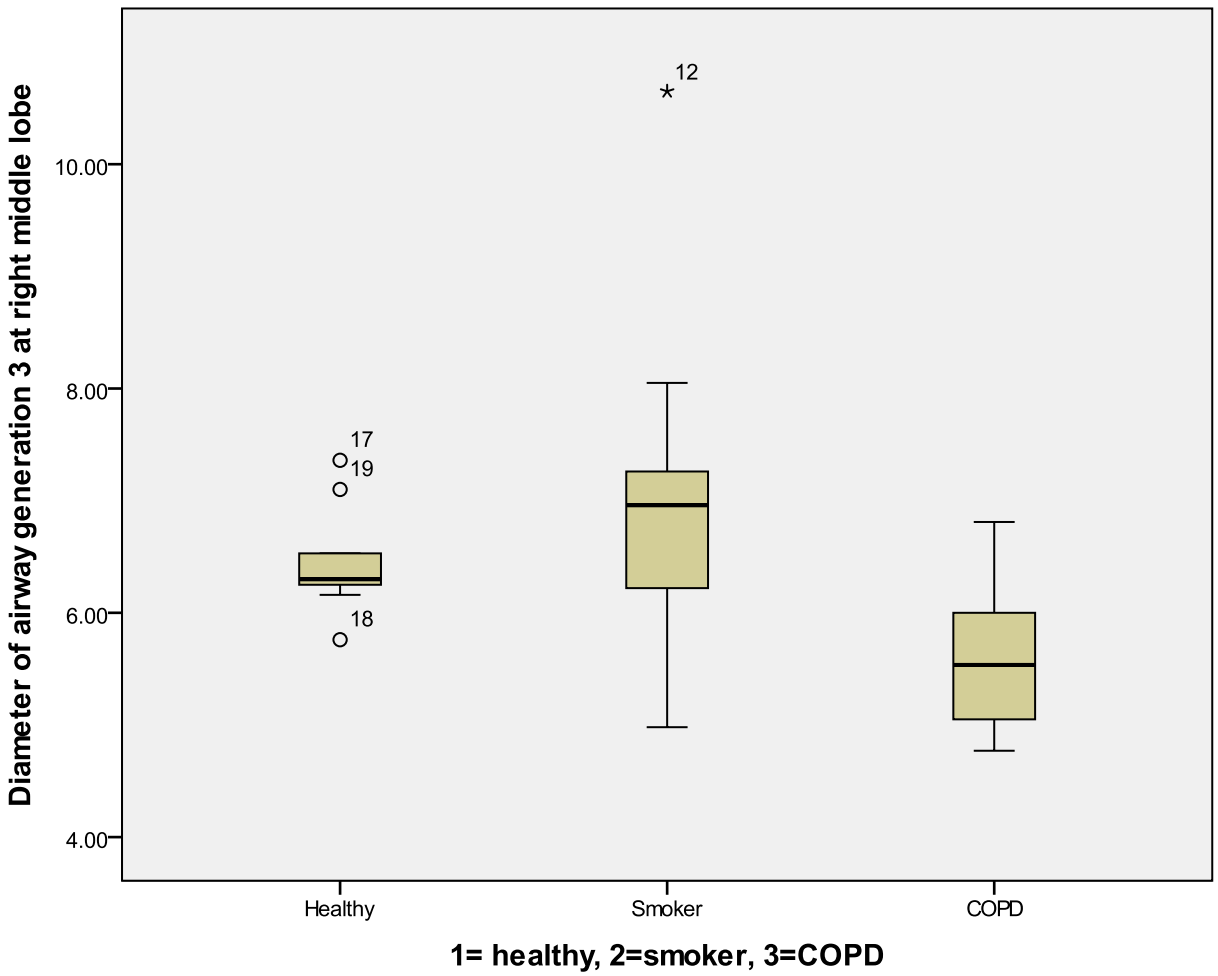

Normal Q-Q Plot of Diameter of airway generation 3 at right middle lobe
for group= Smoker
Expected Normal
Observed Value
3
2
1
0
-1
-2
4
6
8
10
12


Normal Q-Q Plot of Diameter of airway generation 3 at right middle lobe
for group= COPD
Expected Normal
2
1
0
-1
-2
4.5
5.0
5.5
6.0
6.5
7.0
Observed Value


Detrended Normal Q-Q Plot of Diameter of airway generation 3 at right middle lobe
for group= Healthy
Dev from Normal
0.6
0.4
0.2
0.0000
-0.2
-0.4
5.5
6.0
6.5
7.0
7.5
Observed Value


Detrended Normal Q-Q Plot of Diameter of airway generation 3 at right middle lobe
for group= Smoker
Dev from Normal
1.00
0.75
0.50
0.25
0.00
-0.25
4
6
8
10
Observed Value


Detrended Normal Q-Q Plot of Diameter of airway generation 3 at right middle lobe
for group= COPD
Dev from Normal
0.6
0.4
0.2
0.0000
-0.2
-0.4
4.5
5.0
5.5
6.0
6.5
7.0
Observed Value


Diameter of airway generation 3 at right middle lobe
10.00
8.00
6.00
4.00
12
17
19
18
Healthy
Smoker
COPD
1= healthy, 2=smoker, 3=COPD

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Diameter of airway generation 3 at right middle lobe	Healthy	9	6.4633	.48870	.16290	6.0877	6.8390	5.76	7.36
	Smoker	9	7.0178	1.62747	.54249	5.7668	8.2688	4.98	10.65
	COPD	8	5.5938	.69543	.24587	5.0124	6.1751	4.77	6.81
	Total	26	6.3877	1.18579	.23255	5.9087	6.8666	4.77	10.65

Branching angle of airway generation 4 at lingula lobe	Healthy	9	148.3522	8.65323	2.88441	141.7008	155.0037	131.44	159.02
	Smoker	9	149.6189	5.22599	1.74200	145.6018	153.6359	142.65	157.50
	COPD	8	157.3900	6.95809	2.46006	151.5729	163.2071	144.38	167.64
	Total	26	151.5715	7.88631	1.54663	148.3862	154.7569	131.44	167.64
internal perimeter of airway generation 3 at right middle lobe	Healthy	9	20.3078	1.54061	.51354	19.1236	21.4920	18.10	23.13
	Smoker	9	22.0422	5.11189	1.70396	18.1129	25.9716	15.65	33.45
	COPD	8	17.5725	2.18185	.77140	15.7484	19.3966	14.99	21.39
	Total	26	20.0665	3.72440	.73041	18.5622	21.5709	14.99	33.45

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Diameter of airway generation 3 at right middle lobe	2.451	2	23	.108
Branching angle of airway generation 4 at lingula lobe	.581	2	23	.567
Internal perimeter of airway generation 3 at right middle lobe	2.452	2	23	.108

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Diameter of airway generation 3 at right middle lobe	Between Groups	8.667	2	4.334	3.763	.039
	Within Groups	26.485	23	1.152		
	Total	35.152	25			
Branching angle of airway generation 4 at lingular lobe	Between Groups	398.427	2	199.214	3.962	.033
	Within Groups	1156.420	23	50.279		
	Total	1554.848	25			
Internal perimeter of airway generation 3 at right middle lobe	Between Groups	85.416	2	42.708	3.758	.039
	Within Groups	261.363	23	11.364		
	Total	346.778	25			

Multiple Comparisons

Dependent Variable		(I) 1= healthy, 2=smoker, 3=COPD	(J) 1= healthy, 2=smoker, 3=COPD	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Diameter of airway generation 3 at right middle lobe	Bonferroni	Healthy	Smoker	-.55444	.50586	.853	-1.8606	.7517
			COPD	.86958	.52143	.327	-.4768	2.2159
		Smoker	Healthy	.55444	.50586	.853	-.7517	1.8606
			COPD	1.42403*	.52143	.036	.0777	2.7704
		COPD	Healthy	-.86958	.52143	.327	-2.2159	.4768
			Smoker	-1.42403*	.52143	.036	-2.7704	-.0777
	Games-Howell	Healthy	Smoker	-.55444	.56642	.607	-2.1227	1.0138
			COPD	.86958*	.29494	.030	.0862	1.6530
		Smoker	Healthy	.55444	.56642	.607	-1.0138	2.1227
			COPD	1.42403	.59561	.084	-.1827	3.0308
		COPD	Healthy	-.86958*	.29494	.030	-1.6530	-.0862
			Smoker	-1.42403	.59561	.084	-3.0308	.1827

Multiple comparisons

Branching angle of airway generation 4 at lingular lobe	Bonferroni	Healthy	Smoker	-1.26667	3.34263	1.000	-9.8974	7.3640
			COPD	-9.03778*	3.44550	.046	-17.9341	-.1414
		Smoker	Healthy	1.26667	3.34263	1.000	-7.3640	9.8974
			COPD	-7.77111	3.44550	.102	-16.6674	1.1252
		COPD	Healthy	9.03778*	3.44550	.046	.1414	17.9341
			Smoker	7.77111	3.44550	.102	-1.1252	16.6674
	Games-Howell	Healthy	Smoker	-1.26667	3.36963	.925	-10.1514	7.6180
			COPD	-9.03778	3.79100	.075	-18.8935	.8180
		Smoker	Healthy	1.26667	3.36963	.925	-7.6180	10.1514
			COPD	-7.77111	3.01437	.056	-15.7354	.1931

Internal perimeter of airway generation 3 at right middle lobe	Bonferroni	Healthy	Smoker	-1.73444	1.58910	.859	-5.8375	2.3686	
			COPD	2.73528	1.63801	.325	-1.4941	6.9646	
		Smoker	Healthy	1.73444	1.58910	.859	-2.3686	5.8375	
			COPD	4.46972*	1.63801	.036	.2404	8.6991	*
		COPD	Healthy	-2.73528	1.63801	.325	-6.9646	1.4941	
			Smoker	-4.46972*	1.63801	.036	-8.6991	-.2404	
	Games-Howell	Healthy	Smoker	-1.73444	1.77967	.609	-6.6608	3.1919	
			COPD	2.73528*	.92670	.029	.2747	5.1958	
		Smoker	Healthy	1.73444	1.77967	.609	-3.1919	6.6608	
			COPD	4.46972	1.87044	.084	-.5765	9.5159	
		COPD	Healthy	-2.73528*	.92670	.029	-5.1958	-.2747	
			Smoker	-4.46972	1.87044	.084	-9.5159	.5765	.

The mean difference is significant at the 0.05 level

Appendix 10: Example of correlation and multiple regression tests

Descriptive Statistics

	Mean	Std. Deviation	N
Number of crackles per breathing cycle at anterior left region of chest wall	3.4509	1.51013	23
Branching angle of airway generation 2 at left lower lobe	156.7565	8.69206	23
airway wall thickness of airway generation 4 at left upper lobe	1.4257	.16686	23

Correlation

	Number of crackles per breathing cycle at anterior left region of chest wall	Branching angle of airway generation 2 at left lower lobe	airway wall thickness of airway generation 4 at left upper lobe
Pearson correlation Number of crackles per breathing cycle at anterior left region of chest wall	1	-.416	.468
Branching angle of airway generation 2 at left lower lobe	-.416	1	.013
airway wall thickness of airway generation 4 at left upper lobe	.468	.013	1

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	airway wall thickness of airway generation 4 at left upper lobe	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
2	Branching angle of airway generation 2 at left lower lobe	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	airway wall thickness of airway generation 4 at left upper lobe	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
2	Branching angle of airway generation 2 at left lower lobe	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a. Dependent Variable: Number of crackles per breathing cycle at anterior left region of chest wall

Model Summary[c]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.468[a]	.219	.182	1.36567	.219	5.901	1	21	.024	
2	.631[b]	.398	.338	1.22910	.178	5.926	1	20	.024	1.292

a. Predictors: (Constant), airway wall thickness of airway generation 4 at left upper lobe

b. Predictors: (Constant), airway wall thickness of airway generation 4 at left upper lobe, Branching angle of airway generation 2 at left lower lobe

c. Dependent Variable: Number of crackles per breathing cycle at anterior left region of chest wall

ANOVA[c]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	11.005	1	11.005	5.901	.024[a]
	Residual	39.166	21	1.865		
	Total	50.171	22			
2	Regression	19.957	2	9.979	6.605	.006[b]
	Residual	30.214	20	1.511		
	Total	50.171	22			

a. Predictors: (Constant), airway wall thickness of airway generation 4 at left upper lobe

b. Predictors: (Constant), airway wall thickness of airway generation 4 at left upper lobe, Branching angle of airway generation 2 at left lower lobe

c. Dependent Variable: Number of crackles per breathing cycle at anterior left region of chest wall

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	-2.592	2.504		-1.035	.312	-7.799	2.615		
	airway wall thickness of airway generation 4 at left upper lobe	4.239	1.745	.468	2.429	.024	.610	7.867	1.000	1.000
2	(Constant)	8.843	5.210		1.697	.105	-2.025	19.710		
	airway wall thickness of airway generation 4 at left upper lobe	4.288	1.571	.474	2.730	.013	1.012	7.564	1.000	1.000
	Branching angle of airway generation 2 at left lower lobe	-.073	.030	-.422	-2.434	.024	-.136	-.011	1.000	1.000

ANOVA[c]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	11.005	1	11.005	5.901	.024[a]
	Residual	39.166	21	1.865		
	Total	50.171	22			
2	Regression	19.957	2	9.979	6.605	.006[b]
	Residual	30.214	20	1.511		
	Total	50.171	22			

a. Dependent Variable: Number of crackles per breathing cycle at anterior left region of chest wall

Excluded Variables[c]

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	Length of airway generation 3 at right middle lobe	.098[a]	.447	.659	.100	.799	1.252	.799
	Branching angle of airway generation 2 at left lower lobe	-.422[a]	-2.434	.024	-.478	1.000	1.000	1.000
2	Length of airway generation 3 at right middle lobe	-.086[b]	-.406	.690	-.093	.691	1.446	.691

a. Predictors in the Model: (Constant), airway wall thickness of airway generation 4 at left upper lobe

b. Predictors in the Model: (Constant), airway wall thickness of airway generation 4 at left upper lobe, Branching angle of airway generation 2 at left lower lobe

c. Dependent Variable: Number of crackles per breathing cycle at anterior left region of chest wall

Collinearity Diagnostics[a]

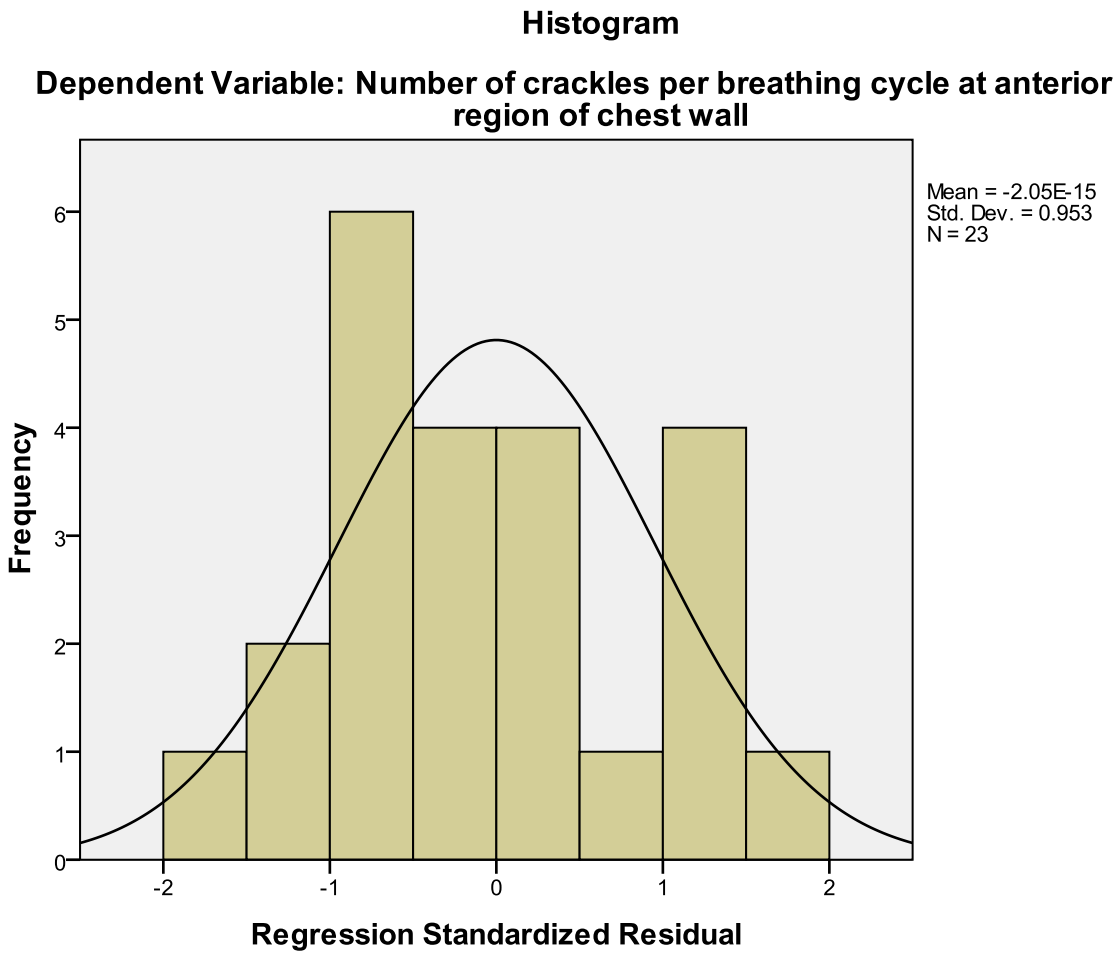
Model	Dimension	Eigenvalue	Condition Index	Variance Proportions		
				(Constant)	airway wall thickness of airway generation 4 at left upper lobe	Branching angle of airway generation 2 at left lower lobe
1	1	1.994	1.000	.00	.00	
	2	.006	17.529	1.00	1.00	
2	1	2.989	1.000	.00	.00	.00
	2	.009	18.065	.03	.93	.07
	3	.001	46.463	.97	.07	.93

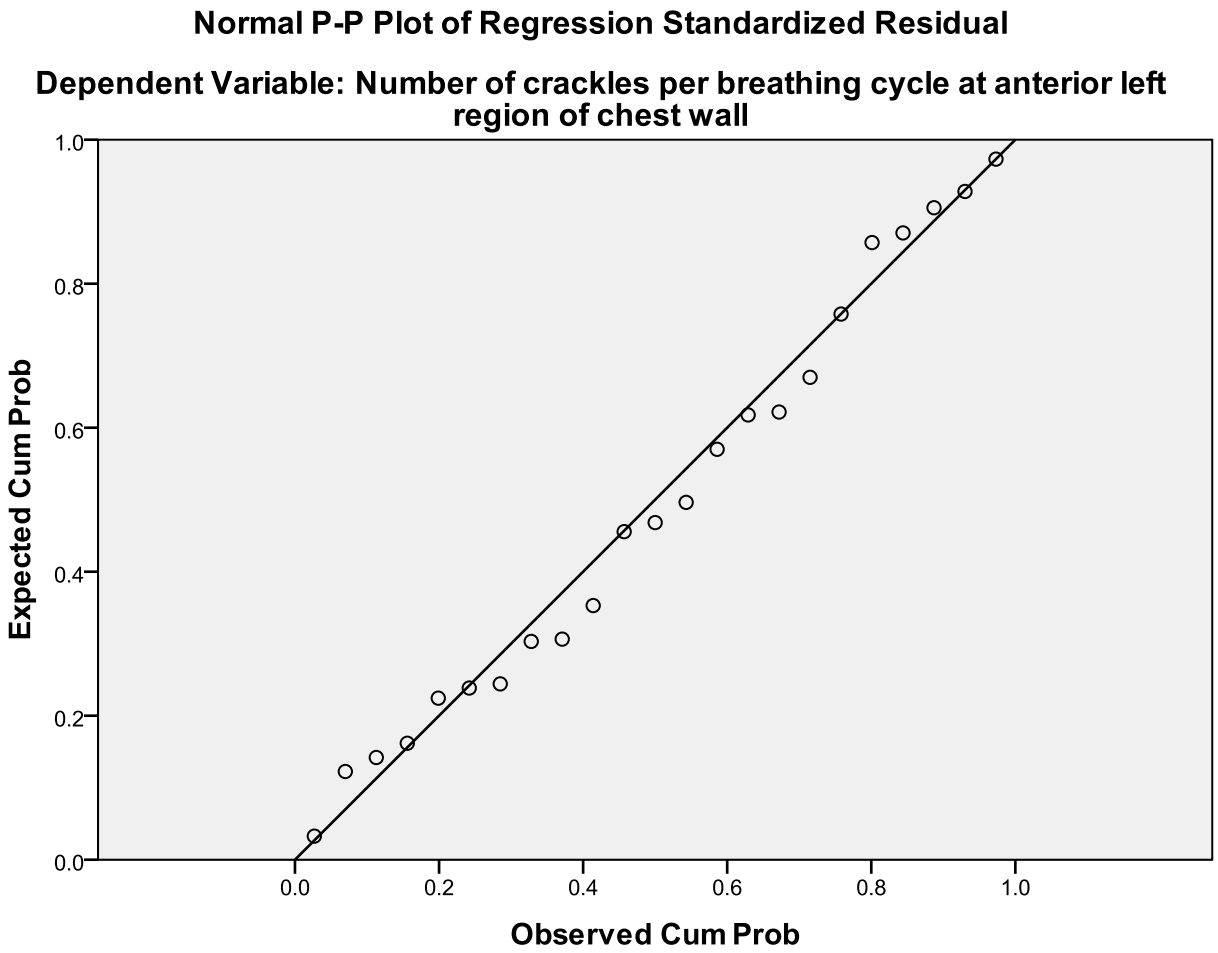
a. Dependent Variable: Number of crackles per breathing cycle at anterior left region of chest wall

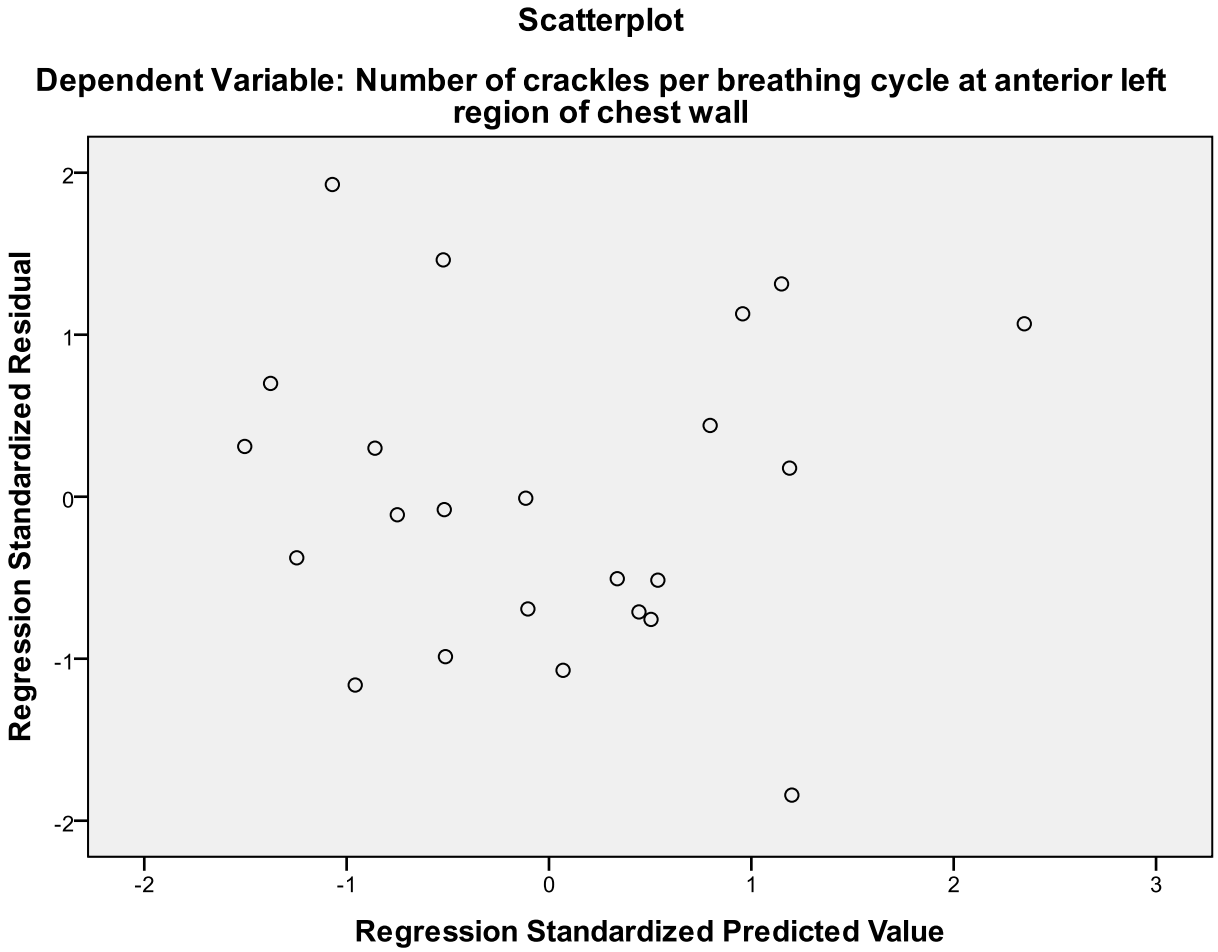
Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	2.0188	5.6880	3.4509	.95244	23
Residual	-2.26396	2.36872	.00000	1.17190	23
Std. Predicted Value	-1.504	2.349	.000	1.000	23
Std. Residual	-1.842	1.927	.000	.953	23

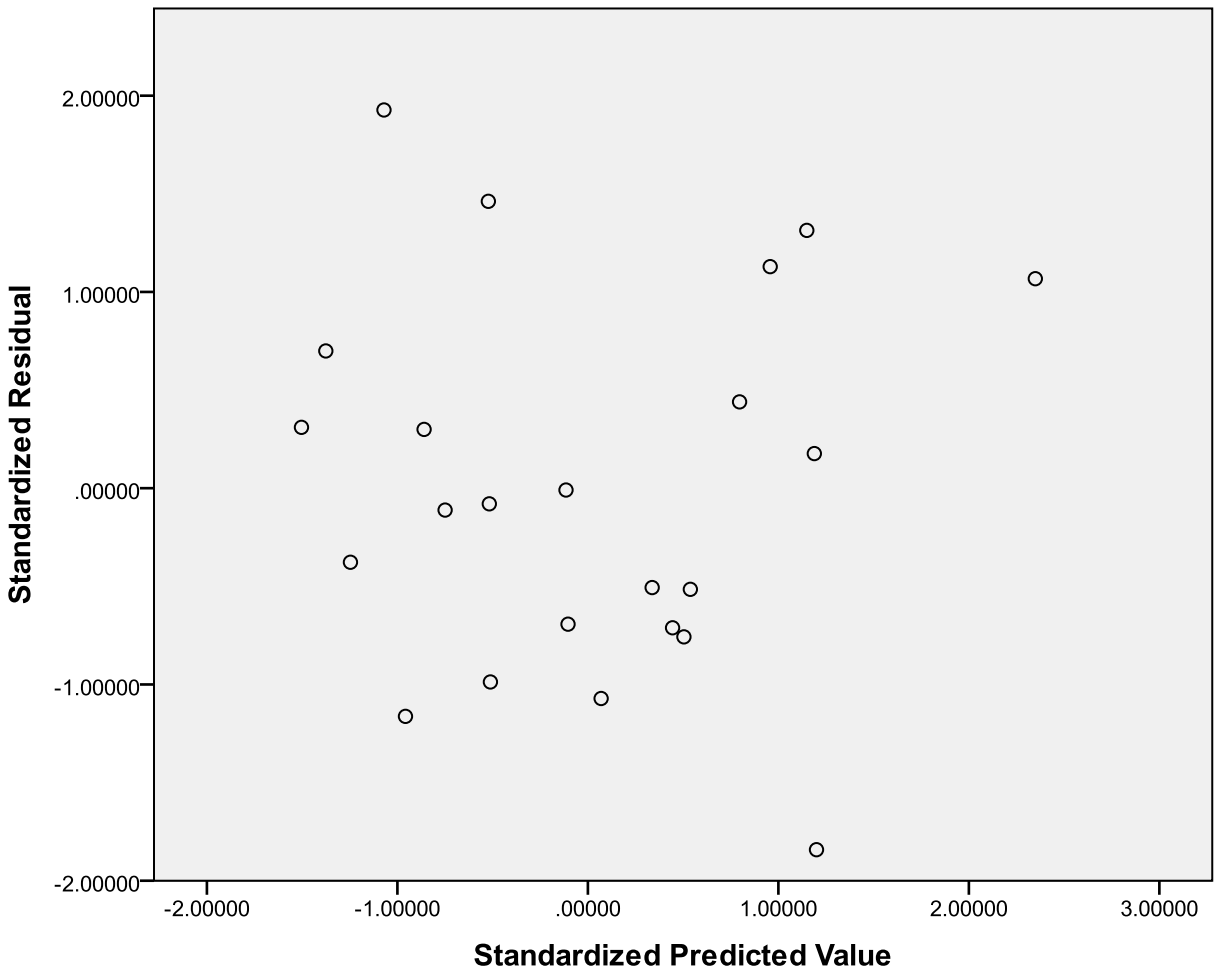
a. Dependent Variable: Number of crackles per breathing cycle at anterior left region of chest wall

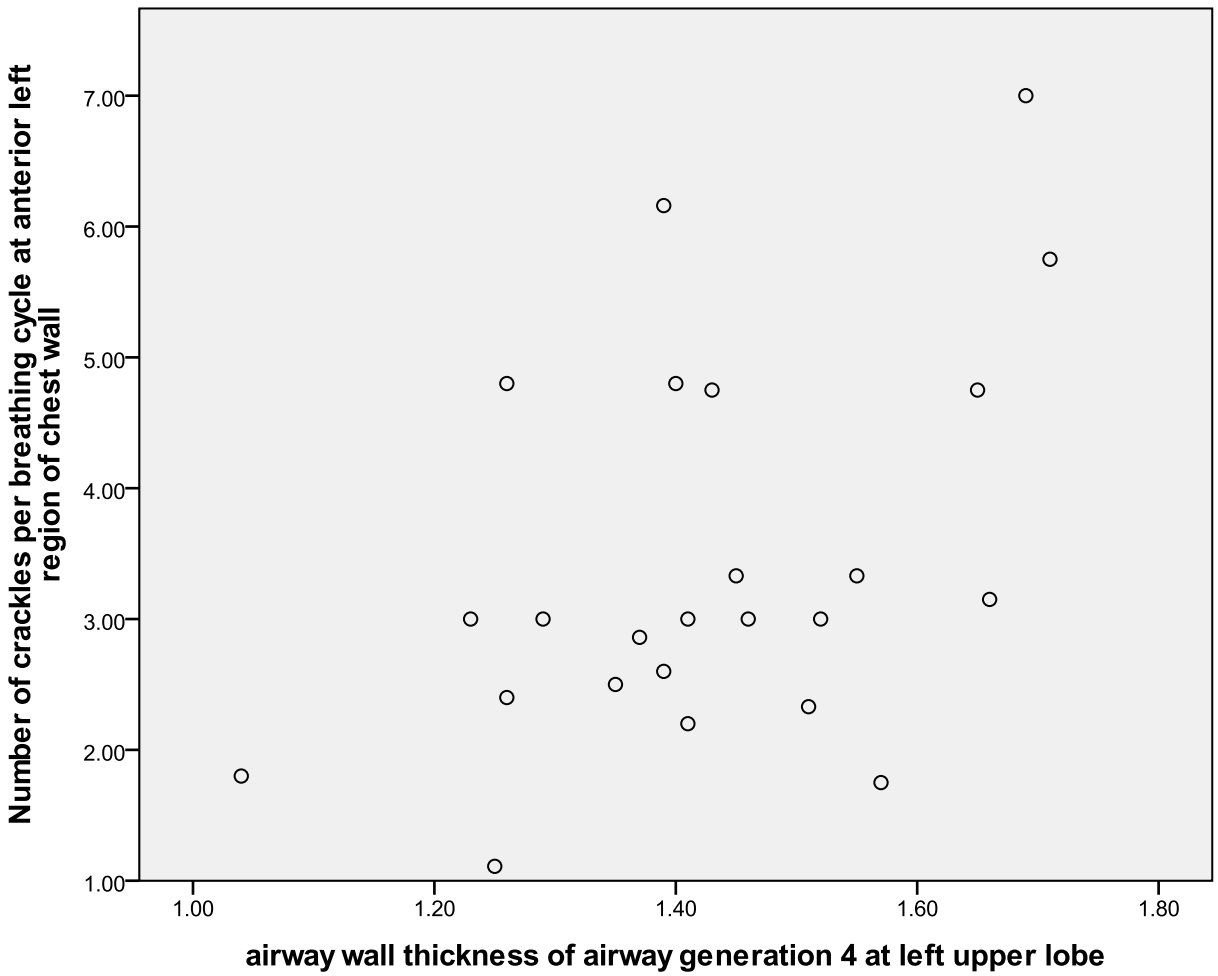

Histogram
Dependent Variable: Number of crackles per breathing cycle at anterior left region of chest wall
Mean = -2.05E-15
Std. Dev. = 0.953
N = 23
Frequency
0
1
2
3
4
5
6
-2
-1
0
1
2
Regression Standardized Residual

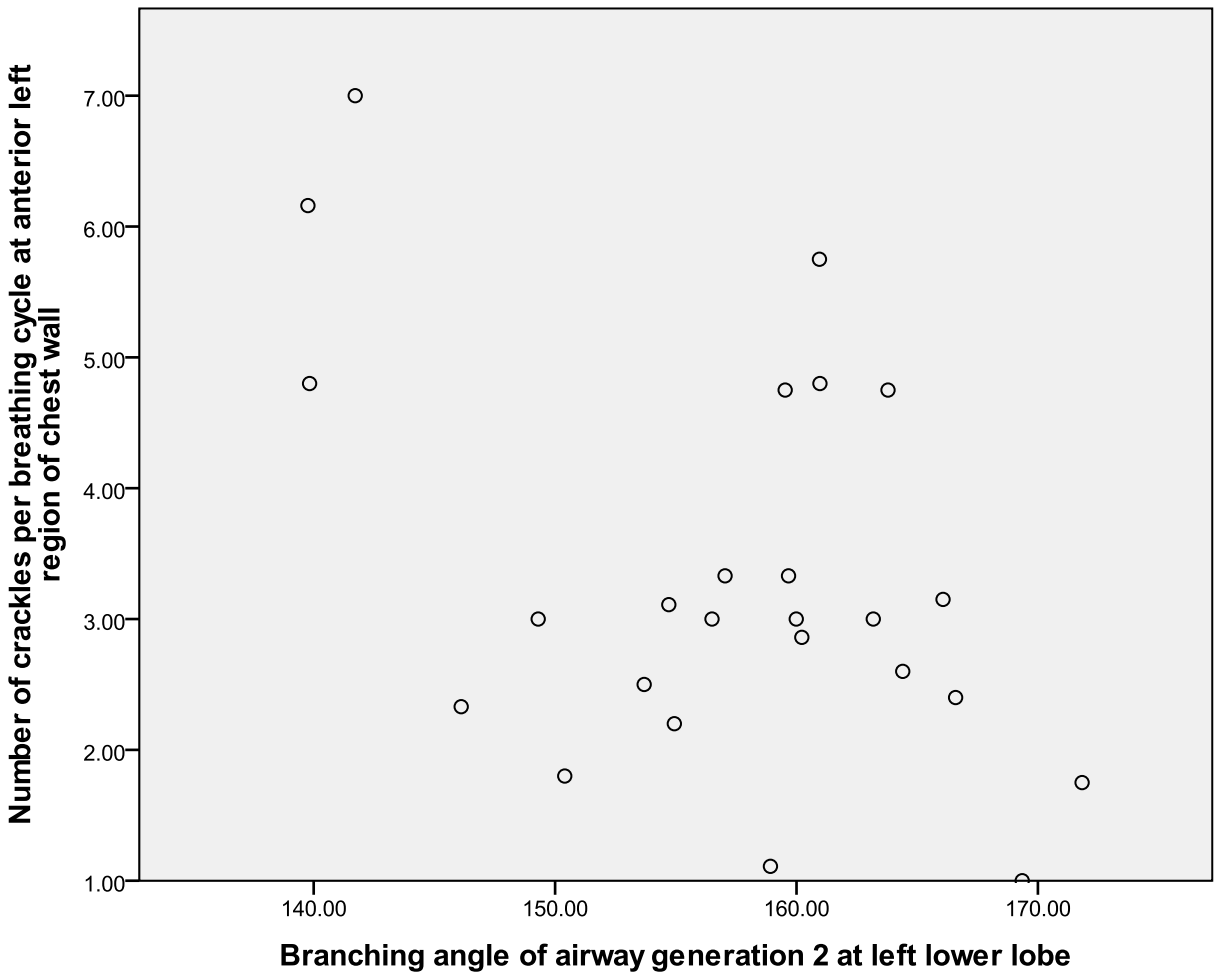

Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Number of crackles per breathing cycle at anterior left region of chest wall
Expected Cum Prob
Observed Cum Prob
0.0
0.2
0.4
0.6
0.8
1.0


Scatterplot
Dependent Variable: Number of crackles per breathing cycle at anterior left region of chest wall
Regression Standardized Residual
Regression Standardized Predicted Value
2
1
0
-1
-2
-2
-1
0
1
2
3

Appendix 10


Standardized Residual
2.00000
1.00000
.00000
-1.00000
-2.00000
-2.00000
-1.00000
.00000
1.00000
2.00000
3.00000
Standardized Predicted Value


Number of crackles per breathing cycle at anterior left region of chest wall
7.00
6.00
5.00
4.00
3.00
2.00
1.00
1.00
1.20
1.40
1.60
1.80
airway wall thickness of airway generation 4 at left upper lobe


Number of crackles per breathing cycle at anterior left region of chest wall
7.00
6.00
5.00
4.00
3.00
2.00
1.00
140.00
150.00
160.00
170.00
Branching angle of airway generation 2 at left lower lobe

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