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QUANTIFYING CRACKLES IN THE LUNG OF SMOKING AND NON-SMOKING YOUNG ADULTS

By

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ABSTRACT

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Crackle sounds are associated with a variety of lung disorders. Smoking is also associated with many of the changes in the lung and airways leading to crackles. However, studying crackles as an indication of pathologic changes related to cigarette smoking in the lung is an underdeveloped area of research which needs to be explored. This study was undertaken to investigate whether differences in the crackles' characteristics (duration of two cycle deflection (2CD) and number of crackles per breathing cycle (NCBC)) in the lung of smoking and non-smoking young adults could be found and to quantify these differences, if present, using a digital stethoscope and computer aided lung sound analysis (CALSA). Sixty male subjects (30 smokers and 30 non-smokers) with an average age of 26.6 years (SD ± 4.7) were recruited, drawn from students at the University of Southampton in the United Kingdom. The lung sound data were recorded on one occasion using a digital stethoscope connected to a laptop running MATLAB to record and store the lung sounds from seven anatomical sites on the chest. The 2CD and NCBC per site in 25 second recordings were calculated using data from each of the anatomical sites used for recording lung sounds (excluding the trachea). No statistically significant differences in NCBC per site were found between smokers and non-smokers at any anatomical location. The 2CD per site data revealed some statistically significant differences at both anterior sites (anterior left: $F(2, 57) = 9.40$, $P = 0.00$; anterior right: $F(2, 57) = 9.51$, $P = 0.00$)) and both lateral sites (middle left: $F(2, 57) = 4.2$, $P = 0.02$; middle right: $F(2, 57) = 4.36$, $P = 0.02$)). The hypothesis that lung crackle's 2CD differ between smokers and non-smokers has been supported but the hypothesis that NCBC differ between smokers and non-smokers has not been supported.

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Declaration of Authorship

I, **Mohammed Alzahrani** declare that this thesis and the work presented in it are my own and have been generated by me as the result of my own original research.

Quantifying Crackles in the Lung of Smoking and Non-smoking Young Adults

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;

2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;

3. Where I have consulted the published work of others, this is always clearly attributed;

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5. I have acknowledged all main sources of help;

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List of abbreviations

AL - anterior left
ANOVA - analysis of variance
AR - anterior right
ATS - American Thoracic Society
BMI - body mass index
CALSA - computer aided lung sound analysis
CORSA - computerised respiratory sound analysis
COPD - chronic obstructive pulmonary disease
CHF - congested heart failure
CTC - crackle transmission coefficient
DF - degrees of freedom
FEV_1 - forced expiratory volume in the first second
FEV_1_p - forced expiratory volume within the first second percentage predicted
FVC - forced vital capacity
FVC_P - forced vital capacity percent from predicted
FEV_1/FVC - ratio between forced expiratory volume in the first second and forced vital capacity
HRCT - high-resolution computed tomography
Hz - Hertz
IDW - initial deflection width
ILD - interstitial lung disease
IPF - interstitial pulmonary fibrosis
LDW - largest deflection width
LL - lateral left
LR - lateral right
Max - maximum
Min - minimum
msec - millisecondes
NCBC - number of crackles per breathing cycle
PL - posterior left
PN - Pneumonia
PR - posterior right
SD - standard deviation
T - Trachea
TEWA - time expanded waveform analysis
2CD - two cycle's deflection
WHO - World Health Organization

CHAPTER 1

Introduction

The aim of this research is to investigate whether a digital stethoscope and computer aided lung sound analysis (CALSA) can be used to compare crackle characteristics in the lung between smokers and non-smokers. This chapter presents a brief introduction to the background of the research followed by the proposed analytical approach for the present study. The thesis outline is reported at the end of the chapter.

1.1 General background

The rationale behind this research was to explore the potential for computerised lung sound analysis to provide a useful tool for early diagnosis of smoking-related changes in the lung. Although cigarette smoking is decreasing in the United Kingdom it continues to be more common among adults aged 20 to 34 than among other age groups (General Household Survey, 2006). In the 2001 European Community Respiratory Health Survey, the proportion of current smokers exceeded that of past smokers among young adults in most European countries (Cerveri et al, 2001). The survey involved 1,500 young adults of both sexes aged 20-44 years in 13 Western European countries.

Cigarette smoking is associated with changes in the lung parenchyma and airways that may lead to a number of pulmonary diseases and pathologic changes characterised by inflammation, airway obstruction and destruction of the lung parenchyma (Amin et al, 2003). It has been reported previously that smokers have a higher tendency to lower (as compared to upper) respiratory tract infection and the coughing continues more than non-smokers having the same infection (Murin et al, 1997). This might lead to progressive pathological changes in the small airways that may be an important cause of airflow obstruction and may progress to the development of Chronic Obstructive Pulmonary Disease (COPD) (Hogg, 2004; Cosio et al, 1980). Diagnosis of lung disorders by the use of chest X-ray or lung function test is dependent on the severity of the damage. Therefore, most of the common chronic lung diseases such as COPD and interstitial lung diseases (ILD) are not easily diagnosed in their early stages. Early diagnosis is desirable because

initial changes to the lungs caused by smoking may be reversible with optimal management (Celermajer et al, 1993).

It has been noted that most smokers start to smoke before the age of 18 (Warren et al, 2008). It is more likely for young smokers to become heavy smokers in future when compared to people who start at an older age (Taioli and Wynder, 1991). Future morbidity and mortality attributed to tobacco probably will increase (Warren et al, 2008), this has led to a call for effective programmes to be developed and implemented soon. In order to be effective, such programmes need evidence relating to the damaging effects of smoking on young people. A recent study by Bize et al (2009) reviewed the use of personal biomarkers (carbon monoxide measurements, spirometry and arterial damage) for measuring the harmful effects of smoking in different age groups and found little evidence for the effectiveness of most of these biomedical tests in the quit rate of smoking in their review. Further, Bize et al (2009) reported lung function results for smokers and non-smokers of the same age group in terms of their relative lung age, this resulted in a higher quit rate for that group than for those who were given the same lung function test results without relating them to lung age. Nevertheless, it has been reported that simple lung function tests like spirometry do not detect changes until significant damage to the airways has already occurred (Parkes et al, 2008). It has also been reported that spirometry rarely detects differences between smokers and healthy non-smokers under the age of 45 (Ferguson et al, 2000), which suggests that spirometry testing might not be sensitive to the early changes in the lung of young adult smokers.

There is, therefore, a need for more sensitive measures to assess the early effects of smoking in young adults. It is known that lung sounds heard via standard auscultation can provide useful information about lung states, but standard auscultation is too subjective to allow quantification of the sounds. More detailed characterisation of individual lung sounds can be achieved using a digital stethoscope to record lung sounds and CALSA to analyse them (Marques et al, 2009a; Kiyokawa and Pasterkamp, 2002). The term CALSA was suggested and used by Marques et al (2009a) to describe the process of recording, storing and analysing lung sounds using a digital stethoscope and computer where it was found possible to quantify both normal and added lung sounds.

It is therefore proposed in this research to use CALSA to quantify one type of added sound (crackles) in recordings of lung sounds taken from smoking and non-smoking young adults (aged 20-35 years). Crackles are reported to be discontinuous added respiratory sounds that can be coarse (moist) when they are due to the movement of sputum in large airways. Fine crackles are reported to be created by small airways snapping open as pressure equalizes in the distal airways (Forgacs, 1978), a detailed description of this added lung sound is presented in the next chapter (Chapter 2).

Smokers are defined in this research as those who at least smoke five cigarettes daily for one year. Non-smokers were defined as those who had never smoked. These definitions followed Dicpinigaitis (2003) where he studied the cough reflex sensitivity in cigarette smokers and found it significantly diminished compared to non-smokers. It was hypothesised that the crackle characteristics on the smokers with the same smoking history might be different from non-smokers. In the following section the core idea of this research is explained, which was to compare crackle characteristics in a population of young adult smokers and non-smokers.

1.2 Analytical approach

Crackles are associated with a variety of lung diseases such as pulmonary fibrosis, bronchiolitis, congestive heart failure and pneumonitis (Hoevers and Loudon, 1990; Flietstra et al, 2011; Piirilä, 1992; Vyshedskiy et al, 2011). Smoking is also associated with a number of changes in the lung and airways, such as peripheral airways inflammatory processes (Hogg et al, 1994) and interstitial lung disease (ILD) (Moon et al, 1999). Moreover, it has been found in previous studies (Sovijärvi et al, 2000 a; Murphy et al, 1984) that the number of crackles per breathing cycle (NCBC) is directly related to the severity of disease in patients with interstitial lung disorders and asbestosis. Murphy et al (2004) have found that an increased NCBC is associated with a greater likelihood of pneumonia. While these conditions are associated with crackles, it is not yet known if smoking is related to crackles in the absence of lung disease. Figure 1 shows that the relationship between cigarette smoking and some lung pathologies is well established in the literature; there is also literature addressing lung pathologies due to lung disease associated with crackles but the association between crackles and smoking has not yet been considered.

In this research, the relationship between cigarette smoking as a factor in some pulmonary disorders and crackles characteristics were studied using CALSA. The crackles characteristics are including frequency, timing, transformation and the number. In this study, two characteristics were investigated: the frequency and the number of crackles per breathing cycle (NCBC). The frequency refers to the number of occurrences of crackles in hertz (frequency in hertz (Hz). Measuring the duration of the two cycle deflection (2CD) of each crackle is used as indication for the frequency where the duration of one cycle is inversely related to the frequency (Hz=1/cycle), shorter duration means a higher frequency of the crackle, namely, higher pitched crackles. However, it was hypothesized in this research that crackle characteristics are different between smokers and non-smokers due to pathological changes in the lung and airways caused by smoking which led to the development of the following aims and the objectives.

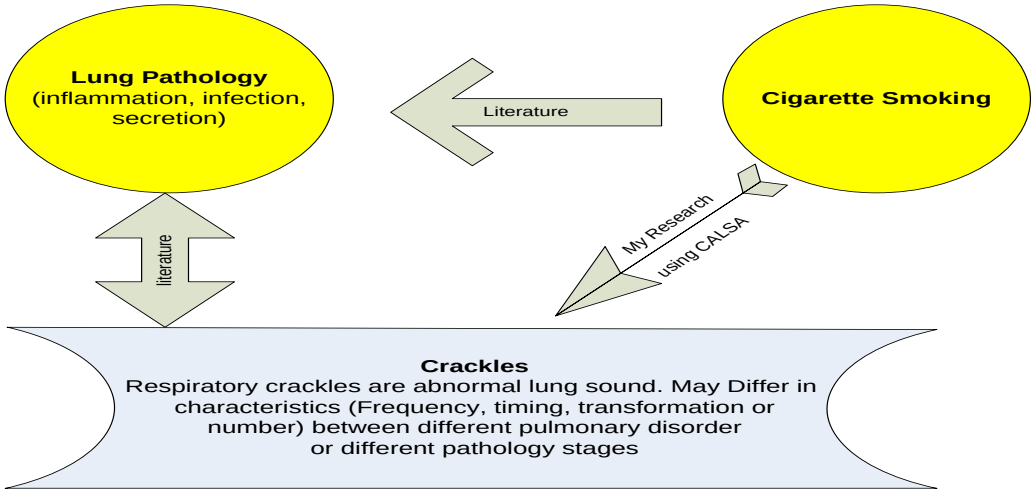


Figure 1: Proposed theoretical model of the research

Note: the frequency in this diagram is measured in hertz; the calculation of the two cycle deflection (2CD) for each crackle is an indication for the frequency (Hz=1/cycle); the number is calculated as number of crackles per breathing cycle (NCBC).

1.3 The research aim and objectives

The aim of this research was to compare crackle characteristics, using CALSA, between smokers and non-smokers. There were two key objectives for this research:

- To evaluate whether there is a statistically significant difference in the duration of the two cycle deflection (2CD) of crackles between smoking and non-smoking young adults using CALSA.

- To evaluate whether there is a statistically significant difference in the NCBC between smoking and non-smoking young adults using CALSA.

If significant differences are detected, this information could be used to provide evidence of the adverse effects of smoking in young adults as part of smoking prevention and health education programmes. In addition to diagnostic purposes, the characteristics of crackles could be used as an early indicator to predict future changes in the lung if considered in longitudinal studies. A further major benefit could be the closer monitoring of the treatment of patients suffering from conditions such as COPD, bronchiolitis, congestive heart failure (CHF), pneumonia (PN) and asbestosis. Crackles' characteristics had been proposed in early studies (Vyshedskiy et al, 2005; Murphy et al, 2004; Marques, 2008) as a tool to be used in diagnosing and monitoring of some chronic lung diseases (ILD, pulmonary oedema, cystic fibrosis).

1.4 Thesis outline

Chapter 1 provides a brief introduction and background to the objectives of the research. Chapter 2 presents a literature review of the subject area which identifies a number of research gaps leading to the development of the research questions. Chapter 3 presents the methodology employed in conducting this research including subject recruitment, data collection and analysis procedures and the power calculation of the sample size. Chapter 4 provides the research results. Chapter 5 discusses the results and relates them to the literature along with a consideration of the research limitations. Finally, Chapter 6 presents the conclusions and the practical applications of the present research findings together with identifying the main areas for future work.

CHAPTER 2

Literature Review

Literature concerning the effect of cigarette smoking on the lung is well established. Abnormal lung sounds (particularly crackles) are used qualitatively to aid the diagnosis of most chronic diseases which are linked to cigarette smoking. Therefore this chapter will review crackles in detail including their generative physical mechanisms and the development of computer aided measurement techniques. The prevalence of crackles in healthy subjects and the expected effects of demographics (age, gender and body mass index) on crackle characteristics will also be reviewed. The review will further identify the impact of cigarette smoking on crackle characteristics as an undeveloped area of research which is identified as a research gap in this review. Finally, the research hypotheses will be derived from the research questions which have been informed by the literature.

2.1 Lung sounds

Listening to lung sounds was first practiced clinically in 1816, when René Laennec started studying the relationship between lung disease and lung sounds (Rapoport, 1986). Lung sounds provide useful information about the structure and functional properties of the lung, as they are believed to relate directly to airway geometry and airflow (Dellinger et al, 2008). They result from the vibrations within the lung and airways and are transmitted to the chest wall (Loudon and Murphy, 1984). Pasterkamp et al (1997) found lung sounds to be sensitive to airway changes and critically dependent on air flow. As a result, changes in lung structure due to disease may affect the amplitude and timing of sound transmission from the airways to the chest surface and generate added lung sounds such as crackles and wheezes.

Historically, a number of different terms have been used to describe lung sounds. In 1816, Laennec used the term ‘râles’ to describe crackle sounds. ‘Râles’ is the French word for rattles and was intended to describe the ‘death rattle’ sound heard when patients are dying and too weak to cough up secretions as reported by Robertson and Coope (1957). The translation of terminologies from French to other languages caused some confusion in

differentiating between lung sounds. Robertson and Coope (1957) were the first to suggest classifying added sounds such as crackles or wheezes to minimise the confusion. Currently, lung sounds are broadly categorised as 'normal' (also known as 'vesicular' lung sounds) and 'added' or 'adventitious' lung sounds. Added lung sounds are further subdivided into crackles, wheezes and rhonchi (Murphy, 2008) (see Table 1).

Table 1: Differences in terms describing added lung sounds

Acoustic characteristics	**Recommended ATS[(1)] terms**	**British terms**	**Laennec's description**
Discontinuous, interrupted explosive sounds Loud, low in pitch	Coarse crackle	Crackle	Escape of water from a bottle held with mouth directly downward
Discontinuous, interrupted explosive sounds, Less loud than above and of shorter duration; higher in pitch than coarse rales or crackles	Fine crackle	Crackle	Crepitation of salts in a heated dish. Noise emitted by healthy lung when compressed in the hand
Continuous sounds Longer than 250 msec[(2)] high-pitched; dominant frequency of 400 Hz or more.	Wheeze	High-pitched wheeze	Prolonged whisper of various intonations; chirping of birds; sound emitted by suddenly separating 2 portions of smooth oiled stone.
Continuous sounds Longer than 250 msec low-pitched; dominant frequency about 200 Hz or less; a snoring sound	Rhonchus	Low-pitched wheeze	Snoring; bass note of a musical instrument; cooing of a wood pigeon

(1) ATS: American Thoracic Society
(2) msec: millisecond
This table is to show the terms for adventitious lung sounds adopted by the American Thoracic Society and British researchers along with the origin descriptions.
Source: Adopted from Murphy (2008)

Added lung sounds have a number of sources and their conditions may change from breath to breath in the presence of airway secretions. The presence of added lung sounds is reported most of the time to indicate a pulmonary disorder (Sovijärvi et al, 2000a). Wheezes are believed to reflect changes in the airways whereas crackles are believed to reflect the status of the lung parenchyma and/or the airways (Pasterkamp et al, 1997).

As crackles are a common physical finding in patients with ILD (Loudon and Murphy, 1984), COPD (Epler et al, 1978) Pneumonia (Piirilä, 1992; Murphy et al, 2004; Piirilä et al, 1991) and are considered to be an important early presenting symptom in most lung disorders (Sovijärvi et al, 2000a), the focus of this research has been on crackles. Cigarette smoking is acknowledged to cause harmful changes in the lung which might lead to these reported diseases.

2.2 Lung sound analysis: history and development

Displaying lung sounds visually started in 1924 using a condenser microphone and oscilloscope that displayed normal and abnormal lung sounds (Murphy, 1981). In 1970, the calibrated amplitude plot enhanced the visual presentation of the overall amplitude of sounds and helped in studying the inspiration and expiration amplitude differences (Murphy, 1981). However, the first attempt to obtain objective characteristics of lung sounds was made possible by time expanded waveform analysis (TEWA). TEWA, a scientific method for differentiating the various lung sounds, was implemented by Murphy et al (1977). They plotted sound amplitude versus time at scale of 400 mm per second which allowed visualization of different categories of lung sounds.

However, during the last three decades, many studies have been undertaken to better detect and differentiate the signals from various lung sounds. However, early work lacked standardized guidelines for data recording, storage, signal processing and analysis of the lung sound signal which made it difficult to compare results from different researchers. This led to slow commercial development of the equipment used for respiratory sound analysis (Mussell, 1992). Several efforts have been undertaken to improve these issues. The Computerized Respiratory Sound Analysis Project (CORSA) was an action project within the BIOMED1 programme, financed by the European Community to solve the above mentioned problems.

The main objective of the CORSA project was to develop guidelines for the research and the practice of lung sound digitalisation and analysis (Sovijärvi et al, 2000 b). The CORSA project was intended to facilitate the development of standardized lung sound analysis equipment and promote research into the understanding of respiratory sounds. Now, by following CORSA guidelines CALSA can be used to quantify lung sounds, make permanent records of the measurements taken and produce graphical representations that will assist in the diagnosis and management of patients suffering from chest diseases (Sovijärvi et al, 2000 b). Following guidelines and recommendations makes it easier to compare one study to other studies and to follow the recommendations of each. The recommendations of CORSA for environmental and subject conditions and breathing manoeuvres in respiratory sound recordings are listed in Appendix A1. The development of crackle recording techniques, analysis procedures and counting methods will be discussed in the following section.

2.3 Crackles

Crackles are categorized as discontinuous added lung sounds. They are described as intermittent, non-musical and brief sounds which were thought to be generated during inspiration as a result of sudden opening of the airways (Forgacs, 1978). They were originally believed to be associated with air bubbling through secretions in the airways. However, crackles are also heard in patients with ‘dry’ lung diseases like fibrosing alveolitis (in which sputum is usually absent) (Forgacs, 1967).

Forgacs (1969) reported on his observations that crackles were present more during inspiration than expiration. He hypothesised that most crackles are generated due to the sudden opening of previously closed airways, by an explosive equalization of pressure. The explosive sound could be generated by air bubbling through secretions in the airways (Forgacs, 1969).

2.3.1 Origin, mechanism and location

Forgacs’ work, during the 1960s and 1970s, revealed new insights into the nature and origin of normal and added lung sounds. He defined crackles as a sequence of short interrupted sounds with a wide spectrum of frequencies. Crackles could differ in frequency (frequency in hertz (Hz) = 1/cycle (seconds)) low or high; number, scanty or

profuse; amplitude, loud or faint; and timing, inspiratory or expiratory, regular or random. Further, he discovered the chronological occurrences of crackles during breathing (Forgacs, 1969). The pattern of crackle occurrences was determined by listening to recordings of breathing played at a reduced speed.

On the other hand, Fredberg and (Fredberg and Holford, 1983) proposed an alternative view of crackle production. They developed a mathematical model of fine crackles in an attempt to predict their behaviour. This model was constructed around the idea of the differences in static elastic stress distribution (i.e. the mechanical forces affecting the lungs and airways) in surrounding lung tissue. They hypothesise that static elastic stress distribution is different around closed and open airways, which they refer to as a stress anomaly. When an airway opens, they hypothesised the stress anomaly disappears. These changes in elastic stress near the airway can be detected as crackles. As a result, they proposed that fine crackles are produced by vibration in the walls and interstitium of the peripheral airway. They managed to produce crackles that were consistent with the published observations of real crackles in shape, duration, amplitude, frequency, content and distribution through idealizing the crackle-generating event and transmission medium. This view focuses upon dynamic events that occur in and near the airway wall when elastic and surface forces are in transition between static equilibrium states, whereas Forgacs' hypothesis focuses upon airway gas dynamics that follow airway opening, to the exclusion of the dynamics of the opening process itself. Nevertheless, Forgacs' thoughts and Fredberg and Holford's interpretation suggested the same conclusion; fine crackle generation is related to the sudden opening of a closed airway. This theory has been accepted in most recent lung sound research (Alencar et al, 2001; Piirilä et al, 2000; Piirilä et al, 1991). They are believed to be generated by pressure equalization change in elastic stress after a sudden opening or closing of airways (Sovijärvi et al, 2000b), or when there is inflammation or oedema in the lung (Davie et al, 1997). Crackles are explosive and transient sounds and their frequency might be affected by the diameter of the airways, which is related to the pathophysiology of the surrounding tissue: the smaller the airways diameter the higher the frequency of the crackles (Piirilä and Sovijärvi, 1995a).

The appearance and the timing of crackles could be used as an early indication for the presence of respiratory disease (Sovijärvi et al, 2000b). The frequency of the sound

allows direct estimation of the sound origin (Kompis et al, 2001) where the high frequency sounds travelled through the airway branching structure whereas the low frequency sounds appear to be generated in the large airways. Expiratory crackles are usually much less frequent compared to the inspiratory crackles. Furthermore, as disease progresses, crackles tend to occur first in the basal areas and later in the upper zones of the lungs. Therefore, the timing of crackles in the respiratory cycle could be used to monitor the progress of the lung diseases.

The closure of small airways is assumed to be related to pleural pressure exceeding airway pressure. This happens in healthy people when they exhale sufficiently to reduce lung volume to approximately the level of the residual volume (Thacker and Kraman, 1982; Leblanc et al, 1970). As a result of changes in lung mechanics, as part of the ageing process, crackles could also happen in older people during normal tidal breathing (Janssens, 2005). Ageing may result in some lung unit closure in the gravity-dependent lung regions during normal tidal breathing (Leblanc et al 1970), which could potentially result in preferential ventilation of regions that are less gravity dependent. This closure will cause the lower lobes of the lung not to be ventilated properly until reaching a proper opening pressure, whereas the upper lobes are much better ventilated (Janssens, 2005; Leblanc et al, 1970). This observation could explain the mechanism behind hearing more crackles at the gravity dependent parts of the lung (Sovijärvi et al, 2000 a).

Several studies (Piirilä and Sovijärvi, 1995a; Sovijärvi et al, 2000 a; Kaisla et al, 1991) have shown that there is a relationship between crackle occurrence and the severity of pulmonary diseases. The theory behind crackle generation is the airways opening and closing. Therefore, they are more likely to be heard in conditions in which the elastic recoil pressure of the lung is increased (i.e. there is a decreased lung compliance and the lung are more difficult to inflate). This occurs in several acute and chronic disorders which give rise to inflammation, pulmonary oedema or infiltrative cells in the lung (Piirilä and Sovijärvi, 1995a; Sovijärvi et al, 2000 a). Thus, the assessment of crackles could help in diagnosis and follow up of pulmonary disorders (Loudon and Murphy, 1984).

The number and distribution of crackles per breathing cycle has been reported to be associated with the process and severity of the disease in patients with congestive heart failure (CHF), pneumonia (PN), asthma, COPD and interstitial pulmonary fibrosis (IPF)

(Murphy, 2008; Murphy et al, 2004; Piirilä, 1992). Piirilä et al (1991) studied crackle features and found differences between COPD, fibrosis alveolitis and heart failure. Standard auscultation would be unable to assess all these characteristics accuretly (for example: number of crackles, duration, or frequency), but CALSA has the potential to identify patterns objectively.

2.3.2 Parameters and classification

Until Forgacs, in the 1970s, studied how the normal and added lung sounds are generated, there was no clear acoustic definition for any type of added lung sounds. He suggested the analysis of crackles based on time or frequency and the quantitative description of intensity and spectral content of the signals time. At this time Forgacs described fine crackles as high pitched (high frequency) and coarse crackles as low pitched (low frequency). Crackles are defined acoustically as a series of short intermittent sounds within the 100 to 2,000 Hz frequency spectrum; the duration of the two cycles' deflection (2CD) is less than 20 msec (Forgacs, 1978; Sovijärvi et al, 2000a; Sovijärvi et al, 2000b). The American Thoracic Society (ATS) classified crackles according to the mean of the initial deflection width (IDW) and 2CD (Figure 2). The IDW is the duration between the beginning of the crackle and the first deflection; the 2CD is the time from the onset of the crackle to the point where the waveform of the crackle has completed two cycles. They are both measured in milliseconds.

The IDW and 2CD for the fine crackles according to ATS are 0.7 and 5 msec and coarse crackles are 1.5 and 10 msec respectively (Table 2). The fine and coarse crackles were classified by CORSA to have a mean of 2CD < 10 msec and > 10 msec respectively.

Table 2: Crackles classification.

Description	2CD [1]	IDW [2]
ATS [3]		
Fine crackles	5 msec[5]	0.7 msec
Coarse Crackles	10 msec	1.5 msec
CORSA [4]		
Fine Crackles	< 10 msec	---
Coarse Crackles	> 10 msec	---

(1) - (2CD): the duration of two-cycle deflection
(2) - IDW: initial deflection width
(3) - ATS: American Thoracic Society
(4) -CORSA: Computerized Respiratory Sound Analysis.
(5) - msec: milliseconds

As this research is applying the guidelines of CORSA the crackles will be defined following the CORSA definition. Following guidelines allows the study to be comparable to the other studies using the same guidelines. Holford (1982) applied the method of TEWA and proposed the use of the IDW and the 2CD of the crackle to differentiate fine from coarse crackles (Figure 2). Sovijärvi et al (2000a) also used IDW and 2CD in his study. Hoevers and Loudon (1990) used the large deflection width (LDW), which represents the duration from the beginning the crackle to the end of the crackle signals, in addition to the other two time-domain parameters (IDW, 2CD) to describe crackles. They found LDW better a parameter than IDW in classifying crackles into fine and coarse but their study lacked specificity where the crackles were extracted from teaching tape with unknown condition or origin. These measures were not applied to crackles from patients with known conditions.

Munakata et al (1991) conducted a study on 16 patients with IPF and 10 patients with chronic bronchitis with daily sputum production. The patients with pulmonary fibrosis were judged clinically to have fine crackles that were heard only during inspiration and no sputum production and the chronic bronchitis patients to have mainly coarse crackles with expectoration of sputum. All pulmonary fibrosis patients had the same diagnostic criteria or had histological confirmation of the diagnosis: progressive-dyspnoea without spirometric evidence of airway obstruction, bilateral crackles over the lung, and bilateral interstitial shadows on the chest radiograph. Histological confirmation of the diagnosis by open lung biopsy was obtained in five of the 16 patients. Lung sounds were recorded with an electric condenser microphone. Sampling was limited to a single inspiratory phase for each patient. Five crackles from one inspiratory phase for each patient were sampled

randomly and analysed using time expanded waveform analysis and the Fast Fourier Transform Analysis. They found that the IDW (Mean ± SD: 1±0.3) and the 2CD (4.4±0.14) in pulmonary fibrosis patients were shorter than those from chronic bronchitis (1.88±0.5) and (7.74±0.32) respectively. These differences were statistically significant at level of 1% significance ($P < 0.001$). The 2CD of fine crackles are shorter than coarse crackles. This could be explained by the nature of each disease. The pulmonary fibrosis disease affects the peripheral airways which are smaller and narrower and chronic bronchitis affects the larger airways. The size of the airways might have an impact on the frequency of the crackles originated in them. The lower frequency crackles were thought to be generated in the peripheral airways and this had been confirmed experimentally in animals (Munakata et al, 1986).

The duration of one cycle is inversely related to the frequency (Hz=1/cycle), shorter duration means a higher frequency of the crackle, namely, higher pitched crackles. On the other hand, longer duration mean lower frequency of the crackles, hence lower pitched crackles. The crackles' 2CD could be measured using CALSA. This feature of crackles has been chosen to be measured in this research because it was found to be more stable and reliable than the other parameter, IDW (Marques et al, 2009a).

2.3.2.1 Reliability issues

There are few published data relating to the reliability of the crackles' characteristics. Miller et al. (2005) defined reliability as the extent of agreement between the results of a successive measurement of the same item carried out with the same method, same observer, same instrument, same location, same condition of use, and repeated over a short time. An acceptable level of measurement reliability is essential because it is considered a fundamental requirement of any physiologic measures that affect decision making (Finch et al 2002).

Many studies have shown high intra-subject reliability of digital recording of the lung sounds in healthy people. Mahagnah and Gavriely, (1994) have studied the breath sounds recorded on the trachea and at four locations over the chest wall from five healthy men. Each subject was studied twice with a time interval of one week. The measurements were done in duplicate, with a 30-min interval between recordings to investigate the variability of the spectral pattern of normal lung sounds. They concluded that the values of the

variability were not significant between records of the same day nor the records of different days. Sanchez and Vizcaya, (2003) have studied tracheal sounds in 7 subjects and lung sounds in 10 adults. The acoustic measurements were done in five occasions over a month for tracheal sounds and on seven occasions over a year for lung sounds to investigate the temporal variability in normal adults they concluded that the spectral pattern of tracheal and lung sounds are stable with low intra-subject variability. However, these studies have analysed lung sounds in small samples of mainly healthy subjects. Moreover, the reliability of the specific parameters of crackles and wheezes (IDW, 2CD) were not adequately determined in previous studies. The only authors to explore the reliability of the detection of added lung sounds were Hoevers and Loudon (1990) by exploring the agreement between the two physicians in detecting IDW, LDW and 2CD; and Marques et al (2009a) by assessing the test-retest reliability of added lung sounds using CALSA.

Hoevers and Loudon (1990) reported on the inter-observer and intra-observer reliability of two physicians' measuring crackles' parameters: IDW, LDW and 2CD from a 'teaching tape'. The crackles were displayed as a waveform on a computer screen and the physicians had to identify each crackle parameter with a cursor. These researchers concluded that the agreement between physicians was higher when detecting the crackles' based on their LDW and 2CD than on their IDW. However, the accuracy of the interpretation was not discussed but the agreement between the two physicians was reported.

Marques et al (2009a) found crackle 2CD to be more reliable and stable (i.e., has less measurement error) than IDW. Fifty-four subjects (37 with bronchiectasis, 17 with cystic fibrosis) were recruited from out-patient clinics for their study. Three repeated lung sound recordings were taken at seven anatomical sites with a digital stethoscope connected to a laptop computer. The intra-subject reliability of crackle IDW and 2CD was found to be 'good' to 'excellent', estimated by the analysis of variance, intra-class correlation coefficient (IDW: 0.76, 0.85; 2CD: 0.8, 0.94), Bland and Altman 95% limits of agreement (IDW: 0.50, 0.47 msec; 2CD: 2.12, 1.87 msec) and smallest real difference (IDW: 0.30, 0.66 msec; 2CD: 1.57, 2.42 msec).

2.3.3 Crackles recording

Standard auscultation limited the usefulness of lung sounds because of difficulties in terms of the descriptions and variability in human hearing. In addition, it is difficult for clinicians to remember exactly all the characteristics of lung sounds in all chest location he or she auscultates. However, signal processing methods combined with digital recording has improved the potential diagnostic capabilities of lung sounds. In the following subsections the different methods used to record crackles in previous studies along with the recording sites and the breathing manoeuvres adopted are described.

2.3.3.1 Recording techniques

Electret single microphones and air-coupled condenser microphones have been used in previous studies to record crackles (Nath and Capel, 1980; Shirai et al, 1981a; Piirilä, 1992). They had been used with belts or rubber tapes to attach them to the proper site while subjects are sitting upright. In some studies microphones were connected to a stethoscope and held by hand (Workum et al, 1982; Bettencourt et al, 1994; Piirilä and Sovijärvi, 1995a). The signals from the microphones, in analogue form, were recorded on magnetic or cassette tape (Thacker and Kraman, 1982), FM recorder (Piirilä, 1992) or digitalised and stored in a computer in digital form (Walshaw et al, 1990). The magnetic tapes were replayed later and sampled onto a computer to be processed for analysis. Digitising and storing directly onto a computer was found to minimize the artefacts caused by the quality of the tape recording.

Recent studies have employed a multichannel lung sound analyser (STG 16) to amplify, filter, digitise and store the data on a computer (Murphy et al, 2004; Vyshedskiy et al, 2005; Vyshedskiy et al, 2009; Flietstra et al, 2011). This analyser contains 16 small electret microphones mounted in the chest pieces of a stethoscope. Fourteen microphones, incorporated into a soft foam pad, are attached posteriorly while subjects are in a supine position. One microphone is used to record the heart and one to record tracheal sounds. This technique is applicable for use with the subject in a supine position and only records from posterior sites (primarily over the lower lobes of the lung). Using multichannel microphones with foam pads is beneficial in that the microphones are attached properly to the skin, otherwise microphone movement relative to the skin, might create artefacts and affect the results. Unfortunately, the front and lateral sites are not recorded by this tool.

2.3.3.2 Sites of recording

During early studies, anatomical recording sites varied as researchers generally chose the locations at which crackles were easiest to hear. The CORSA guidelines (Appendix A) recommend specific recording locations (i.e. trachea: on the trachea at the sternal notch; right and left posterior and basal area of the chest, five centimetres laterally from the paravertebral line and seven centimetres below the scapular angle (in adults); right and left anterior area of the chest at the second intercostal space on the mid-clavicular line; right and left lateral area of the chest at the fourth or fifth intercostal space on the mid-auxiliary line (Rossi et al, 2000).

The middle lobes have been ignored in previous studies (Murphy et al, 2004; Vyshedskiy et al, 2005; Vyshedskiy et al, 2009; Vyshedskiy et al, 2011) which have focused on recordings made at posterior chest locations. On the other hand, CORSA guidelines included the middle lobes and refer to them as lateral left and lateral right. The anatomy of the middle lobe bronchus is considered to play a major role in the pathology of middle lobe syndrome, which is characterised by recurrent or chronic atelectasis of the right middle lobe (Culiner, 1966). The bronchus of this lobe is longer than average with a sharp angle at the bifurcation and a narrower airway diameter than other bronchi in different lung segments, which makes the middle lobe more vulnerable to inflammatory disease than other parts of the lung (Albo and Grimes, 1966). It has been reported that the middle lobes are isolated from the other lung lobes and do not benefit from collateral ventilation due to the deep fissure that ensures fewer parenchymal bridges (Ayed, 2004). Cigarette smoke irritation and the subsequent inflammatory process might also lead to enlargement of the lymph nodes arranged about the middle lobe bronchus near its origin, preventing bronchial drainage and thereby causing secretions to accumulate in these lobes (Culiner, 1966). Middle lobe syndrome is considered to be the end result of this process (Ayed, 2004).

2.3.3.3 Breathing patterns during recording

Four main different breathing manoeuvres have been used while recording lung sounds i) Tidal breathing (Nath and Capel, 1980; Walshaw et al, 1990); ii) breathing more deeply at volumes between functional residual capacity and residual volume (Thacker and Kraman, 1982; Workum et al, 1982); iii) breathing as for (i) or (ii), with added breath holds at end

of inspiration or expiration (Walshaw et al, 1990); and iv) breathing deeper than normal with open mouth (Murphy et al, 1984; Vyshedskiy et al, 2009; Marques et al, 2009a). Each manoeuvre has its own advantages and disadvantages; for example during normal breathing the air volume is lower which cause the detected lung sound signals to be weak. While the advantages of normal breathing would be that subjects may not have to make extra efforts for breathing. Breathing more deeply (for longer times) can lead to better detection of lung sound signals but the subjects might lower their carbon dioxide (CO_2) level which can cause cerebral arterial constriction and lead them to feel dizzy. Inspiratory breath holds might support opening atelectatic areas in the lung and improve the likelihood of secretion movements from distal airways to proximal airways. During expiratory breath holds, there might be a greater likelihood of peripheral area atelectasis due to low volumes needed to keep alveoli opened (Duggan and Kavanagh, 2005).

The flow level is lower during nose breathing and has a negative effect on standardization and signal capturing of lung sounds (Rossi et al, 2000) which might have an impact on the accuracy of breathing cycle detection through algorithms. Most recent studies have used breathing deeper than normal with the open mouth technique (Marques et al, 2009a; Murphy et al, 2004; Vyshedskiy et al, 2005; Vyshedskiy et al, 2011). Several studies have used a pneumotachograph to record and control airflow and to aid breathing cycle detection (Shirai et al, 1981b; Walshaw et al, 1990; Piirilä et al, 1991).

2.3.4 Analysis and counting

When using standard auscultation technique, some of the characteristics of crackles such as number of crackles and the frequency can be described subjectively. It would be valuable therefore if measurements of such characteristics could be used to differentiate between lung sounds objectively. Using this analysis it was documented that crackles begin with a short initial deflection followed by deflection with greater amplitude. Thus, the following criteria to describe the true crackle was developed: 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 et al, 1989) (see Figure 2).

Now, using TEWA, automated crackle counters have been developed to overcome the limitations of human hearing in distinguishing between individual crackles. Crackles could be defined and categorised according to certain parameters (Figure 2). The parameters of each crackle could be identified by the computer and then quantified. This will help in counting and comparing this sound objectively.

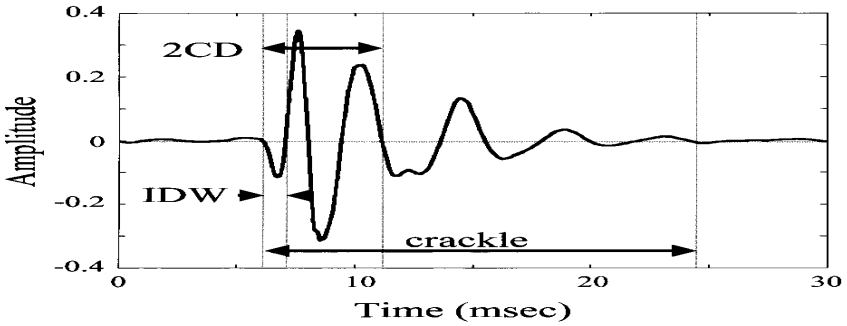


Figure 2: Plot of crackles in time-expanded waveform analysis (TEWA).

- IDW or initial deflection width represents the duration between the beginning of the crackle and the first deflection.
- 2CD (duration of the two-cycle deflection) represents the duration from the beginning the crackle to the time at which the waveform finished two complete cycles.

Adopted from (Kawamura et al, 2003)

2.3.4.1 Crackles counting methods

Murphy et al (1989) developed an automated method to count crackles visualized in the time expanded waveform according to specific criteria based on amplitude, duration and frequency. Two chest physicians and three other observers experienced in lung sounds listened to the recordings independently and estimated the number of crackles per breath to validate the system. The results from the system correlated well with the physicians' results and with the visual count made from the time expanded waveform. The crackle count by the computer based method was higher than those made by the physicians. Many algorithms to automatically detect crackles were developed later (Kaisla et al, 1991; Vannuccini et al, 1998).

Vannuccini et al (1998) proposed a new method to detect and analyse crackles in digitalised lung sounds. The method was based on two steps: (1) a threshold value which was applied to the first derivative absolute value of lung sound to locate the *zone of interest* and then (2) in this zone a crackle was detected if certain conditions were verified. The details of conditions can be seen in Vannuccini's article. An expert observer scanned the recording and pointed out the crackles following Murphy's criteria (see Section 2.3.4). This method showed a high value of sensitivity and specificity (84% and

89% respectively) when validated using 200 inspiratory crackles detected from 15 cryptogenic fibrosis alveolitis patients. It was used in recent studies, Marques et al (2009a) and will be employed in this present research.

2.3.4.2 Breathing cycle detection

The detection of breathing cycles is important because it allowed the results to be comparable between studies. In addition, the timing of crackles in the breathing cycle (early vs late or expiratory vs inspiratory) could have an indication of the crackles origin and the disease stage (Nath and Capel, 1980). The pneumotachograph is considered to be the most accurate method and is considered to be the gold standard for measuring the airflow to and from the lung (Brouwer et al, 2007). Using a mouthpiece or a face mask during recording is considered as one of the disadvantages of this technique where it causes patient discomfort (Akre et al, 2000) and makes it impractical to be used clinically. It might be impossible to be used with children or patients with neurological impairments or unconsciousness where the cooperation of the subjects is very important. These aspects have also been acknowledged in previous studies (Yadollahi and Moussavi, 2007; Moussavi et al, 2000). Measuring flow at the mouth may be difficult to be performed at the bedside during routine physical examination or in the outpatient clinics.

Breathing cycle detection without airflow measurements has been successfully achieved (Moussavi et al, 2000). Twenty-one healthy subjects aged 4-51 years of age were studied by Moussavi et al (2000) using a computer program based on MATLAB to determine respiratory phases. They used six accelerometers to record respiratory sounds in six locations of the chest. The accelerometer is a device that measures vibration. It converts the acceleration of air movement along the axis of accelerometer into an electrical signal. It is popular in lung sound research and can be calibrated on a vibration table so their output is quantified. The accelerometers were attached by double sided adhesive tape rings. The principle of the program was to measure the difference between breath sound intensity during inspiration and expiration. They found that inspiratory and expiratory sound intensities increased with increasing airflow. The inspiratory and expiratory sound intensity was found to be significantly different in all frequency bands for most of the recording locations (on the midclavicular line: the 2nd intercostal space on the left and 3rd intercostal on the right). They concluded that using only acoustical sensors provided accurate timing of the breaths and simplified instrumentation significantly. However, this

was in healthy subjects breathing normally and may not extrapolate to patients with respiratory problems.

In a recent study using CALSA, as an outcome measure for respiratory therapy, a pneumotachograph was not used. They meant to assess the potential of CALSA to be used as an outcome measure for respiratory interventions in a clinical environment so the breathing cycles were identified from recorded data without pneumotachograph. To overcome this difficulty, the subjects were instructed to breathe deeper than normal by the researcher, who carefully observed their performance (Marques, 2008). The researcher observed the breathing pattern of the subjects during recording to try to get symmetrical breathing which could help in better detection of the breathing cycles. This breathing pattern helped in better detection of the breathing cycles using a semiautomatic algorithm developed for this purpose. The same breathing pattern and the semiautomatic algorithm will be adopted in this present research.

2.3.5 Crackles relationship with anthropometrics

The detection of crackles during auscultation might not be always considered a sign of lung pathology. Some studies have reported the presence of crackles in healthy subjects who did not have any current or previous pulmonary problems (Thacker and Kraman, 1982; Workum et al, 1982). They reported that crackles were detected in normal subjects who inhaled slowly from near residual volume while auscultation was performed. Moreover, they found that expired volumes during auscultation play a role in the occurrence of crackles. This might happen during the reinflation of the lung which had undergone partial segmental collapse as a result of reducing lung volume (Thacker and Kraman, 1982). Thacker and Kraman (1982) reported the occurrence of the crackles in the lateral right site (right middle lobe) in about 50% of the subjects in their study. They did not study the left middle lobe because they did not have the proper technology to minimize the interference of the heart sound. The study was on healthy people (eight women and 44 men) aged 22-39 years.

Some demographic characteristics – gender, age and body mass index (BMI) – were found to have an effect on the pulmonary function in previous studies (Jones and Nzekwu, 2006; Janssens, 2005; Canoy et al, 2004; Xu et al, 1994). The relationship between crackles and demographics are an underdeveloped area of research. In the

following subsections, studies showing the possible effect (indirect) of the demographics on crackles will be discussed.

2.3.5.1 Gender

Many authors under different formulations have identified that cigarette smoking is associated with low levels of lung function parameters in female smokers. Several factors might cause these differences, for example gender-related differences in airway geometry, different smoking behaviours, and differences in environmental and occupational exposures (Becklake and Kauffmann, 1999). There are gender differences in lung anatomical development and airway behaviour across the human lifespan (Becklake and Kauffmann, 1999). In a recent study among Norwegian adolescents, researchers found that the impact of smoking leads to a higher risk of respiratory symptoms in girls than boys (Tollefsen et al, 2007). In a longitudinal study on the effect of cigarette smoking on lung function in adolescent boys and girls, researchers found that cigarette smoking has an impact on mild airway obstruction, where a slower growth in lung functions was more visible in girls (Gold et al, 1996). In addition, findings regarding the differences in effects of smoking between males and females on the lung function in previous studies have varied.

The frequency spectra of vesicular breath sounds were found to differ significantly ($p < 0.05$) between men and women. Women had a larger proportion of higher frequencies than men in a study of the relationship between normal lung sounds, age, and gender (Gross et al, 2000). Lung sound was recorded in four locations in the posterior thorax of 162 subjects. The data were analysed according to age, sex, and smoking habit. Dicpinigaitis (2003) suggested, in his study on cough reflex, sensitivity to be gender specific when analysing the measurements among different populations, where it was reported in early studies that the cough reflex is significantly more sensitive in women. Therefore, gender-specific comparison is pursued in this present research because of the documented gender differences as reported in this section. Many gender differences were reported in many aspects so the gender effect should be considered in the analysis.

2.3.5.2 Age

For issues related to smoking and lung disorders, older people were involved in studies more than other age groups. In the present research, this is noted to be a research gap for further work. Therefore, this present research is focused to investigate the added lung sounds among young adults (20-35 years of age).

The majority of studies on smoking and its health impact have been conducted among middle-aged or older people (Elders et al, 1994). In some studies, healthy young adults were included as a control group. A recent study by Kataoka and Matsuno (2008) used auscultation and computed tomography scan (CT scan) to examine the prevalence of pulmonary crackles among patients with cardiovascular disease stratified by decade. In 6-12 months observation they reported an increase in the risk of pulmonary crackles with age - three times for every decade older than 45 years - in people without structural or functional heart disease. Minimal interstitial changes in some patients with crackles were detected using high-resolution computed tomography (HRCT) scan, suggesting that other factors could cause crackles rather than simply aging. They also observed that age-related pulmonary crackles are likely to be fine and basilar in posterior location.

In a study in smokers older than 45 years without respiratory symptoms using spirometry, it was found that the abnormality rates in lung function parameters are relatively high in men and women. However, this abnormality rate was found to be similar in current and former smokers as compared to non-smokers in subjects younger than 45 years of age (Ferguson et al, 2000).

Changes in breathing sounds in pathologic processes have long been known and studied. These changes are much more pronounced, with the result that slight age-dependent changes can be neglected in the automatic detection of lung diseases. “Electronic auscultation” could be introduced as a routine clinical technique for the objective diagnosis of lung diseases and their progress, without consideration of the age of the patient (Gross et al, 2000). However, age might not have an effect on lung sounds without other factors, particularly in young people. Murphy (1984) reported an increase in the prevalence of crackles with both exposure and age while studying crackles as an early detection of asbestosis. This could be related to the duration of exposure which was found

closely correlated to age; in addition, they did not find any effect of age on the prevalence of crackles in the control group.

2.3.5.3 Body mass index (BMI)

BMI is the weight in kilograms divided by the squared height in metres² (kg/m²). It is used as an indication for the obesity in many previous respiratory function studies (Canoy et al, 2004; Lazarus et al, 1997; Gross et al, 2000; Jones and Nzekwu, 2006). According to the World Health Organization (WHO) classification, a BMI of <18.5 is underweight, 18.5 - 24.9 is normal, and more than 25.0 is overweight (Schachter et al, 2001). Population surveys using BMI have generally reported lower levels of ventilatory function among subjects with a high BMI (Schoenberg et al, 1978) and another study has found that an increasing BMI is associated with an accelerated loss of ventilatory function (Chen et al, 1993).

However, obesity is associated with wheeze which might result from extra thoracic obstruction, which might lead to obstructive sleep apnoea and breathlessness more than other symptoms in healthy people (Shepard et al, 1991). In 2005, King et al concluded that obesity is associated with reduced lung volume, which is linked with airway narrowing. The mechanisms causing airway narrowing and differences in obesity are unknown. Their measurements included: presence of asthma (wheeze and airway hyper-responsiveness), functional residual capacity and airway conductance. These variables were measured in 276 randomly selected subjects aged 28–30 years to see the effects of body weight on airway calibre. There is no clear evidence from the literature that shows association between BMI and lung sounds but it was found that a BMI above 30 kg/m2 could decrease both function residual capacity and expiratory reserve volume by approximately 1% for each unit increase in BMI (Jones and Nzekwu, 2006). With BMI increasing globally (Finucane et al, 2011) and the reported relationship between function residual capacity and airway resistance, the frequency of complaints of shortness of breath in people with high BMI could be expected to increase. In addition, inactivity and obesity have been identified as risk factors when interacted with smoking behaviour (Decramer et al, 2011).

2.3.6 Crackles relationship with pathology

The origin and description of crackles have been discussed in Section 2.3.1. The main focus of this research is crackles in smokers and non-smokers, but there is no literature published specifically about smoking and lung sounds. However, there is a body of evidence related to various lung diseases and associated lung sounds. In this section, literature related to lung diseases and crackles will be reviewed.

A study by Elper et al (1978) reviewed the medical records of the ILD patients going back over 28 years. There were 272 ILD patients diagnosed by open lung biopsy compared to 335 patients whom were diagnosed clinically. Crackles were reported to be heard in more than 60 percent of the patients using an ordinary stethoscope. Elper et al found that the incidence of crackles did not differ significantly between the two groups. They reported the correlation in timing, quality and number of crackles differences to clinical, physiologic, radiologic and pathologic features in the ILD patients. They found a significant correlation at 1% level of significance ($P < 0.01$) between the functional impairment as estimated from lung biopsy and the presence of fine crackles. They also reported a significant correlation at 5% level of significance ($P < 0.05$) between the microscopic honeycombing distribution and severity, from the chest X-ray, with the presence of fine crackles.

Baughman et al (1991) also assessed crackles in ILD. They recruited two types of ILD (sarcoidosis ($n = 17$) and cryptogenic fibrosing alveolitis ($n = 11$)) patients. Standard auscultation was performed by two clinicians on all patients. They reported that crackles were present in all patients with cryptogenic fibrosing alveolitis but only in two patients with sarcoidosis. They used HRCT to study these differences and found that crackles were associated with subplural fibrotic changes found in the lower lobes. Moreover, this difference in the prevalence of crackles between the two conditions might be related to the distribution of parenchymal fibrosis. The had not studied the middle lobes where the recording of lung sound was conducted on 5 anatomical sites (at the 2 bases, at the 2 apices and at the mouth). Cryptogenic fibrosing alveolitis is a chronic lung disease characterised initially by the presence of inflammatory cells within the alveoli. Thus the middle lobe anatomy could play a role in the progress of this disease as discussed in Section 2.3.3.2.

The location, timing and quality of crackles might differ from one lung disorder to another. Nath and Caple (1980) described the timing of crackles during inspiration in three different diseases (bronchiectasis, fibrosing alveolitis, and obstructive chronic bronchitis). Their recordings were conducted using a crystal suction microphone mounted in an aluminium cup fixed on the chest wall on the right or left lung base where the lung sounds were best heard, while the subject was sitting upright. The patients were asked to breathe normally and then to take slow vital capacity breaths. In some patients the recordings were repeated after coughing and on bending forwards without altering the location of the microphone. Different breathing technique might have different effects on the crackle generation. Nath and Caple found that the crackle timing in chronic bronchitis is in the early phase of inspiration; in alveolitis, starts early or mid-phase and continues to the end of inspiration and in bronchiectasis is confined to early and mid-inspiration and when present in late inspiration they did not continue to the end of the inspiration. On the other hand, the occurrence of expiratory crackles was assessed by (Walshaw et al, 1990) in patients with fibrosing alveolitis and was found to occupy the mid-late third of the phase.

However, until the beginning of the 1990s no study reported using computerised lung sound analysis to compare crackles in different pulmonary diseases. Piirilä et al (1991) compared the waveform of crackles and their timing in the respiratory cycle in three lung diseases (fibrosing alveolitis (FA) (n = 10), bronchiectasis (BE) (n = 10), and COPD (n = 10) and CHF (n = 10). An acoustically isolated chamber, using two microphones (one for each lung) encased in plastic supports and attached with rubber belts, was employed to record lung sound. The patients were asked to breathe deep and slow inspiration and expiration while sitting upright through a pneumotachograph with peak flow of 1 litre/second. The number of crackles in the inspiratory cycle was greatest in BE patients with a mean ± SD of (8.5±5.1) then in patients with FA (7.6±3.7) and in patients with COPD (2.9±1.5). The IDW of the crackles was significantly shorter in patients with FA (1.3±0.2) than in patients with BE (1.8±0.2) and COPD (2.1±0.3) and HF (2.1±0.3). The 2CD was significantly shorter in patients with FA (7.7±1.3) than in the other patient groups (BE (10.6±1.0), COPD (11.6±1.1) and HF (11.8±1.3). These results suggest that analysis of crackles may be helpful in the diagnosis of pulmonary disorders.

Piirilä (1992) also investigated the frequency and waveform of crackles and their timing within the respiratory cycle in pneumonia patients (n = 11). The aim was to assess if these characteristics changed during the clinical course of pneumonia. Air-coupled condenser microphones, with a high pas filter of 95 Hz, were used to record lung sounds on the posterior basal regions of both lung. The patients were sitting upright for recording while breathing through a pneumotachograph with a maximum flow of about 1 l/s. The recording was conducted twice within the space of more than two days between recordings. The conclusion of the study was that the crackles varied markedly during the clinical course of pneumonia. The duration of the LDW became shorter and the timing of the crackles shifted towards the end of inspiration. In addition, IDW and 2CD had a tendency to shorten when the patient was recovering from pneumonia, but the change was not significant. These findings could help in the diagnosis of pneumonic crackles.

Most of the recent studies have reported the same methodology of recording lung sound (Vyshedskiy et al, 2005; Murphy et al, 2004; Flietstra et al, 2011; Vyshedskiy et al, 2011). The recording method was described in Section 2.3.3.1. Crackles were counted using automatic validated methods Murphy et al (1989) and defined following CORSA standards. Lung sounds were analysed by performing TEWA rapidly for each channel. The crackles' scores were calculated separately for inspiration and expiration (crackles per respiratory phase).

Prior to discussing the finding of these studies the recording methodology will be discussed. Multichannel lung sound analysers can only be used on the posterior sites ignoring the lateral and the anterior sites as described in Section 2.3.3.1. In the posterior sites, the closest lobes to the surface are the lower lobes of the lung. However, the middle lobes' anatomy differs from the other lobes in the lung in a way that might make them more susceptible to infection. These are closest to the surface at lateral locations. The radiograph in the Murphy et al, (2004) study showed right middle lobe opacification and the microphone closest to this site recorded abnormality. Some previous studies documented crackles in the middle lobes (lateral sites) which might be associated with initiation of the middle lobe syndrome. However, in most pulmonary diseases, crackles appear in the basal (posterior) pulmonary areas first, then as the disease progresses, in the other (anterior, lateral) pulmonary areas (Smyllie et al, 1965).

However, the use of multichannel lung sound analyser enables researchers to calculate the crackle transmission coefficient (CTC). The development of the CTC came about as a result of observing the apparent differences in crackle transmission among different crackles observed on TEWA by Vyshedskiy et al (2005). The thought that crackle transmission might have clinical value was the rationale behind developing a method to examine the crackles transmission systematically. For example, in patients with pneumonia, the crackling sound was transmitted throughout a considerable area of the chest. However, using this methodology requires the subjects to be on the bed during the recording time.

(Flietstra et al, 2011) investigated if crackles of interstitial pulmonary fibrosis (IPF) differ in their transmission and frequency from crackles of congestive heart failure (CHF) and pneumonia (PN). They compared the features of the IPF patients (n = 39) to the lung sounds of the CHF patients (n = 95) and the PN patients (n = 123). They focused on the differences between crackles of IPF and those of CHF and PN. In contrast to the crackles in the patients with PN, the crackles in the patients with IPF were transmitted over a much smaller area. They also found the crackles in IPF are significantly different in the crackle frequency ($P < .001$) from other two diseases. This suggests that the crackles in this disease are created in smaller airways than those of CHF and PN. The results of IDW and 2CD were not reported, but the crackle frequency was reported. However, classifying crackles objectively to fine and coarse crackles, according to CORSA, is by using the 2CD.

Murphy et al (2004) conducted a study to improve the diagnostic accuracy of the physical findings used to diagnose pneumonia, which they considered generally to be low. They have found that an increased NCBC is associated with a greater likelihood of pneumonia in a sample of 100 pneumonia patients aged 69 ± 18 years (58% female) and 100 control subjects (had no clinical evidence of pneumonia) aged 69 ± 7 years (48% female). In pneumonia patients the inspiratory crackles classified as coarse in 63% and as both (coarse and fine) in 99% and 50% of the expiratory crackles were coarse. In the control group, only subjects older than 60 years had crackles. This contradicts many studies reporting crackles in subjects younger than this age (Marques et al, 2009b; Thacker and Kraman, 1982).

However, most of the reviewed diseases in this section could be directly or indirectly related to smoking. For example, it has been reported that an increased risk of community-acquired pneumonia is associated with the smoking status of patients (Almirall et al, 1999). The review of this section might conclude that detecting the early impact of smoking on crackles characteristics might develop a better understanding of the onset or progress of lung diseases related to smoking. The association between smoking and lung diseases will be discussed in the following section (Section 2.3.7).

2.3.7 Crackles relationship with cigarette smoking

The smoking history is reported in Pack-years, the unit of measurement for the amount of cigarettes smoked over a long period of time (pack-years = the number of cigarettes smoked per day multiplied by the number of years smoked divided by 20) (Sovijärvi et al, 2000). However, cigarette smoking is associated with a number of health problems, including various respiratory disorders (Skurnik and Shoenfeld, 1998). Longer exposure to cigarette smoking might cause complicated health problems. One reason for this is that tobacco smoke contains complex toxic gases which are inhaled into the lung. When these toxic substances pass through the airways they can stimulate both local and systemic inflammatory responses by stimulating alveolar macrophages to release the pro-inflammatory cytokines and chemocytokines such as neutrophil chemotactic factors, including interleukin 8 (IL-8) and lipid mediator (LTB-4), to phagocytise these particles (Attili et al, 2008; Hogg, 2004). Consequently, these macrophage cells release protease to break down connective tissue in the lung parenchyma and also stimulate mucus hyper-secretion around damaged tissue. This leads to problems such as airway obstruction, lung fibrosis and emphysema. In emphysema, the walls of the air sacs (alveolar septae) are destroyed; consequently, the individual air spaces (alveoli) become larger but irregular and decreased in number (Screaton and Koh, 2004; Moon et al, 1999). This might have an impact on the air movement in the distal airways and causes some of them to close and open irregularly depending on the air volume, which might generate crackles.

Researchers have been investigating the effects of cigarette smoking on the lung for a considerable period of time. Hogg et al (1968) for example, studied excised lungs of five people with normal lungs and nine with diseased lungs, seven with emphysema, one with bronchiectasis and one with bronchiolitis. They measured small airway pressure to investigate the site and mechanism of airway obstruction. The data showed that the major

site of airway obstruction in the lung of patients with COPD was in the peripheral airways (Hogg et al, 1968). They reported also that the changes seen in the small airways could be reversible or irreversible. Mucus plugging and acute inflammation of the airways could be reversible but fibrosis, distortion and narrowing of the airways might not. The researchers had not reported any information about the age of these cases. Moreover, the method used to measure the small airway pressure was used in living dogs. It is appropriate for post-mortem human lungs but it is difficult to be validated.

There is a possible association between cigarette smoking and an increased risk of developing emphysema. Ogushi et al (1991) performed broncho-alveolar lavage in a population of 12 young adult non-smokers and eight young adult current cigarette smokers. Neither group showed evidence of disease by history, physical examination, chest X-ray or lung function tests. The average age of the non-smokers was 29±2 years and of the smokers was 30±3 years and the average smoking history of the smokers was 15±4 pack-year. Bronchoalveolar lavage was performed on the subjects of the two groups. They concluded that cigarette smoking is associated with a decrease in the lower respiratory tract leukocyte elastase inhibitory capacity, which increases the chances of exposing the lung to elastolytic destruction, leading to an increased risk of developing emphysema.

It has been reported, in an early study by Gold et al (1996) that the effects of cigarette smoking on lung function in adolescent boys and girls, that smoking five cigarettes or more per day, is associated with a slower growth rate of lung function parameters as measured by forced expiratory volume in the first second (FEV_1) compared to non-smoking subjects (those who had never smoked). The association between passive smoking and reduced lung function measures in adult subjects has been reported in many studies, even though these associations were different between studies (Frette et al, 1996; Lebowitz et al, 1987). It was reported in previous studies by Hill et al (2007) that exposure to passive smoking increases the risk of developing lung cancer, ischaemic heart disease and stroke, with growing evidence of increasing the risk of various respiratory diseases. Jaakkola et al (1991) concluded in their longitudinal survey of a young population (aged 15-40 years) that exposure to environmental tobacco smoke at home and work does not lead to major ventilatory deterioration. Whereas Eisner (2009), defined passive smoking as an exposure to as little as one hour per day of tobacco smoking and

concluded in his study that it can cause an acute decline in lung function. He further stated that longer-term exposure can induce asthma, excessive decline in lung function, and possibly COPD. However, crackles have not been studied in relation to cigarette smoking (passive or active) but if we consider the changes in the lung which have an effect on the lung function parameters, FEV_1 and forced vital capacity (FVC), it could be hypothesised that the changes could be an indication of the changes in the small airways and lung parenchyma which might be presented by crackles.

Cigarette smoking is associated with changes in the lung parenchyma and airways that may lead to a number of pulmonary diseases and pathologic changes. Remy-Jardin et al (1993) detected parenchymal abnormalities using HRCT on healthy smokers who had normal chest X-ray and normal lung function test parameters. Moreover, smoke-related lung damage could be characterised by inflammation, airway obstruction and destruction of the lung parenchyma (Amin et al, 2003). Nevertheless, most of the common chronic lung diseases such as COPD are not easily diagnosed in their early stages but abnormal lung sounds are often found in the later stages. This present research seeks to add to the body of the knowledge concerning the early detection of pathological changes in the lung due to cigarette smoking.

2.3.7.1 Cough reflex

The effect of cigarette smoking on cough reflex sensitivity (protective function preventing foreign material from entering the respiratory tract and easing the mucus mobilization from the airways) had been relatively underexposed. However, Dicpinigaitis (2003) found cough reflex sensitivity in smokers is significantly diminished compared to non-smokers at 5 % level of significance. This might affect the clearance of secretion leading to secondary pulmonary infection which might lead to damages to lung parenchyma and the airways. The study included 20 male smokers and 50 male non-smokers where the smokers were defined as those who smoke at least five cigarettes daily for one year.

Rubin et al (1992) reported that long-term cigarette smoking induced changes in the character of airway mucus (volume and mucociliary mobility) which might play a role in moderating cough reflex sensitivity. They compared the physical and transport properties of tracheal mucus from 16 asymptomatic smokers and 18 non-smokers. Significant differences were identified in the composition of mucus (larger volume of mucus, lower

solids content and a lower degree of rigidity) from asymptomatic smokers compared to the mucus of non-smokers at 5% level of significance. These differences could be assumed to play a major role in composing a barrier, shielding the superficial airway cough receptors from tussive stimuli. However, crackles could originate in environments like this and their characteristics could be changed with time in case of deterioration leading to small airway diseases.

2.3.7.2 Small airway disease related to smoking

Cigarette smoking has an effect on most of the structural components of the respiratory system and leads to both obstructive and ILD (Ryu et al, 2001; Attili et al, 2008). Diagnosis of these disorders by chest X-ray or lung function test is dependent on the severity of the damage. The concept of early and reversible damage to the lung by cigarette smoking is the rationale for searching for a method to detect the early changes in the lungs of smokers in this research.

The lungs of young smokers and controls of comparable age were studied by Niewoehner et al (1974) from a population of sudden non-hospital deaths. There were 39 cases (19 smokers and 20 non-smokers). The mean age was 25±1.2 years did not differ significantly between the groups at 5% level of significance. The history of smoking was 20±4.1 pack years. The history was obtained by personal interview with close relatives. The most characteristics lesion in the peripheral airways of the young smokers was respiratory bronchiolitis which was present in all smokers but only in five of non-smokers. This study showed an association between cigarette smoking and pathologic changes in the peripheral airways in young smokers with significant (at 5% level) increase in the inflammatory cells. They speculate that the respiratory bronchiolitis could be precursor of emphysema and obstructive airway diseases.

2.3.7.3 Chronic obstructive airway disease

COPD is a condition in which inflammation in the small airways of the lung leads to impaired ventilation of the lung. The morbidity and mortality rates are higher in people with COPD compared to those without it (Sin and Man, 2005). Abnormalities in small airways are very important for diagnosing COPD but pulmonary function tests alone might not accurately detect these abnormalities when they are small (Macklem, 1972).

COPD affects 210 million people in the world, according to a recent estimation of the WHO, including 44 million people in Europe. It is presently the fourth leading cause of death and it will become the third leading cause of death by 2030. It is currently the first leading cause of healthcare expenditure in Europe (Decramer et al, 2011).

Deveci et al (2004) studied the airway wall thickness in three groups (COPD patients, healthy current smokers and healthy non-smokers) using modified HRCT. They found that the airway wall thickness is greater in COPD patients than in healthy current smokers and healthy non-smokers. Moreover, the wall thickness is found to be increased in healthy smokers who had normal spirometry measurement compared with normal controls. Wall thickness is inversely related to the degree of airflow obstruction, and positively related to the smoking history (pack-years). Deveci et al (2004) reported that the obstruction in patients with COPD may be due to thickening of the walls of both the large and small airways.

Crackles patterns were used by Piirilä et al (1991) to differentiate four diagnostic groups (fibrosing alveolitis, bronchiectasis, COPD and heart failure). In their study, they managed to differentiate between the groups by observing the timing and the wave-form characteristics using CALSA. There were 10 COPD patients (two with chronic bronchitis and eight with emphysema) aged 63±6 years. They found an average of 2.9±1.5 crackles per breathing cycle with an average 2CD of 11.6±1.1 milliseconds. They performed lung sound recording in only two sites (lower posterior). Crackles in COPD patients occurred earlier and occupied 22±10 percent of the respiratory cycle.

2.3.7.4 Interstitial lung disease

ILD includes a number of lung disorders which affect the lung tissue and space around the alveoli within the lung, including; desquamative interstitial pneumonia, respiratory bronchiolitis-associated interstitial lung disease, pulmonary Langerhans' cell histiocytosis, and idiopathic pulmonary fibrosis (Ryu et al, 2001). In the epithelium of the central airways, cigarette smoke causes loss of cilia, mucous gland hyperplasia and an increase in the number of goblet cells (Skurnik and Shoenfeld, 1998) which might increase the prevalence of crackles in this disease. Cigarette smoking has an inflammatory effect on the lung tissues and airways (Amin et al, 2003). However, crackles are a

common physical finding in patients with ILD (Loudon, 1984) and are considered to be an important and early presenting symptom (Sovijärvi et al, 2000 a).

Respiratory bronchiolitis (RB-ILD) usually affects current smokers of 30-40 years of age with a 30 pack-year or greater history of cigarette smoking. Male smokers are affected by this disorder more than the female. It might be difficult to stabilize symptoms in most patients with ILD without smoking cessation (Attili et al, 2008). Positive relationships between airway wall thickening and smoking history (pack-years) could have an impact on crackles generation and characteristics. However, narrowing of the small airways and loss of cartilaginous support of bronchi could be the main cause of crackles in most COPD patients. Therefore, crackles could be detected in the early stages of this disease as a result of losing the cartilaginous support due to elastolytic destruction section.

2.4 Summary: a highlight of research gaps

This chapter started with a brief history and description of three types of added lung sounds: crackles, wheezes and rhonchi. The crackles or discontinuous added lung sounds are the main interest of this research. There are two types of mechanism suggested in literature for crackles generation: one is the sudden opening and closing of airways and the other is the air bubbling through secretions. However, the features of crackles vary from one lung pathology to other lung pathologies.

As crackles are brief sounds, they have been difficult to study until the advent of computer based systems for data analysis which provide means to quantify their features. The IDW and 2CD are now used as the key parameters of crackles. These time domain parameters (IDW and 2CD) have been used widely to classify crackles into fine and coarse. In addition to classifying the crackles (fine or coarse) they were used in developing automated methods for counting crackles in early 1990s. However, recently 2CD has been reported to be more stable over short periods of time than IDW (in patients with bronchiectasis and cystic fibrosis).

The review has shown a significant relationship between crackles and a number of different lung diseases, for example, ILD, COPD, bronchiectasis, fibrosing alveolitis, chronic bronchitis, pneumonia, and asbestosis. It is not yet known whether lung sounds

can be used to diagnose respiratory disorders without the aid of additional tests such as chest X-rays and pulmonary function tests.

Cigarette smoking affects the lung in two ways: through direct damage to lung parenchyma and airways, or through enhancing the severity of existing disease. It is not yet known whether cigarette smokers with no diagnosed respiratory disease have detectable damage to their lungs or if the history of smoking will have an impact on the crackles detected. Standard lung function tests are generally not sensitive enough to detect abnormality until significant damage has occurred. It is hypothesised that lung sound recordings may provide a more sensitive measure of lung health.

Therefore this research was designed to answer the following research question:

> *Are there any differences in crackles characteristics between smoking and non-smoking young adults?*

This question led to the following hypotheses:

> H1: There will be statistically significant differences in the crackle 2CD between smoking and non-smoking young adults.
>
> H2: There will be statistically significant differences in the number of crackles per breathing cycle between smoking and non-smoking young adults.

If either of the hypotheses is accepted, the effect of smoking history on the crackles characteristics could be assessed by dividing the smokers group to heavy and light smokers according to the pack-year.

The methodology used to answer these questions and to test these hypotheses will be presented in the next chapter.

CHAPTER 3

Methodology

3.1 Introduction

This chapter describes and justifies the methodology used in this research. It explains the recruitment process, including criteria for subject selection. The data collection procedures are described along with the data preparation process, including justification for the variable analysis choices and the chapter concludes with the data analysis plan.

3.2 Research aim

The aim of this research was to compare crackle characteristics, using CALSA, between smokers and non-smokers. There were two key objectives for this research:

- To evaluate whether there is a statistically significant difference in the 2CD of crackles between smoking and non-smoking young adults using CALSA.
- To evaluate whether there is a statistically significant difference in the NCBC between smoking and non-smoking young adults using CALSA.

3.3 Research design

A cross-sectional design was employed in this observational research involving two groups of subjects: smokers and non-smokers. The subjects were invited individually to attend a single data collection session for 45 minutes. Full ethical approval and sponsorship were received from the Governance Department at the University of Southampton prior to starting data collection.

3.4 Setting

The research was carried out in the School of Health Sciences at the University of Southampton. The data collection was started in the autumn of 2008 after receiving the ethical approval from the school's ethical committee.

3.5 Subject inclusion/exclusion criteria

3.5.1 Inclusion criteria for smokers

Subjects had to be:

- young adults aged 20-35;
- current smokers; and
- have a smoking history of more than five cigarettes per day for at least one year (Dicpinigaitis, 2003)

3.5.2 Inclusion criteria for non-smokers

Subjects had to be:

- young adults aged 20-35; and
- have never smoked actively

3.5.3 Exclusion criteria for both smokers and non-smokers

Subjects who possessed any of the following qualities would be excluded from the research:

- abnormal lung function test result (FEV_1 < 80% of predicted and /or FEV_1/FVC <75%);
- have a history of lung disease;
- have had an upper respiratory tract infection in the last four weeks before recording time; or
- were currently receiving treatment from a clinician.

3.6 Recruitment procedure

Initially, a letter was sent to the Heads of School in the university seeking permission to contact the students in their respective disciplines (Appendix B1). An email was then sent to the students explaining the purpose of the research and inviting them to reply to the researcher if they wished to participate (this research recruited prior to the

School of Health Sciences adoption of a rule preventing email contact for recruitment). An information sheet and contact details were sent to those who showed an interest and a date and time to attend for data collection was arranged on an individual basis.

Moreover, another letter was sent to the heads of the student societies at the University of Southampton to be delivered to the societies' members during their regular meetings (Appendix B2). An information sheet and contact details were sent to those who showed an interest and a date and time to attend for data collection was arranged on an individual basis.

3.7 Sample size

Initially, the plan was to study both genders and the data to be collected from male and female subjects. The response from the female smokers was not adequate and resulted in small number of this group. The decision then was taken to exclude the female gender from this research to avoid bias in results due to the differences reported between male and female either in lung anatomy or smoking behaviour (Section 2.3.5.1). A sample size of more than 30 subjects in each group (smokers and non-smokers) was based on the number estimated by Hopkins (2000) to be appropriate for the smallest real difference calculation which was proposed as a measure of sensitivity to change.

The data were collected by the researcher from 43 male subjects (30 smokers and 13 non-smokers) aged between 20 and 35. Another 17 non-smoker male subject's data were chosen randomly, using the Excel program (Appendix C), from the lung sound data base available in the School of Health Sciences at the University of Southampton after matching the age with the data collected by the researcher. These data were needed to make up the total sample size of the non-smokers male subjects equal to the sample size of the smokers male subjects (30 subjects).

The mean and standard deviation of the 2CD were used to calculate the proper sample size (Appendix D). Based on a sample size calculation, 32 subjects (16 smokers and 16 non-smokers) would be required to get results that reflect the target population. However, this research was conducted on 60 subjects (30 smokers and 30 non-smokers) resulting on high probability (estimated power =0.98) that the test will reject the null hypothesis when

the null hypothesis is false (Appendix E). The high estimated power suggests that chances to make false negative decision could lower.

3.8 Equipment

The equipments used in this research were meant to be portable and usable in non-clinical sittings:

i. Weighting scale (ordinary portable bathroom scale ED-302)
ii. Height measure (ordinary tape measure)
iii. Digital stethoscope (ANR2 Think Labs)
iv. portable Spirometry (Alpha Spirometry, Vitalograph, Ireland)
v. Laptop computer

3.9 Data collection

Subjects were each invited to a laboratory, within the School of Health Sciences, on one occasion. After the study had been explained to them in detail, they were given the opportunity to ask questions and then asked to sign a consent form (Appendix F). A formal screening process was conducted to ensure their eligibility for the study. This involved asking questions about, history of smoking, recent cold or flu and history of any lung disease before starting the main data collection procedure. Three sets of data were collected for this project, i.e., baseline data, lung sound data and lung function data, as described in the following sections.

3.9.1 Baseline data

Baseline data were collected first. This included age, height, weight, and smoking data:

Age: age was measured in completed number of years since birth. Most of the previous studies about the effect of smoking on lungs have involved older age groups (those over 40-50 years old) as reported in Section 2.8.2. In this study, all subjects were aged between 20 and 35 years. This is the age group in which cigarette smoking is increasing more than other age groups. In literature, this age group is defined as consisting of young adults and had been considered as a single group in most of the studies. Therefore it is considered as a single group in this study.

Height, weight and BMI: height was measured in centimetres (cm) and the weight in kilograms (Kg). They were used to calculate the BMI which is frequently used as a proxy measure for obesity in epidemiological studies (Chen et al, 1993). Moreover, BMI of 25 kg/m^2 is defined as the overweight cut-off point according to the WHO classification (Section 2.8.3).

Smoking data: the data were collected on smoking status, number of cigarettes smoked per day and number of years having smoked. There are two groups according to the smoking status as defined in Section 1.1. The groups were given numeric codes: non-smokers=0 and smokers=1. The smoking history was then calculated in pack-years (Section 2.3.7).

3.9.2 Lung sound data

The lung sound data were collected using a digital stethoscope (ANR2 Think Labs) which includes a metal part that allows sound to be heard (stethoscope head), amplifier to help connection with a laptop, two movable metal prongs attached to the tubing which allows the stethoscope to be positioned in both ears (lower binaural tubes), the ear tips, and the tubing which allows sound to transmit up through the amplifier then ear tubes and is made from a flexible material (Figure 3).

Figure 3: Digital stethoscope used for data collection

The lower part of the tubing attaches to the stethoscope head and the upper part attaches to the ear tubes through an amplifier which was connected to a laptop running MATLAB®.

The recording of the lung sound took place on seven sites (the trachea and six chest locations) at a sampling frequency of 44.1 KHz (44,100 times per second). The stethoscope positions and sampling frequency were chosen according to CORSA guidelines (Rossi et al, 2000) (Appendix A2).

Each subject was then asked to sit on a chair with his/her hands on his/her knees. The recording lasted for 25 seconds at each chest location which would normally include four to six breathing cycles. The data were stored in (.wav) format in a separate file for each subject and each chest location including the date and the time of recording.

Subjects were requested to breathe slightly deeper than normal during recordings despite CORSA guidelines recommending tidal breathing, as breathing deeper than normal during recording might have an impact on the detection of the breathing cycle and crackles, where the spectral content of the breath sound depends mostly on the lung volume (Rossi et al, 2000; Murphy et al, 2004). Therefore, breathing deeper than normal would help make the analysis of the sound files easier and to better detect the breathing cycles. Frequent rests were given between recordings to prevent "over breathing" leading to dizziness or other symptoms of low carbon dioxide. The lung sounds obtained were digitised and stored in the computer for analysis. The laptop computer was protected by a password and all data were stored anonymously using codes.

3.9.3 Lung function data

Spirometry measures the amount of air (volume) taken in and exhaled as a function of time and is a reliable, valid, bedside measure (Miller et al, 2005) and has often been used in the last decades as a health indicators in clinics and research studies. The most common measurements used for lung function through spirometry are listed below with the acceptable values according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines:

- FEV_1: Forced expiratory volume in one second. This is the volume of air that can be forced out within one second. Normal values of more than 80% predicted.

- FVC: Forced Vital Capacity is the maximum volume of air that can be blown out in one breath, normal value >80%.

- FEV_1/FVC: Forced expiratory ratio of the maximum volume of air that can be blown out in one breath, this is the proportion that can be blown out in one second, acceptable value > 70%.

These tests can identify abnormalities of lung function, such as obstructive or restrictive patterns, by measuring the FEV_1 and FVC and then calculating the FEV_1/FVC ratio after considering age, sex and height (Crapo, 1994). In obstructive diseases, expiration is slow, giving a low FEV_1 and thus a low FEV_1/FVC (i.e. less than 70%). In restrictive diseases, FEV_1 and FVC are reduced to a similar extent, so that the FEV_1/FVC ratio does not change much (i.e. greater than 70%) (Ward, 2005). It has been reported by Epler et al (1978) that the differences in lung function parameters (FEV_1, FVC, and FEV_1/FVC ratio) between smokers and non-smokers below the age of 45 have been neglected. The spirometry test results were used in many researches on healthy populations for the purpose of excluding subjects with abnormal lung function (FEV_1% predicted < 80% and FEV_1/FVC ratio < 70%).

To assess the presence of airway obstructions or restrictions in the subjects of this study, lung function data were collected using portable spirometry (Alpha Spirometry, Vitalograph, Ireland). During a spirometry test, a patient places their mouth over the mouthpiece of the spirometer while standing, takes a deep breath in, and then blows out as forcefully as possible. The spirometry tests were performed in accordance with American Thoracic Society guidelines (Appendix G) to measure FEV_1, FEV_1% predicted, FVC, FVC% predicted and FEV_1/FVC ratio. The machine was calibrated every day before starting to collect data. The ambient temperatures when the recordings took place were recorded because they are important variables in pulmonary function tests (Miller et al, 2005). Each subject performed three measurements and the highest FEV_1 and FVC were recorded. The need for repeated maximal expiratory effort is observed to cause strong coughs in many subjects. High airflow in the bronchi during coughing found to be important in clearing the airways from excess mucus and to affect the calibre of the bronchi diminishes during the compressive phase of cough (Piirilä and Sovijärvi, 1995b) which might have an effect on the lung sound. Therefore, lung function test was performed after the lung sound recordings. Recently, a study by Vyshedskiy et al (2011) reported that there are no significant differences in crackle frequency nor crackle number between breathes in patients with IPF, CHF and PN even when patients do coughs between manoeuvres. They reported, on the other hand, that crackles were disappeared after three deep breaths in patients undergoing a spinal anaesthesia during hydrocoel surgery. This could be related to mini atelectasis resolving or secretions clearance.

After collecting all information, the lung sound files were processed for analysis. This process included the analysis of crackles and breathing cycles from the sound files. This information was used to test the hypotheses of the study.

3.10 Lung sound files analysis

The data from each of the six chest locations were used. Tracheal data were not included in this analysis as this is not a common site for detecting crackles. However, the site and data were used in this study as a visual test for the correct functioning of the recording equipment. All data obtained will be added to the data base of the Institute of Sound and Vibration Research at the University of Southampton. According to Forgacs' (1967) theory, crackles are possibly due to the opening and closing of the small airways, which means that crackles are generated in the peripheral region and are related to that area (Section 2.3). However, all transmitted sounds are affected by the anatomy of the airways between the source and the recording site (Petak et al, 2006), so crackles detected in the trachea did not originate there.

The data analysis involved checking the sound files for the number and quality of records for each subject before running them through the "My Gui" program for analysis. The "My Gui" program is a special program developed by the Institute of Sound and Vibration Research at the University of Southampton for recording, saving and analysing lung sounds. This program integrates two algorithms: an algorithm to detect breathing cycles and an algorithm to detect crackles. The lung sound files were processed using this program.

Initially, the raw data sound file in (.wav) format was processed through the 'My Gui' program. The data was plotted in MATLAB (Figure 4).

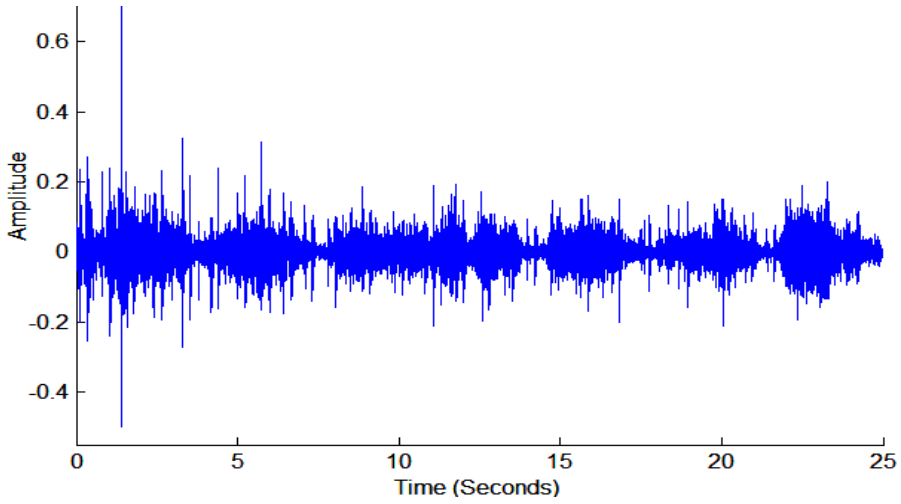


Figure 4: Example of lung sounds original raw data plot of 25 seconds in MATLAB

The data then was down-sampled. Down-sampling is the process of reducing the sampling rate of a signal to reduce the size of the record. Since down-sampling reduces the sampling rate, the resulting digital signal might have aliasing. To avoid the data aliasing, a low-pass filter is used as an anti-aliasing filter to reduce the bandwidth of the signal before the signal is down-sampled; the overall process (low-pass filter, then down-sample) is called decimation. A fourth-order Butterworth low-pass filter with 3-dB cut-off frequency at 2 kHz was applied to the data and then the signal was down-sampled from 44,100 data points per second to 8,820 data points per second.

The frequency range [150,450] Hz was used to recognize the breathing cycle using the logarithmic scale of the power spectrum which showed the highest power of breathing occurs in this frequency range. The remaining frequency range was removed from the processed data. To achieve this, a 7^{th} order median filter to smooth the peaks and remove undesirable noise was used (Figure 5).

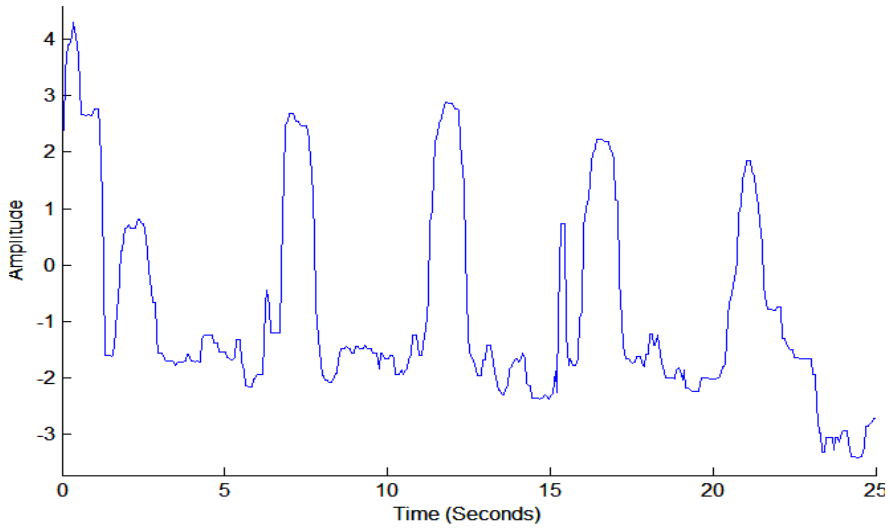


Figure 5: Example of smoothed raw data plot of 25 seconds in MATLAB

3.10.1 Detecting breathing cycles

The breathing cycle's detection could provide valid information related to the timing of the crackles occurrences (inspiratory or expiratory; late or early) and provide a tool for comparison with other studies. In this research, the detection of the breathing cycles was performed without the use of a pneumotachograph despite this being considered the gold standard (Brouwer et al, 2007). It has been discussed in Section 2.3.4.2 why the flow was not monitored at the mouth as performed in other studies; instead a semi-automatic algorithm developed in the Institution of Sound and Vibration at the University of Southampton was used. This algorithm has been used in another study (Marques, 2008) and found to be suitable for daily clinical practice.

The breathing cycles were detected by defining the start and the end of one breathing cycle, while listening to the recording, by the researcher. The maximum and minimum thresholds for the same cycle were also identified: the highest peak was considered the maximum threshold and the lowest peak the minimum threshold. These thresholds were then used to detect the breathing cycles within each file automatically (Figure 6). The disadvantage of this algorithm is that: it depends on the individual judgement of the start and end of the breathing cycle; the threshold value is chosen visually (as an average of the maximum and minimum values) which makes it subject to error. Choosing four points of the first breathing cycle (start, end, maximum and minimum) manually might be subject to individual error which will affect the automatic detection. This might lead to repetition

of the manual process many times while listening to the record until reaching the proper result. However, the algorithm used in this study is the only one available.

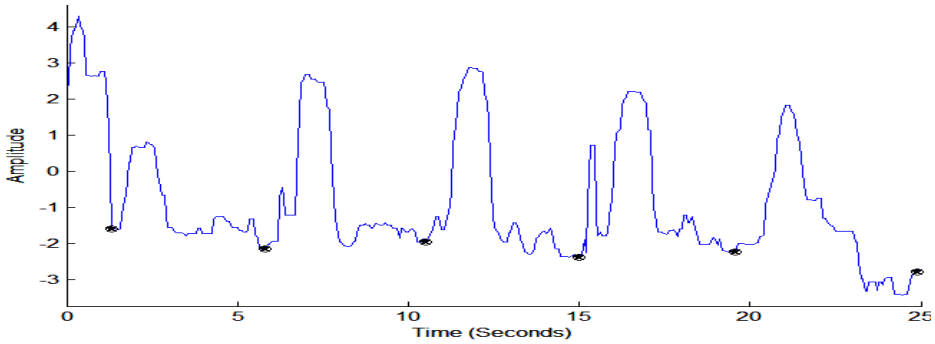


Figure 6: Example of the breathing cycles detection during 25 seconds of data (one breathing cycle between two black dots)

3.10.2 Detecting crackles

There are many published algorithms for crackle detection (Vannuccini et al, 1998; Kaisla et al, 1991; Murphy et al, 1989) with different strengths and weaknesses. However, the algorithm developed by Vannuccini et al (1998) has been used in this study. The sensitivity (ability to reliably identify a genuine crackle as a crackle) and specificity (not to label anything a crackle, when it is not genuinely a crackle) of this algorithm was discussed in Section 2.3.4.1. Moreover, the test-retest reliability of the algorithm was studied by Marques et al (2009a) on lung crackle characteristics in cystic fibrosis and bronchiectasis patients. They concluded that crackle IDW and 2CD characterized by CALSA have good test-retest reliability. The algorithm has also been used in recent studies (Marques et al, 2009a; Marques, 2008).

After the breathing cycles were detected, the crackles were plotted in the signal and a waveform with the breathing cycle detection and the crackles per breathing cycle obtained (Figure 7).

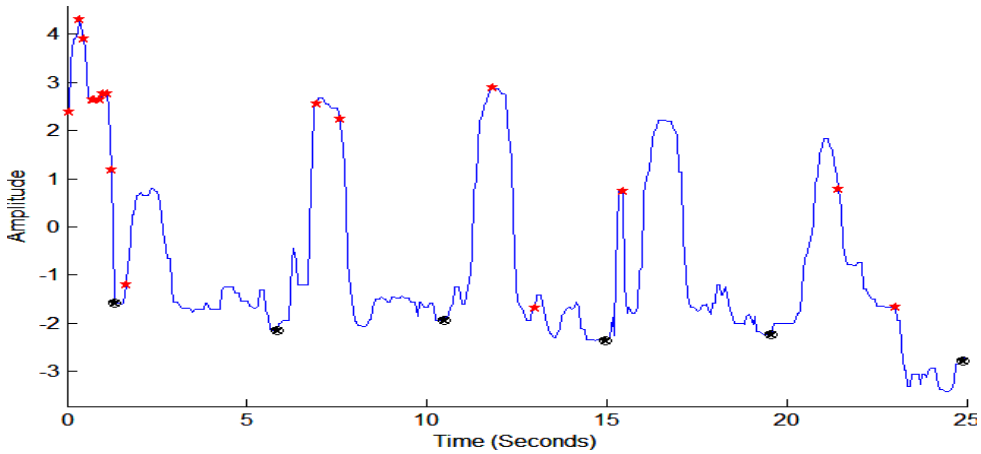


Figure 7: Example of the breathing cycles detection (black dots) with the crackles plotted in each breathing cycle (red stars) during 25 seconds of data plot.

3.10.3 Lung sound analysis results

Three files were generated, one file with a MATLAB extension (.mat) and two files with an Excel extension (.xls), for each recording. The first Excel file saved the information associated with the crackles' variables (number of crackles, IDW and 2CD). The second Excel file was created with the information related to the detection of the crackles per breathing cycle. Each Excel file included information of the breathing cycle beginning (seconds); the breathing cycle ending (seconds) and the 2CD value (msec) of each crackle. The analysis of crackles was made on 25 second long files of recorded lung sound.

In the first Excel file, the researcher manually checked the number of crackles and the 2CD in each crackle. The 2CD was chosen here to represent the crackle's frequency in comparing crackles between non-smoker and smoker subjects. Based on the findings that the 2CD was more reliable and stable, in other words has less error measurement, 2CD might be more responsive to airways pathology than other parameters IDW (Section 2.3.2). However, the total time of the 2CD in 25 seconds was divided by the total number of crackles in each file to acquire the average 2CD in each location. Six variables were produced from this file: 2CD from anterior left location (AL_2CD), 2CD from anterior right location (AR_2CD), 2CD from lateral left location (LL_2CD), 2CD from lateral right location (LR_2CD), 2CD from posterior left location (PL_2CD) and 2CD from posterior right location (PR_2CD).

In the second Excel file, the researcher manually counted the number of breathing cycles and the number of crackles in each breathing cycle. The total counted number of crackles was divided by the total counted number of breathing cycles to acquire the average NCBC in each site in each file and was termed NCBC. Six variables were also generated from this file: NCBC in the anterior left location (NCBC_AL), NCBC in the anterior right location (NCBC_AR), NCBC in the lateral left location (NCBC_LL), NCBC in the lateral right location (NCBC_LR), NCBC in the posterior left location (NCBC_PL) and NCBC in the posterior right location (NCBC_PR).

An Excel file was generated to save the information related to the NCBC and 2CD of the crackles in the six locations of each subject. Information was exported to SPSS version 17 for further analysis.

3.11 Statistical data analysis

The data from 60 male subjects (30 non-smokers, 30 smokers) were used in this analysis. All baseline data, lung function data and lung sound data collected from smoking and non-smoking subjects were compared to each other. The mean and standard deviation (SD) were used for descriptive purposes in this study unless indicated otherwise. All data were entered into SPSS version 17 and descriptive statistics were used to characterise the sample.

An evaluation of the data distribution in each dependent variable was performed. Testing the hypothesis that the data in each variable follows a normal distribution was completed using the Shapiro-Wilk normality test of distribution (Oztuna et al, 2006). Testing the distribution is important because a number of statistical tests and procedures (for example the T-test and ANOVA) assume that data follow a normal distribution. In the case of rejecting a hypothesis, proper transformation usually needs to be carried out (Field, 2004). However, 95% confidence level was used to test the hypothesis of a normal frequency distribution for all dependent variables in this research. The results of testing the hypothesis that variables follow a normal distribution were:

- accepted with NCBC_PL and all 2CD (AL_2CD, AR_2CD, LL_2CD, LR_2CD and PR_2CD) variables; but

- rejected with NCBC_AL, NCBC_AR, NCBC_LL, NCBC_LR and NCBC_PR

All NCBC variables that did not follow normal distribution were transformed to achieve normality using the square root and logarithmic transformation (log10).

A database was created in SPSS, with the data related to 2CD and NCBC detected by CALSA in each recording position. After concluding the normality of the data could be assumed, then one-way analysis of variances (ANOVA) was performed to compare the crackles in smokers and non-smokers.

3.11.1 Lung sound data analysis: one-way analysis of variance

The differences between smokers and non-smokers were assessed using the data from crackles' 2CD and crackles' NCBC using ANOVA. It was reported by Bland (2000) that if there are only two groups, one way analysis of variance is another way to do a two sample t test. Moreover, the F statistic in ANOVA is the square of the t statistic and the two give the same level of probability significance.

The factor of smoking status with 2 levels (smokers and non-smokers) was used initially. In case of finding significant differences in the variables mean between the two groups, the smoker group was further divided to two groups (light smokers and heavy smokers) using the median of four pack-years because the data in this variable was not normally distributed and the median is used primarily to describe data with skewed distributions. Therefore, it was considered in this research to categorize the smoking history according to the median: light smokers equal or below median and heavy smokers above median. The post hoc of Fisher's Least Significant Difference (LSD) test was used in conjunction with ANOVA to determine which specific group pairs are statistically different from each other.

In addition, the analysis of covariance (ANCOVA) was used to test the interaction of BMI and age as a covariate factors with smoking statues on the significant results differences detected on crackles' 2CD or NCBC. ANCOVA is a technique which attempts to make allowance for imbalances between groups and in this instance would try

to determine whether there is a difference between smokers and non-smokers, independent of any age or BMI differences between the groups that may exist

3.11.1.1 Crackles' two cycle deflection duration

For each chest location, comparisons were made between smokers and non-smokers. The test variable was 2CD and the dependent variables were (AL_2CD, AR_2CD, LL_2CD, LR_2CD, PL_2CD, and PR_2CD). Where statistically significant differences found between the two groups (smokers and non-smokers) a further analysis was used to compare non-smokers, light smokers and heavy smokers.

3.11.1.2 Number of crackles per breathing cycle

For each chest location, comparisons were made between smokers and non-smokers. The test variable was NCBC. The dependent variables were (NCBC_AL, NCBC_AR, NCBC_LL, NCBC_LR NCBC_PL and NCBC_PR). Where statistically significant differences were found between the two groups (smokers and non-smokers) a further analysis was used to compare non-smokers, light smokers and heavy smokers.

3.12 Summary

The methodology used in this research has been explained and justified. The recruitment steps, data collection procedures and data preparation processes before starting the analysis have been described. The test for normal frequency distribution was conducted using the Shapiro-Wilk test. Transformation was carried out for the non-normally distributed variables. The chapter ended with a section on detailed data analysis plans.

The results from both descriptive and inferential statistics from this data analysis are presented in the next chapter.

CHAPTER 4

Results

4.1 Introduction

This chapter presents the results of the data analysis. A general description of the whole sample is presented followed by the descriptive characteristics of each group (smoker and non-smoker). The statistical hypothesis of the differences in crackles' 2CD and NCBC, between smokers and non-smokers, is tested using an analysis of variance (ANOVA) technique followed by the LSD and ANCOVA tests in the case of finding significant results.

4.2 The sample

Data were analysed from 60 male subjects in this study (30 smokers and 30 non-smokers). Descriptive statistics of the mean and the standard deviation of age, height, weight and BMI of the whole sample are shown in Table 3.

The age of the subjects was normally distributed between 20 and 35 years of age in this sample. The range of BMI was between 18.6 and 35.3. The descriptive statistics of lung function test parameters ($FEV_{1,}$ FVC and FEV_1/FVC) for all subjects are also presented in Table 3. These parameters were in the normal ranges (exclusion criteria: Section 3.5.3) for all subjects included in this study.

Table 3: Descriptive statistics for the study sample (n = 60).

Variables	Mean	Standard Deviation (SD)
Age	26.6	4.7
Weight (Kg)	75.5	14.1
Height (cm)	174.54	8.1
Body Mass Index BMI (Kg/m^2)	24.8	3.9
Forced vital capacity percent of predicted (FVC_P)	108.6	20.1
Forced expiratory volume in the first second percent of predicted (FEV_1_P)	109	19.9
FEV_1_FVC_Ratio	84.2	4.2

4.3 Group characteristics

The sample of 60 subjects was divided into two groups: smokers group (n = 30) and non-smokers group (n = 30).

The characteristics of the two groups are shown in Table 4. There are no significant differences seen in the age and BMI between the two groups at 5 % level of significance. The average number of breathing cycles detected in this study was 5 breaths with a range between three and eight breaths in 25 seconds recording time.

The lung function test parameters did not differ significantly between the two groups at 5% level of significance.

Table 4: The basline characteristics of the two groups

	Smokers (n = 30)		Non-Smokers(n = 30)	
	mean	SD	Mean	SD
Age	27.1	5.6	26.1	3.6
Body Mass Index (BMI) (Kg/m^2)	25.7	4.5	23.9	3.1
History of cigarettes smoked (Pack-years)	4 (1 – 20)*		-	-
Breathing Cycles detected in 25 seconds recording	5.3	1.2	5.1	1.0
FVC_P	110	17.1	105	23
FEV_1_P	109	17.0	107	22
FEV_1_FVC_Ratio	84	4.1	84	4.3

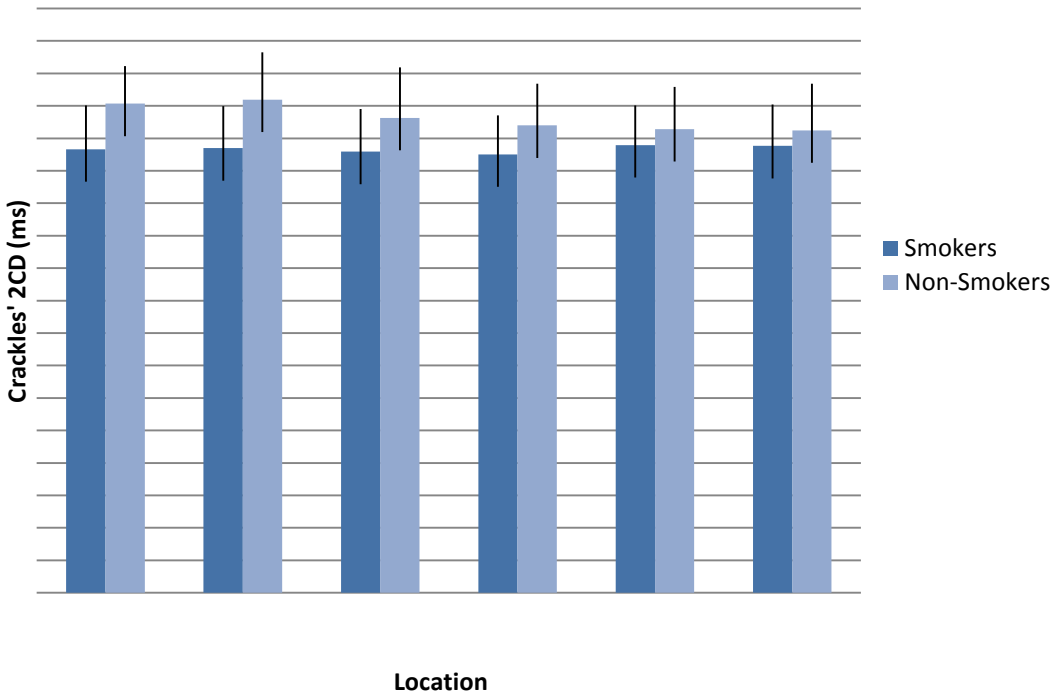
SD: standard deviation
FVC_P: Forced vital capacity percent % of predicted
FEV_1_P: Forced expiratory volume in the first second % of predicted
* The median and the range are reported here because the data is not normally distributed in this variable.

4.4 Crackle characteristics

Two crackle characteristics have been analysed in this study: the crackles' 2CD and the NCBC. Results from 2CD and NCBC at the six chest locations were analysed. The following sections present the results from the analysis.

4.4.1 Crackles' two cycle deflection duration

Crackles' 2CD was analysed and the mean was calculated in each location and compared between smokers and non-smokers. The result of the 2CD mean comparison is shown in Figure 8.



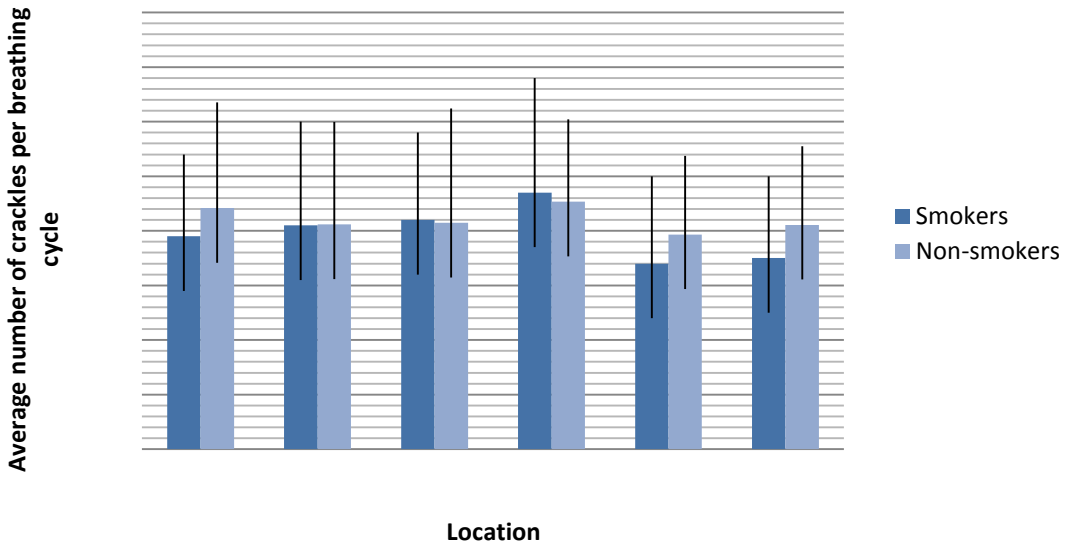
2CD: duration of two cycle deflection; AL: anterior left site; AR: anterior right site; LL: lateral left site; LR: lateral right site; PL: posterior left site; PR: posterior right site.

Figure 8: Average crackles' 2CD (msec) at six locations on the chest; smokers (n = 30), non-smokers (n = 30). * Starred results are significant, p<0.05. Error bars represent ± standard deviation.

The p-value shows that the differences were significant at 5 % level of significance in four locations. This significance allows further analysis which is performed later in Section 4.5 using the post hoc LSD test in two groups of smokers and one non-smoker group.

4.4.2 Number of crackles per breathing cycle

Results from the analysis of NCBC at six chest locations were analysed from 30 non-smoker and 30 smoker subjects. There were no significant differences between smokers and non-smokers in the NCBC at any location. Figure 9 shows the results of NCBC.



AL: anterior left site; AR: anterior right site; LL: lateral left site; LR: lateral right site; PL: posterior left site; PR: posterior right site.

Figure 9: Average number of crackles per breathing cycle detected by CALSA at six sites on the chest of smokers (n = 30) and non-smokers (n = 30). Error bars represent ± standard deviation.

4.5 Crackle differentials between smokers and non-smokers

This section shows the results of testing the study hypothesis at 5% significance level. Two dependent variables (crackles' 2CD and the NCBC) were tested in six chest sites between smoking and non-smoking young male adults. To test the hypothesis analysis of variances (ANOVA) was used followed by post hoc LSD test to further explore the differences between the group of non-smokers (n = 30) and two groups of smokers (light smokers (n = 16) and heavy smokers (n = 14). The smoker group was divided into two groups using the pack-years median value (light smokers group (n = 16) and heavy smokers group (n = 14) (Section 3.11). The pack-years median was four pack-years so the smoking history ≤ 4 pack-years was defined as 'light smoker' and > 4 defined as 'heavy smoker' in this study.

Further, the analysis of covariance was used to test the interaction of BMI and age as a covariate factors with smoking history on the significant differences detected on crackles' 2CD.

4.5.1 Hypotheses testing results

4.5.1.1 Hypothesis one

H1: There will be a statistically significant difference in the crackle 2CD between smoking and non-smoking young male adults.

To test this hypothesis ANOVA was performed on the data (obtained from the six sites on the chests) of the three groups: light smokers group (n = 16) and heavy smokers group (n = 14) and non-smokers (n = 30) followed by post hoc LSD test and ANCOVA.

Anterior left site: H1 accepted (Table 5)

Table 5: Testing Hypothesis 1 anterior left

AL_2CD		DF	F-statistic	P-value
	Between Groups	2	9.40	0.00
	Within Groups	57		
	Total	59		

AL_2CD: two cycle deflection of crackles in anterior left site; DF: degrees of freedom

- **Post hoc (LSD) test:**

The 2CD values in both the light smoker (Mean = 13.79 ± 1.57) and heavy smoker (Mean = 13.52 ±1.09) groups are significantly less than those in the non-smoker group (Mean = 15.07 ± 1.16), but the light smoker group did not differ significantly from the heavy smoker group.

- **Covariance test (ANCOVA)**

There is a main effect of smoking history on AL_2CD ($F(3, 56) = 8.70$, $p < 0.05$); no main effect of BMI ($F(3, 56) = 0.37$, $p = 0.55$) and no main effect of age ($F(3, 56) = 0.28$, $p = 0.60$). There is no significant interaction.

Anterior right site: H1 accepted (Table 6)

Table 6: Testing Hypothesis 1 anterior right

AR_2CD		DF	F-statistic	P-value
	Between Groups	2	9.51	0.00
	Within Groups	57		
	Total	59		

AR_2CD: duration of two cycle deflection of crackles in anterior right site; DF: degrees of freedom; P-value: the probability value.

- **Post hoc (LSD) test:**

The 2CD values in both the light smoker (Mean = 13.97 ± 1.21) and heavy smoker groups (Mean = 13.40 ± 1.36) are significantly less than those in the non-smoker group (Mean = 15.19 ± 1.46), but the light smoker group did not differ significantly from the heavy smoker group.

- **Covariance test (ANCOVA)**

There is a main effect of smoking history on AR_2CD ($F(3, 56) = 8.55$, $p < 0.05$); no main effect of BMI ($F(3, 56) = 0.12$, $p = 0.74$) and no main effect of age ($F(3, 56) = 0.45$, $p = 0.49$). There is no significant interaction.

Lateral left site: H1 accepted (Table 7)

Table 7: Testing Hypothesis 1 lateral left

LL_2CD		DF	F-statistic	P-value
	Between Groups	2	4.2	0.02
	Within Groups	57		
	Total	59		

LL_2CD: duration two cycle deflection of crackles in lateral left site; DF: degrees of freedom; P-value: the probability value.

- **Post hoc (LSD) test:**

The 2CD values in the light smoker group (Mean = 13.42 ± 1.20) is significantly less than those in the non-smoker group (Mean = 14.63 ± 1.55), but the heavy smoker group (Mean = 13.79 ± 1.45) did not differ significantly from the light smoker and non-smoker groups.

- **Covariance test (ANCOVA)**

There is a main effect of smoking history on LL_2CD ($F(3, 56) = 3.50$, $p < 0.05$); no main effect of BMI ($F(3, 56) = 0.60$, $p = 0.44$) and no main effect of age ($F(3, 56) = 1.60$, $p = 0.21$). There is no significant interaction.

Lateral right site: H1 accepted (Table 8)

Table 8: Testing Hypothesis 1 lateral right

LR_2CD		DF	F-statistic	P-value
	Between Groups	2	4.36	0.02
	Within Groups	57		
	Total	59		

LR_2CD: duration of two cycle deflection of crackles in lateral right site; DF: degrees of freedom; P-value: the probability value.

- **Post hoc (LSD) test:**

The 2CD values in the heavy smoker group (Mean = 13.26 ± 1.41) is significantly less than those in the non-smoker group (Mean = 14.40 ± 1.29), but the light smoker group (Mean = 13.72 ± 0.98) did not differ significantly from the heavy smoker and non-smoker groups.

- **Covariance test (ANCOVA)**

There is a main effect of smoking history on LR_2CD ($F(3, 56) = 3.72$, $p < 0.05$); no main effect of BMI ($F(3, 56) = 0.37$, $p = 0.55$) and no main effect of age ($F(3, 56) = 0.45$, $p = 0.50$). There is no significant interaction.

Posterior left site: H1 rejected (Table 9)

Table 9: Testing Hypothesis 1 posterior left

PL_2CD		DF	F-statistic	P-value
	Between Groups	2	1.27	0.29
	Within Groups	57		
	Total	59		

PL_2CD: duration of two cycle deflection of crackles in posterior left site; DF: degrees of freedom; P-value: the probability value.

Posterior right site: H1 rejected (Table 10)

Table 10: Testing Hypothesis 1 posterior right

PR_2CD		DF	F-statistic	P-value
	Between Groups	2	1.39	0.26
	Within Groups	57		
	Total	59		

PR_2CD: duration of two cycle deflection of crackles in posterior right site; DF: degrees of freedom; P-value: the probability value.

4.5.1.2 Hypothesis two

H2: There will be a statistically significant difference in NCBC between smoking and non-smoking young male adults.

To test this hypothesis ANOVA was performed on the data (obtained from the six sites on the chest) of the two groups: smokers (n = 30) and non-smokers (n = 30).

Anterior left site: H2 rejected (Table 11)

Table 11: Testing Hypothesis 2 anterior left

NCBC_AL		DF	F-statistic	P-value
	Between Groups	1	1.20	0.28
	Within Groups	58		
	Total	59		

NCBC_AL: number of crackles per breathing cycle in anterior left site; DF: degrees of freedom; P-value: the probability value

Anterior right site: H2 rejected (Table 12)

Table 12: Testing Hypothesis 2 anterior right

NCBC_AR		DF	F-statistic	P-value
	Between Groups	1	0.01	0.91
	Within Groups	58		
	Total	59		

NCBC_AR: number of crackles per breathing cycle in anterior right site; DF: degrees of freedom; P-value: the probability value.

Lateral left site: H2 rejected (Table 13)

Table 13: Testing Hypothesis 2 lateral left

NCBC_LL		DF	F-statistic	P-value
	Between Groups	1	.11	0.74
	Within Groups	58		
	Total	59		

NCBC_LL: number of crackles per breathing cycle in lateral left site; DF: degrees of freedom; P-value: the probability value.

Lateral right site: H2 rejected (Table 14)

Table 14: Testing Hypothesis 2 lateral right

NCBC_LR		DF	F-statistic	P-value
	Between Groups	1	0.00	0.96
	Within Groups	58		
	Total	59		

NCBC_LR: number of crackles per breathing cycle in lateral right site; DF: degrees of freedom; P-value: the probability value.

Posterior left site: H2 rejected (Table 15)

Table 15: Testing Hypothesis 2 posterior left

NCBC_PL		DF	F-statistic	P-value
	Between Groups	1	2.14	0.15
	Within Groups	58		
	Total	59		

NCBC_PL: number of crackles per breathing cycle in posterior left site; DF: degrees of freedom; P-value: the probability value.

Posterior right site: H2 rejected (Table 16)

Table 16: Testing Hypothesis 2 posterior right

NCBC_PR		DF	F-statistic	P-value
	Between Groups	1	2.50	0.12
	Within Groups	58		
	Total	59		

NCBC_PR: number of crackles per breathing cycle in posterior right site; DF: degrees of freedom; P-value: the probability value.

4.6 Summary of the results

This chapter presented the results of this research. There was statistically significant difference found in the crackle 2CD between smoking and non-smoking young male adults:

- left upper site with $F(2, 57) = 9.40$, $p < 0.05$,
  Post hoc test showed that the crackle 2 CD in non-smokers is statistically different compared to the other two groups of smokers (light, heavy), at 0.05 level of significance.
- right upper site $F(2, 57) = 9.51$, $p < 0.05$,
  Post hoc test showed that the crackle 2 CD in non-smokers is statistically different compared to the other two groups of smokers (light, heavy), at 0.05 level of significance.
- left middle site $F(2, 57) = 4.20$, $p < 0.05$ and
  Post hoc test showed that the crackles 2 CD in non-smokers is statistically different compared to the light smokers group at 0.05 level of significance
- right middle site $F(2, 57) = 4.36$, $p < 0.05$.
  Post hoc test showed that the crackles 2 CD in non-smokers is statistically different compared to the heavy smokers group at 0.05 level of significance

There comparisons between the two groups of smokers (light and heavy) in this study were not significant in all sites.

There was no significant interaction found between BMI or age of the subjects with the smoking history on the results.

The sample evidence did not however, support the hypothesis regarding NCBC differences between smoking and non-smoking young male adults.

The next chapter discusses the results and links them to the literature review, as well as reviewing the limitations of the study.

CHAPTER 5

Discussion

5.1 Introduction

This chapter discusses the findings of this research which was conducted on smoking and non-smoking young male adults with normal lung function test parameters and no history of lung diseases. No statistically significant differences between smokers and non-smokers were detected in crackle numbers, but some differences were seen in crackle 2CD at anterior and lateral sites.

The arguments presented in this chapter are best explained by referring to the theoretical model of this research developed in Chapter 1 (Figure 1). By following Figure 1, it is evident that smoking has an impact on lung pathology (Amin et al, 2003; Sovijärvi et al, 2000a; Skurnik and Shoenfeld, 1998; Piirilä and Sovijärvi, 1995; Cosio et al, 1980) but that there has been little research into the relationships between lung sounds and pathology (Murphy et al, 2004; Piirilä et al, 2000; Piirilä et al, 1991). The lung sounds and lung pathology in connection with cigarette smoking has not been previously explored. Thus, this research was designed to explore the differences in lung sounds, particularly crackle characteristics, between smoking and non-smoking young adults.

Baseline characteristics of the subjects might have an effect on outcomes in many disciplines of research. Keeping this in mind, it could be argued that many variables are set as the baseline information of the samples depending on their possible effect on the final results. Hence, the discussion in the following two sections is based on the research question developed from the literature presented in Chapter 2. The hypothesised baseline variables that might have an impact on crackle characteristics in addition to cigarette smoking are identified as: gender, age and BMI. As very few female smokers were recruited, it was decided to exclude their data from the analysis and focus on males only, to avoid possible bias in the results due to the differences reported between male and female either in lung anatomy or smoking behaviour as discussed Chapter 2. The discussion of the statistical power calculation to detect the crackle differentials for the

selected sample is presented after discussing the findings on 2CD and the NCBC measurements. Finally, the chapter ends with a discussion of the study limitations.

5.2 Baseline information

5.2.1 Age

According to the General Household Survey in 2006, cigarette smoking in the UK continues to be more common among adults aged 20 to 34. Programmes of smoking-prevention education are needed (Taioli and Wynder, 1991) because initiating smoking at an early age might lead to heavy smoking. In order to be effective, such programmes need evidence relating to the damaging effects of smoking. Previous research has been conducted with middle-aged or elderly people to explore the impact of cigarette smoking on lungs (Elders et al, 1994). In contrast, the young adult group (20 - 35 years old) has been investigated in this present research. This might have a twofold advantage: it not only fills the gap identified in the subject literature, but investigates possible early effects of smoking in young adults. Reporting these effects earlier might have a positive impact on the quit rate of cigarette smoking. Although ageing could have an impact on the crackles, it did not have a significant effect on the present results. The age of the subjects in both groups (smokers and non-smokers) was young and similar. The aim of this study was to explore the differences in crackles characteristics between smokers and non-smokers, the effect of age on crackle characteristics could be investigated in future studies.

5.2.2 Body mass index

There was no relationship between BMI and crackles characteristics in this study. In addition, BMI showed no significant effect on the differences between the crackles characteristics in the present results. Previous studies (Jones and Nzekwu, 2006; Lazarus et al, 1997; Schoenberg et al, 1978) have reported lower levels of ventilatory function among subjects with a high BMI. In this line of argument, King et al, 2005, found association between obesity and reduced lung volume, which was linked with airway narrowing in healthy young adults (28-30 years old). Because Lung volumes might have an impact on lung sounds and crackles specifically, it would be interesting to look at some individual cases of obese subjects and their lung sounds. There were only two

subjects considered to be obese in this study (BMI >30 kg/m^2). The two subjects were smokers and the crackles' 2CD mean value did not differ significantly from the other smokers. However, the effect of BMI on the crackle characteristics need to be explored in the future studies.

5.2.3 Lung function test

There was no statistically significant difference found between smokers and non- smokers in the lung function parameters. The result is consistent with the study by Ferguson et al (2000) where they reported that spirometry rarely detects differences between smokers and healthy non-smokers under the age of 45 years. A slower growth in lung function parameters (FVC, FEV1) was reported on adolescent smokers, both boys and girls, by Gold et al (1996) but their study was longitudinal. A longitudinal study to explore any relationship between lung function parameters and crackles characteristics, in smokers and non-smokers, need to be designed for further study.

5.3 Crackles characteristics

5.3.1 Crackles' two cycle deflection duration

The findings that 2CD was consistently shorter in smokers than non-smokers will now be discussed. This difference showed statistical significance in anterior and lateral sites but not in posterior sites. The 2CD is a measure of duration and is related to frequency. All the crackle records were classified as coarse crackles i.e. > 10 msec duration, which suggests that they were generated from the central airways. In smokers, the average 2CD is shorter; suggesting that some of the crackles were less coarse, as possibly generated more peripherally.

This indicates that there might be secretions or inflammatory processes widely spread over the peripheral airways of the smokers whereas in non-smokers, secretions might be spread over the central airways. Smoke-related lung damage is characterised by inflammation, airway obstruction and destruction of the lung parenchyma (Amin et al, 2003). However, Dicpinigaitis (2003) found cough reflex sensitivity in smokers is significantly diminished compared to non-smokers. This might affect the clearance of secretion leading to secondary pulmonary infection which might lead to damages to lung

parenchyma and the airways. Therefore, we may expect smokers to be more vulnerable to chest infections than non-smokers as sputum is less easy to clear. This argument is quite consistent with the finding that smokers have a higher tendency to lower (as compared to upper) respiratory tract infection (Murin et al, 1997).

Piirilä et al (1991) found that the crackles in COPD patients had an average 2CD of 11.6±1.1 msec (detect in posterior sites). The average 2CD for the crackles detected in the posterior sites in this present study was: smokers 13.5±1.0 and non-smokers 14.5±1.2. There is possible agreement between the results and Piirilä et.al (1991) in a way that the crackles in both studies are categorised as coarse crackles with lower durations. Moreover, comparing the 2CD from the findings of the present study and the findings from the COPD patients of Piirilä et.al (1991), the crackles in non-smokers are coarser than the crackles in smokers and the COPD patients. The finer the crackles the more peripheral the expected effected airways which can be seen in the COPD patients and the diseases related to smoking. However, there is association between cigarette smoking and inflammatory cells in the peripheral airways hypothesised to be precursor of COPD in young smokers by Niewoehner et al (1974) and might be related to the differences found between the smokers and non-smokers in this study.

5.3.1.1 Smoking history and 2CD

As a result of finding significant differences in 2CD between smokers and non-smokers a further hypotheses was evolved and tested using the post hoc (LSD test); that there will be significant differences in 2CD between light smoker, heavy smoker and non-smoker groups. Hence the 2CD of the three groups were compared to each other as well as to the non-smoker group. There are no differences in crackles characteristics between both groups of smokers (heavy and light). However, statistically significant differences were observed between both smoker groups and the non-smoker group in both upper sites. The history of smoking (pack-years) is known to have an impact on the severity of the lung disorders related to cigarette smoking (Attili et al, 2008) or to interact with other factors in the production of crackles, asbestosis for example (Murphy et al, 1984). However, the non-significant differences between the light and heavy smokers in this present study might be due to short history of smoking which is directly related to the age group of the subjects. Or it might be due to small sample size causing less power for subgroups analysis. The power calculation (Appendix E) suggested a sample size of 16 in each

group would be required to reject the null hypothesis (crackle`s characteristics of smokers and non-smokers male subjects are similar) when the alternative hypothesis is true (crackle`s characteristics of smokers and non-smokers male subjects are different). After dividing the smokers to light and heavy smokers there were 16 and 14 subjects in each group respectively. As a result the conclusion could be affected by type 2 error.

The statistically significant results from the left and right middle sites were different. In the left middle site the result was strange. The differences between the light smokers group and non-smokers group were statistically significant but heavy smokers group did not differ significantly from non-smokers. On the other hand, the statistical significant differences in the right middle lobe were found between heavy smokers and non-smokers. These different results might be explained by the smaller sample size causing less power for subgroups analysis. However, these findings could suggest that the effect of cigarette smoking might have different effect on different lung sites of the lung and the history of smoking might have an impact on these differences. A study to determine the different effects of cigarette smoking on different sites of the lung and the impact of smoking history on these differences could be indicated.

5.3.2 Crackle numbers

The finding that the NCBC was similar in both groups is interesting because it is in contrast to previous studies (Murphy et al, 2004; Epler et al, 1978; Piirilä, 1992) which have reported an association between number of crackles and specific lung diseases (pneumonia and ILD for example) This may be explained because of methodological differences between this study and their studies (age group, lung function, sample size, the method of detecting crackles, equipment, choice of algorithm and the setting). Or it could be because damage effects of smoking in this group were not large enough to affect crackles generated in terms of number of crackles.

5.4 Study limitation

There are a few limitations in this study. The reliability of the crackles' IDW and 2CD has not been adequately explored. Marques et al (2009a) reported high inter-subject variability of crackle parameters, while the intra-subject variability of crackle parameters

was reported low over short time periods which led to the conclusion that these measures are relatively stable and reliable within individuals.

Literature has discussed the association between passive smoking and reduced lung function measures in adult subjects but these associations have been reported adversely differently between studies (Frette et al, 1996; Lebowitz et al, 1987). The issue is still under debate among researchers and does not give clear conclusive statements. The association between these changes and crackle characteristic changes could be hypothesised. For example there will be differences in crackle characteristics between subjects who are exposed to passive smoking and those who are not. Unfortunately, this study did not consider the passive smoking status as inclusion or exclusion criteria but the passive smoking subjects were all smokers and all non-smoker subjects did not report to be passive smokers according to the criteria in Section 2.8. Therefore, the possible influence of passive smoking on the crackles characteristics differences between smoking and non-smoking groups in this study could be neglected due to data limitation.

Another limitation of this study is the consideration of single gender subjects. The female gender was not included, which will affect the generalization of the results. It was difficult to recruit smokers from both genders but the response from female smokers showed minimal interest in the study.

The age of the subjects is relatively young; more than 75% of the subjects are below 28 years of age, which is at the lower end of the chosen age group.

It was reported by Marques (2008), the originator of the breathing cycle detection algorithm used in this present study, that it has not yet been fully validated. The disadvantage of this algorithm is that: choosing four points of the first breathing cycle (start, end, maximum and minimum) manually might be subject to individual error which will affect the automatic detection. This might lead to repetition of the manual process many times while listening to the record until reaching the proper result. To overcome this disadvantage the researcher has developed an algorithm (Appendix H) which will be validated by the researcher in the future work.

CHAPTER 6

Conclusion

6.1 Introduction

The overall aim of this research was to investigate whether a digital stethoscope and CALSA can be used to compare the characteristics of crackles in the lung between smoking and non-smoking young adults. Due to lack of response from young adult female smokers, the research was restricted to young male adults only. Two crackles' parameters, 2CD and NCBC, were identified to compare the crackles in smoking and non-smoking young adults. This chapter presents the concluding remarks regarding the set aim of this study and the practical applications of its findings and identifies the direction for some future research.

6.2 Conclusions

The information obtained during this research leads to the overall conclusion that CALSA has the potential to detect differences objectively between young adult male smokers and non-smokers in a portable setting. Nevertheless, the aim proposed at the outset for this research has been achieved. The 2CD and NCBC variables were used to compare the crackles in smokers and non-smokers. The differences in the 2CD measurements between smokers and non-smokers are statistically supported (significant at 5%). Testing the NCBC at the same level of significance did not show any significant differences. The marked differences that differentiate smokers from non-smokers monitored in this study provide evidence that CALSA could be a helpful guide in observing the smoking behaviour. These observations suggest that more definitive studies should be done to evaluate the clinical utility of these differences in crackle characteristics.

The conclusion is further supported with the statistical power analysis (Appendix E). Statistically, power is the probability of rejecting the null hypothesis (crackle`s characteristics of smokers and non-smokers male subjects are similar) when the alternative hypothesis is true (crackle`s characteristics of smokers and non-smokers male subjects are different). With a sample of 60 subjects, the power was estimated to be 0.977. The calculated power is quite consistent according to statistical guidelines (power should

be at least 0.8) to detect a reasonable departure from the null hypothesis. Additionally, power is equal to one minus the probability of type II error (0.023). This concludes that there are only 2.3 % chances of failing to reject a false null hypothesis. Overall, the study's conclusions are less effected by statistical errors (type I and type II).

The detection of the breathing cycles was possible using CALSA. However, the detection was conducted in a semi-automatic pattern in this research (i.e., the manual start and end of one breath with threshold had to be defined). After defining the threshold value manually, the breathing cycles were detected automatically then counting the NCBC using CALSA was possible. Further work needs to be done on the breathing cycles' algorithm developed for this research, but complete automatic detection seems to be feasible, even with a single sensor.

In addition this research has shown that using a digital stethoscope to record lung sounds, in non-clinical setting environment, is possible. Signal processing technique was used to successfully analyse these recordings. The breathing cycles and crackles' duration were successfully identified. In sum, using CALSA to identify the characteristics of the crackles in data collected via digital stethoscope in non-clinical setting environment is possible. Nevertheless, identifying the characteristics of crackles using CALSA would be more effective in a clinical setting.

6.3 Study implications within practical settings

This study highlights the importance of smoking prevention and control programs in the younger population. Some of the practical dimensions of using CALSA arising from this research are discussed in this section. It can be used as an objective tool in anti-smoking programs and campaigns. It could be used as an early indication tool in lung health screening programs which can be portable and low cost. For general practitioners and clinicians, CALSA could be used as an objective diagnostic tool. It can also provide objective record of the lung status which can be added to the patient's medical profile for follow up. It could be used as a tool to objectively detect changes in the lung due to environmental pollution effects. Finally, it has the potential of creating predictive models of lung disorders.

The main priorities for further work are itemised in the next section.

6.4 Proposed areas for future work

Research designed to compare the crackles characteristics in female smokers and non-smokers using CALSA. This may be conducted by recruiting female smoker and non-smoker subjects following the methodology in this research.

Research designed to explore the reliability of crackles' characteristics between different age groups, different BMI and different smoking history is necessary to confirm the effectiveness of CALSA in evaluating differences in crackles' characteristics between smokers and non-smokers.

Longitudinal research designed to explore the reliability of crackles characteristics to be used to follow up the progress of the changes due to cigarette smoking in long term.

Research designed to explore the potential of comparing the wheezes characteristics between smokers and non-smokers. This would require the development of a suitable algorithm to analyse wheezes in the lung sound files of smokers and non-smokers.

Research designed to validate the algorithm, proposed by the researcher in the process of this study but not as yet completely developed for breathing cycle detection (Section 5.4). This might be achieved by comparing the algorithms findings to pneumotachograph findings, using data recorded simultaneously.

Research designed to explore the potential to compare the timing of crackles characteristics between smokers and non-smokers. This would require the reliable and accurate algorithm to detect the breathing cycles in the lung sound files of smokers and non-smokers.

Research designed to explore the potential of comparing the crackles characteristics on different sites of the lung between smokers and non-smokers. This would require recruiting a large sample number and apply proper statistical techniques.

These are the main areas that the researcher considers to be essential to allow further development of the idea that CALSA can be used to characterise adventitious lung sounds, and to confirm or reject the hypothesis that CALSA has the potential to objectively evaluate the differences between smokers and non-smokers in early stages.

APPENDICES

APPENDIX A

A.1 CORSA Recommended chest locations to record lung sounds

As recommended by CORSA (successive or simultaneous recordings) depending on the position of the subject and the application (Rossi et al, 2000):

- trachea: on the trachea at the sternal notch;
- right and left posterior and basal area of the chest, five centimetres laterally from the paravertebral line and seven centimetres below the scapular angle (in adults);
- right and left anterior area of the chest at the second intercostal space on the mid-clavicular line (optional);
- right and left lateral area of the chest at the fourth or fifth intercostal space on the mid-auxiliary line (optional).

A.2 CORSA general recommendations and guidelines for recording lung sounds

The following recommendations are taken from environmental and subject conditions and breathing manoeuvres for respiratory sound recordings (Rossi et al, 2000):

- Background noise intensity level preferably < 45 dB (A) or < 60 dB (linear).
- Minimum ambient noise from the environment.
- Minimized generation of non-respiratory sounds including voiced sounds.
- Comfortable room temperature, humidity, lighting and ventilation.
- For short-term recordings, the sitting position of the subject is preferred.
- Tidal breathing, 7-10 respiratory cycles.

APPENDIX B

B1: Letter to the Heads of School at the University of Southampton

Letter to obtain permission to email students

Dear Head of …..School,

I am a doctoral student at the School of Health Sciences, University of Southampton. I am carrying out a study to compare the lungs of the smoking and non-smoking young adults through recording lung sounds using a digital stethoscope and performing lung function test using spirometry. I am particularly interested in looking for any relationship between smoking history and lung sounds. Correlation between lung sound changes and lung function parameters changes will be studied as well.

This e-mail has been sent to seek your permission to give me access to the students' mailing list in your School to send them an invitation to join my study. Attached to this email are an invitation letter and an information sheet that explains in more detail what the study involves.

If you have any questions that are not answered in the information sheet you are very welcome to contact me on 023 8059 5908 or e-mail mga1w07@soton.ac.uk. Alternatively you can contact my supervisor Dr Anne Bruton on 023 8059 5283 or email ab7@soton.ac.uk.

Date: 30/10/2008 Version: 1 Ethics Number: SHPRS-ETHICS 08-029

B2: Letter to the heads of the student societies at the University of Southampton

Text of the talk to the students' societies

Dear president of the…… students' society,

I am a doctoral student at the Faculty of Health Sciences, University of Southampton. I am carrying out a study to compare the lungs of the smoking and non-smoking young adults through recording lung sounds using a digital stethoscope and performing lung function test using spirometry. I will be very great full if deliver this talk to your society during your meeting.

If you have any questions you are very welcome to contact me on 00442380595908 or e-mail mga1w07@soton.ac.uk. Alternatively you can contact my supervisor Dr Anne Bruton on 02380595283 or email ab7@soton.ac.uk.

''A doctoral student at the Faculty of Health Sciences, University of Southampton, is carrying out a study to compare the lungs of the smoking and non-smoking young adults through recording lung sounds using a digital stethoscope and performing lung function test using spirometry. The study is particularly interested in looking for any relationship between smoking history and lung sounds. Correlation between lung sound changes and lung function parameters changes will be studied as well.''

This talk is to ask if any of you would be willing to participate in this study. There is no risk in participating on this study. Information sheet that explains in more detail what the study involves is available with us for those who are interested. If you have any questions that are not answered in the information sheet you are very welcome to use the contact information at the end of the information sheet.

After reading the information sheet, if you are interested in taking part in the study, pleas send an e-mail to the researcher to send you the time and the location of the study.''

Date: Version: Ethics Number:

APPENDIX C

Excel program used for random sampling from lung sound data base

From 154 healthy subjects in the data base there was data from 60 male subjects. Matching the age group, 39 of them aged between 20 to 35 years old. Applying the exclusion criteria of the study, 4 of the 39 were excluded to have FEV_1/FVC ratio below 70%. From the remaining 35 subjects, 17 were chosen randomly using the Excel program as follow:

- Microsoft Excel 2010 was started and the workbook was opened that contains data of the file codes to get a random sample.
- Column A was verified empty, to be used to generate random numbers.
- Click and drag to select the cells in Column A that correspond with the records in the other cells.
- An empty cell for each row of information was selected in the spread sheet.
- The command "=RAND ()" was typed with no quotes in the "Formula" textbox. Then the "Enter" key on keyboard pressed to generate the random numbers into column A.
- All data in the spread sheet along with the corresponding random numbers were selected.
- By using the "Data" tab at the top of the screen, the "Sort" button was clicked from the "Sort and Filter" group in the "Data" ribbon; the "Sort" dialog box opened onto the screen.
- Choosing "Column A" from the "Sort by Column" drop-down list and "Smallest to Largest" from the "Sort by Order" drop-down list then clicking the "OK" button to close the dialog box and return to the spread sheet.
- The top number of rows were chosen to make up the random sample.

APPENDIX D

Sample size calculation details

Stata command:

sampsi 14.6 13.7, sd1(0.86) sd2(0.9) alpha(0.05) power(0.8)

Step 1:

Test H_0: m1 = m2, where m1 is the mean in population 1 (smoking young male adults) and m2 is the mean in population 2 (non-smoking young male adults)

Step 2:

The mean of the crackles' 2CD of smoker subjects = 13.7;
The standard deviation of the crackles' 2CD of smoker subjects = 0.90

The mean of the crackles' 2CD of non-smoker subjects = 14.6;
The standard deviation of the crackles' 2CD of non-smoker subjects =0 .86

Step 3:

Estimated sample size for two-sample comparison of means

Assumptions:

Level of significance = 0.05;

Power = 0.8

Estimated required sample sizes:

n1 = 16

n2 = 16

APPENDIX E

Power calculation details

Stata command:

sampsi 14.6 13.7, sd1(0.86) sd2(0.9) alpha(0.05) n1(30) n2(30)

Step 1:

Test H0: m1 = m2, where m1 is the mean in population 1 (smoking young male adults) and m2 is the mean in population 2 (non-smoking young male adults)

Step 2:

The mean crackles' 2CD of smoker subjects = 13.7;

The standard deviation of crackles' 2CD of smoker subjects = 0.90

The mean crackles' 2CD of non-smoker subjects = 14.6;
The standard deviation of crackles' 2CD of smoker subjects =0 .86

Step 3:

Estimated power for two-sample comparison of means

Assumptions:

Level of significance = 0.05;
Sample size of group 2 (smokers) = 30 Subjects
Sample size of group 1 (non-smoker) = 30 Subjects;

Estimated power

Power **= 0.9773**

APPENDIX F

Consent form

Title of Project: Quantifying differences in lung sounds between smoking and non-smoking young adults

Name of Researcher: Mohammed Alzahrani

	Please initial box
1. I confirm that I have read and understand the information sheet dated……(version....) for the above study and have had the opportunity to ask questions.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.	☐
3. I understand that at the end of the study data collected from me will be securely stored at the University of Southampton for 15 years.	☐
4. I agree to take part in the above study.	☐

_____	_____	_____
Name of Participant	Date	Signature

_____	_____	_____
Researcher	Date	Signature

1 for participant, 1 for researcher.

APPENDIX G

The Guidelines of American Thoracic Society for Spirometry

Adapted from (Miller et al, 2005)

- Open circuit manoeuvre performance method
- Have subject assume the correct posture
- Attach nose clip
- Inhale completely and rapidly with a pause of 1 second at Total Lung Capacity (TLC)
- Place mouthpiece in mouth and close lips around the mouthpiece
- Exhale maximally until no more air can be expelled while maintaining an upright posture
- Repeat instructions as necessary, coaching vigorously
- Repeat for a minimum of three manoeuvres; no more than eight are usually required
- Check test repeatability and perform more manoeuvres as necessary

APPENDIX H

Proposed algorithm to detect the breathing cycles

This proposed algorithm was the product of working with Dr David Simpson, the leader of Demystifying Biomedical Signals: Principles and Applications module, in the Institution of Sound and Vibration at the University of Southampton. It is proposed that counting heart rate algorithm can be modified to detect the breathing cycle in lung sound files collected and saved in (.wav) format. The algorithm was modified and used on lung sound files collected from subjects in this research. It works in a similar way to detect the QRS complexes in ECG signals for heart rate calculation. A possible advantage of this algorithm is the ability of identifying the start and the end of every breath automatically. The code used to implement the proposed algorithm, available upon request, had been tried in different lung sound files and showed reasonable detection but it still need to be developed and validated in the future work.

REFERENCES

Akre, H., Borgersen, A., Mair, I. & Skatvedt, O. (2000). Tracing air flow and diagnosing hypopnoeas in normal subjects. *Physiological Measurement,* 21**,** 221-7.

Albo, R. J. & Grimes, O. F. (1966). The Middle Lobe Syndrome: A Clinical Study,. *Diseases of the Chest,* 50**,** 509-518.

Alencar, A. M., Buldyrev, S. V., Majumdar, A., Stanley, H. E. & Suki, B. (2001). Avalanche dynamics of crackle sound in the lung. *Physical Review Letters,* 87**,** 088101-088101.

Almirall, J., González, C. A., Balanzó, X. & Bolíbar, I. (1999). Proportion of Community-Acquired Pneumonia Cases Attributable to Tobacco Smoking*. *Chest,* 116**,** 375-379.

Amin, K., Ekberg-Jansson, A., Lofdahl, C. G. & Venge, P. (2003). Relationship between inflammatory cells and structural changes in the lungs of asymptomatic and never smokers: a biopsy study. *Thorax,* 58**,** 135-142.

Attili, A. K., Kazerooni, E. A., Gross, B. H., Flaherty, K. R., Myers, J. L. & Martinez, F. J. (2008). Smoking-related Interstitial Lung Disease: Radiologic-Clinical-Pathologic Correlation1. *Radiographics,* 28**,** 1383-1396.

Ayed, A. K. (2004). Resection of the Right Middle Lobe and Lingula in Children for Middle Lobe/Lingula Syndrome. *Chest,* 125**,** 38-42.

Baughman, R. P., Shipley, R. T., Loudon, R. G. & Lower, E. E. (1991). Crackles in interstitial lung disease. Comparison of sarcoidosis and fibrosing alveolitis. *Chest,* 100**,** 96-101.

Becklake, M. R. & Kauffmann, F. (1999). Gender differences in airway behaviour over the human life span. *Thorax,* 54**,** 1119-1138.

Bettencourt, P., Del Bono, E., Spiegelman, D., Hertzmark, E. & Murphy, R. (1994). Clinical utility of chest auscultation in common pulmonary diseases. *American Journal of Respiratory and Critical Care Medicine,* 150**,** 1291-7.

Bize, R., Burnand, B., Mueller, Y. & Cornuz, J. (2009). Biomedical risk assessment as an aid for smoking cessation. *Cochrane Database Systematic Reviews***,** CD004705.

Bland, M. (2000). *An introduction to medical statistics*, Oxford University Press.

Brouwer, A. F. J., Roorda, R. J. & Brand, P. L. P. (2007). Comparison between peak expiratory flow and FEV1 measurements on a home spirometer and on a pneumotachograph in children with asthma. *Pediatric pulmonology,* 42**,** 813-818.

Canoy, D., Luben, R., Welch, A., Bingham, S., Wareham, N., Day, N. & Khaw, K. T. (2004). Abdominal Obesity and Respiratory Function in Men and Women in the EPIC-Norfolk Study, United Kingdom. *American Journal of Epidemiology,* 159**,** 1140-1149.

Celermajer, D., Sorensen, K., Georgakopoulos, D., Bull, C., Thomas, O., Robinson, J. & Deanfield, J. (1993). Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation,* 88**,** 2149-2155.

Cerveri, I., Accordini, S., Verlato, G., Corsico, A., Zoia, M. C., Casali, L., Burney, P. & De Marco, R. (2001). Variations in the prevalence across countries of chronic bronchitis and smoking habits in young adults. *European Respiratory Journal,* 18**,** 85-92.

Chen, Y., Horne, S. L. & Dosman, J. A. (1993). Body weight and weight gain related to pulmonary function decline in adults: a six year follow up study. *Thorax,* 48**,** 375-380.

Cosio, M. G., Hale, K. A. & Niewoehner, D. E. (1980). Morphologic and morphometric effects of prolonged cigarette smoking on the small airways. *American Review of Respiratory Disease,* 122**,** 265-21.

Crapo, R. O. (1994). Pulmonary-Function Testing. *The New England Journal Of Medicine,* 331**,** 25-30.

Culiner, M. M. (1966). The Right Middle Lobe Syndrome, A Non-Obstructive Complex. *Diseases of the Chest,* 50**,** 57-66.

Davie, A. P., Francis, C. M., Caruana, L., Sutherland, G. R. & Mcmurray, J. J. (1997). Assessing diagnosis in heart failure: which features are any use? *QJM: An International Journal of Medicine* 90**,** 335-339.

Decramer, M., Sibille, Y., Bush, A., Carlsen, K., Rabe, K., Clancy, L., Turnbull, A., Nemery, B., Simonds, A. & Troosters, T. (2011). The European Union conference on chronic respiratory disease: purpose and conclusions. *European Respiratory Journal,* 37**,** 738-742.

Dellinger, R. P., Parrillo, J. E., Kushnir, A., Rossi, M. & Kushnir, I. (2008). Dynamic Visualization of Lung Sounds with a Vibration Response Device: A Case Series. *Respiration,* 75**,** 60-72.

Deveci, F., Murat, A., Turgut, T., Altuntas, E. & Muz, M. H. (2004). Airway wall thickness in patients with COPD and healthy current smokers and healthy non-smokers: assessment with high resolution computed tomographic scanning. *Respiration,* 71**,** 602-10.

Dicpinigaitis, P. V. (2003). Cough reflex sensitivity in cigarette smokers. *Chest,* 123**,** 685-8.

Duggan, M. & Kavanagh, B. P. (2005). Pulmonary Atelectasis: A Pathogenic Perioperative Entity. *Anesthesiology,* 102**,** 838-854.

Eisner, M. D. (2009). Secondhand Smoke and Obstructive Lung Disease: A Causal Effect? *American Journal of Respiratory and Critical Care Medicine,* 179**,** 973-974.

Elders, M. J., Perry, C. L., Eriksen, M. P. & Giovino, G. A. (1994). The report of the Surgeon General: preventing tobacco use among young people. *American Journal of Public Health,* 84**,** 543-547.

Epler, G. R., Carrington, C. B. & Gaensler, E. A. (1978). Crackles (rales) in the interstitial pulmonary diseases. *Chest,* 73**,** 333-339.

Ferguson, G. T., Enright, P. L., Buist, A. S. & Higgins, M. W. (2000). Office Spirometry for Lung Health Assessment in Adults. *Chest,* 117**,** 1146-1161.

Field, A. 2004. Discovering Statistics Using SPSS for Windows: Advanced Techniques for Beginners. Sage Publications, Inc., Thousand Oaks, CA.

Finch E, Brooks D, Stratford O W and Mayo N E (2002). Physical Rehabilitation Outcome Measures (Baltimore, MD: Lippincott Williams & Wilkins)

Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek C.J. , (2011). National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *The Lancet,* 377, 557-567.

Flietstra, B., Markuzon, N., Vyshedskiy, A. & Murphy, R. (2011). Automated Analysis of Crackles in Patients with Interstitial Pulmonary Fibrosis. *Pulmonary Medicine* [Online], 2011. Available: http://www.hindawi.com/journals/pm/2011/590506/cta/.

Forgacs, P. (1967). Crackles and Wheezes. *The Lancet,* 290**,** 203-205.

Forgacs, P. (1969). Lung sounds. *British Journal Of Diseases Of The Chest,* 63**,** 1-12.

Forgacs, P. (1978). The functional basis of pulmonary sounds. *Chest,* 73**,** 399-405.

Fredberg, J. J. & Holford, S. K. (1983). Discrete lung sounds: Crackles (rales) as stress--relaxation quadrupoles. *The Journal of the Acoustical Society of America,* 73**,** 1036-1046.

Frette, C., Barrett-Connor, E. & Clausen, J. L. (1996). Effect of active and passive smoking on ventilatory function in elderly men and women. *American Journal of Epidemiology,* 143**,** 757-65.

General Household Survey. (2006). *Cigarette Smoking prevalence* [Online]. National Statistics Website. Available: http://www.statistics.gov.uk/cci/nugget.asp?id=866 [Accessed 30 April 2008].

Gold, D. R., Wang, X., Wypij, D., Speizer, F. E., Ware, J. H. & Dockery, D. W. (1996). Effects of cigarette smoking on lung function in adolescent boys and girls. *The New England Journal Of Medicine,* 335**,** 931-7.

Gross, V., Dittmar, A., Penzel, T., Schuttler, F. & Von Wichert, P. (2000). The Relationship between Normal Lung Sounds, Age, and Gender. *American Journal of Respiratory and Critical Care Medicine,* 162**,** 905-909.

Hill, S. E., Blakely, T., Kawachi, I. & Woodward, A. (2007). Mortality among Lifelong Nonsmokers Exposed to Secondhand Smoke at Home: Cohort Data and Sensitivity Analyses. *American Journal of Epidemiology,* 165**,** 530-540.

Hoevers, J. & Loudon, R. G. (1990). Measuring crackles. *Chest,* 98**,** 1240-1243.

Hogg, J. C. (2004). Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *The Lancet,* 364**,** 709-721.

Hogg, J. C., Macklem, P. T. & Thurlbeck, W. M. (1968). Site and nature of airway obstruction in chronic obstructive lung disease. *The New England Journal Of Medicine,* 278**,** 1355-60.

Hogg, J. C., Wright, J. L., Wiggs, B. R., Coxson, H. O., Opazo Saez, A. & Pare, P. D. (1994). Lung structure and function in cigarette smokers. *Thorax,* 49**,** 473-8.

Holford, S. K. (1982). *Discontinuous adventitious lung sounds: measurement, classification and modeling* [Online]. ScD Thesis, Massachusetts: Massachusetts Institute of Technology. Available: http://dspace.mit.edu/bitstream/handle/1721.1/22395/Holford_Stephen_PhD_1981.pdf. [Accessed December/10/2009].

Hopkins, W. G. (2000). Measures of reliability in sports medicine and science. *Sports Medicine,* 30**,** 1-15.

Jaakkola, M. S., Jaakkola, J. J. K., Ernst, P. & Becklake, M. R. (1991). Ventilatory Lung-Function in Young Cigarette Smokers - a Study of Susceptibility. *European Respiratory Journal,* 4**,** 643-650.

Janssens, J. (2005). Aging of the Respiratory System: Impact on Pulmonary Function Tests and Adaptation to Exertion. *Clinics in chest medicine,* 26**,** 469-484.

Jones, R. L. & Nzekwu, M. (2006). The Effects of Body Mass Index on Lung Volumes. *Chest,* 130**,** 827-833.

Kaisla, T., Sovijärvi, A., Piirilä, P., Rajala, H. M., Haltsonen, S. & Rosqvist, T. (1991). Validated method for automatic detection of lung sound crackles. *Medical & Biological Engineering & Computing,* 29**,** 517-521.

Kataoka, H. & Matsuno, O. (2008). Age-Related Pulmonary Crackles (Rales) in Asymptomatic Cardiovascular Patients. *Annals of Family Medicine* 6**,** 239-245.

Kawamura, T., Matsumoto, T., Tanaka, N., Kido, S., Jiang, Z. & Matsunaga, N. (2003). Crackle analysis for chest auscultation and comparison with high-resolution CT findings. *Radiation Medicine* 21**,** 258-66.

King, G. G., Brown, N. J., Diba, C., Thorpe, C. W., Munoz, P. and Marks, G. B. (2005). The effects of body weight on airway calibre. *European Respiratory Journal,* 25, 896-901.

Kiyokawa, H. & Pasterkamp, H. (2002). Volume-dependent variations of regional lung sound, amplitude, and phase. *Journal of Applied Physiology,* 93**,** 1030-1038.

Kompis, M., Pasterkamp, H. & Wodicka, G. R. (2001). Acoustic Imaging of the Human Chest. *Chest,* 120**,** 1309-1321.

Lazarus, R., Sparrow, D. & Weiss, S. T. (1997). Effects of Obesity and Fat Distribution on Ventilatory Function. *Chest,* 111**,** 891-898.

Leblanc, P., Ruff, F. & Milic-Emili, J. (1970). Effects of age and body position on "airway closure" in man. *Journal of Applied Physiology,* 28**,** 448-51.

Lebowitz, M. D., Holberg, C. J., Knudson, R. J. & Burrows, B. (1987). Longitudinal study of pulmonary function development in childhood, adolescence, and early adulthood. Development of pulmonary function. *American Review of Respiratory Disease,* 136**,** 69-75.

Loudon, R. & Murphy, R. L. (1984). Lung sounds. *American Review of Respiratory Disease,* 130**,** 663-73.

Macklem, P. T. (1972). Obstruction in small airways--a challenge to medicine. *American Journal of medicine,* 52**,** 721-4.

Mahagnah M. and Gavriely N. (1994). Repeatability of measurements of normal lung sounds *American Journal of Respiratory and Critical Care Medicine,* 149 477–81

Marques, A., Bruton, A. & Barney, A. (2009a). The reliability of lung crackle characteristics in cystic fibrosis and bronchiectasis patients in a clinical setting. *Physiological Measurement* 30**,** 903-12.

Marques, A., Bruton, A., Mahoney, K., Kolstoe, K., Moss, R., Thomas, S. & Beverley, Z. 2009b. Inter-individual variability of added lung sounds in a healthy population. *in Proceedings of European Respiratory Societies (ERS) Annual Congress.* Vienna

Marques, A. S. (2008). *The use of computer aided lung sound analysis to characterise adventitious lung sounds: a potential outcome measure for respiratory therapy.* PhD Thesis, University of Southampton.

Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., Van Der Grinten, C. P. M., Gustafsson, P., Jensen, R., Johnson, D. C., Macintyre, N., Mckay, R., Navajas, D., Pedersen, O. F., Pellegrino, R., Viegi, G. & Wanger, J. (2005). Standardisation of spirometry. *European Respiratory Journal,* 26**,** 319-338.

Moon, J., Du Bois, R. M., Colby, T. V., Hansell, D. M. & Nicholson, A. G. (1999). Clinical significance of respiratory bronchiolitis on open lung biopsy and its relationship to smoking related interstitial lung disease. *Thorax,* 54**,** 1009-1014.

Moussavi, Z., Leopando, M., Pasterkamp, H. & Rempel, G. (2000). Computerised acoustical respiratory phase detection without airflow measurement. *Medical & Biological Engineering & Computing,* 38**,** 198-203.

Munakata, M., Homma, Y., Matsuzaki, M., Ogasawara, H., Tanimura, K., Kusaka, H. & Kawakami, Y. (1986). Production mechanism of crackles in excised normal canine lungs. *Journal of Applied Physiology,* 61**,** 1120-1125.

Munakata, M., Ukita, H., Doi, I., Ohtsuka, Y., Masaki, Y., Homma, Y. & Kawakami, Y. (1991). Spectral and waveform characteristics of fine and coarse crackles. *Thorax,* 46**,** 651-657.

Murin, S., Hilbert, J. & Reilly, S. (1997). Cigaret smoking and the lung. *Clinical Reviews in Allergy and Immunology,* 15**,** 307-361.

Murphy, R. L., Del Bono, E. A. & Davidson, F. (1989). Validation of an automatic crackle (rale) counter. *American Review of Respiratory Disease,* 140**,** 1017-1020.

Murphy, R. L., Gaensler, E. A., Holford, S. K., Del Bono, E. A. & Epler, G. (1984). Crackles in the early detection of asbestosis. *American Review of Respiratory Disease,* 129**,** 375-9.

Murphy, R. L. H. (2008). In Defense of the Stethoscope. *Respiratory Care,* 53**,** 355 - 369.

Murphy, R. L. H., Vyshedskiy, A., Power-Charnitsky, V.-A., Bana, D. S., Marinelli, P. M., Wong-Tse, A. & Paciej, R. (2004). Automated lung sound analysis in patients with pneumonia. *Respiratory Care,* 49**,** 1490-1497.

Mussell, M. (1992). The need for standards in recording and analysing respiratory sounds. *Medical & Biological Engineering & Computing,* 30**,** 129-139.

Nath, A. R. & Capel, L. H. (1980). Lung crackles in bronchiectasis. *Thorax,* 35**,** 694-699.

Niewoehner, D. E., Kleinerman, J. & Rice, D. B. (1974). Pathologic changes in the peripheral airways of young cigarette smokers. *The New England Journal Of Medicine,* 291**,** 755-8.

Ogushi, F., Hubbard, R. C., Vogelmeier, C., Fells, G. A. & Crystal, R. G. (1991). Risk-Factors for Emphysema - Cigarette-Smoking Is Associated with a Reduction in the Association Rate-Constant of Lung Alpha-1-Antitrypsin for Neutrophil Elastase. *Journal of Clinical Investigation,* 87**,** 1060-1065.

Oztuna, D., Elhan, A. H. & Tuccar, E. (2006). Investigation of Four Different Normality Tests in Terms of Type 1 Error Rate and Power under Different Distributions *Turkish Journal of Medical Sciences,* 36**,** 171-176

Parkes, G., Greenhalgh, T., Griffin, M. & Dent, R. (2008). Effect on smoking quit rate of telling patients their lung age: the Step2quit randomised controlled trial. *British Medical Journal,* 336**,** 598-600.

Pasterkamp, H., Kraman, S. & Wodicka, G. (1997). Respiratory Sounds . Advances Beyond the Stethoscope. *American Journal of Respiratory and Critical Care Medicine,* 156**,** 974-987.

Petak, F., Habre, W., Babik, B., Tolnai, J. & Hantos, Z. (2006). Crackle-sound recording to monitor airway closure and recruitment in ventilated pigs. *European Respiratory Journal,* 27**,** 808-816.

Piirilä, P. (1992). Changes in Crackle Characteristics during the Clinical Course of Pneumonia. *Chest,* 102**,** 176-183.

Piirilä, P., Lehtola, H., Zitting, A., Kivisaari, L., Koskinen, H., Luukkonen, R., Salo, S. P., Vehmas, T., Nordman, H. & Sovijärvi, A. R. (2000). Lung sounds in asbestos induced pulmonary disorders. *European Respiratory Journal,* 16**,** 901-908.

Piirilä, P. & Sovijärvi, A. (1995a). Crackles: recording, analysis and clinical significance. *European Respiratory Journal,* 8**,** 2139-2148.

Piirilä, P. & Sovijärvi, A. (1995b). Objective assessment of cough. *European Respiratory Journal,* 8**,** 1949-1956.

Piirilä, P., Sovijärvi, A. R., Kaisla, T., Rajala, H. M. & Katila, T. (1991). Crackles in patients with fibrosing alveolitis, bronchiectasis, COPD, and heart failure. *Chest,* 99**,** 1076-1083.

Rapoport, J. (1986). Laennec and the discovery of auscultation. *Israel Journal of Medical Science,* 22**,** 597-601.

Remy-Jardin, M., Remy, J., Boulenguez, C., Sobaszek, A., Edme, J. L. & Furon, D. (1993). Morphologic effects of cigarette smoking on airways and pulmonary parenchyma in healthy adult volunteers: CT evaluation and correlation with pulmonary function tests. *Radiology,* 186**,** 107-15.

Robertson, A. J. & Coope, R. (1957). Rales, Rhonchi, and Laennec. *The Lancet,* 270**,** 417-423.

Rossi, M., Sovijärvi, A. R. A., Piirilä, P., Vannuccini, L., Dalmasso, F. & Vanderschoot, J. (2000). Environmental and subject conditions and breathing manoeuvres for respiratory sound recordings. *European Respiratory Review,* 10**,** 611-615.

Rubin, B. K., Ramirez, O., Zayas, J. G., Finegan, B. & King, M. (1992). Respiratory mucus from asymptomatic smokers is better hydrated and more easily cleared by mucociliary action. *American Review of Respiratory Disease,* 145**,** 545-7.

Ryu, J. H., Colby, T. V., Hartman, T. E. & Vassallo, R. (2001). Smoking-related interstitial lung diseases: a concise review. *European Respiratory Journal,* 17**,** 122-132.

Sanchez I. and Vizcaya C. (2003). Tracheal and lung sounds repeatability in normal adults. *Respiratory Medecin,* 97, 1257-60

Schachter, L. M., Salome, C. M., Peat, J. K. & Woolcock, A. J. (2001). Obesity is a risk for asthma and wheeze but not airway hyperresponsiveness. *Thorax,* 56**,** 4-8.

Schoenberg, J. B., Beck, G. J. & Bouhuys, A. (1978). Growth and decay of pulmonary function in healthy blacks and whites. *Respiration Physiology,* 33**,** 367-393.

Screaton, N. J. & Koh, T. (2004). Emphysema and smoking-related lung diseases. *Imaging,* 16**,** 50-60.

Shepard, J. W., Jr., Gefter, W. B., Guilleminault, C., Hoffman, E. A., Hoffstein, V., Hudgel, D. W., Suratt, P. M. & White, D. P. (1991). Evaluation of the upper airway in patients with obstructive sleep apnea. *Sleep,* 14**,** 361-71.

Shirai, F., Kudoh, S., Shibuya, A., Sada, K. & Mikami, R. (1981a). Crackles in Asbestos Workers - Auscultation and Lung Sound Analysis. *British Journal Of Diseases Of The Chest,* 75**,** 386-396.

Shirai, F., Kudoh, S., Shibuya, A., Sada, K. & Mikami, R. (1981b). Crackles in asbestos workers: auscultation and lung sound analysis. *British Journal Of Diseases Of The Chest,* 75**,** 386-396.

Sin, D. D. & Man, S. F. P. (2005). Chronic Obstructive Pulmonary Disease as a Risk Factor for Cardiovascular Morbidity and Mortality. *Proceedings of the American Thoracic Society* 2**,** 8-11.

Skurnik, Y. & Shoenfeld, Y. (1998). Health effects of cigarette smoking. *Clinics in Dermatology,* 16**,** 545-56.

Smyllie, H. C., Blendis, L. M. & Armitage, P. (1965). Observer Disagreement in Physical Signs of the Respiratory System. *Lancet,* 2**,** 412-3.

Sovijärvi, A., Dalmasso, F., Vanderschoot, J., Malmberg, L. & Stoneman, S. (2000). Definition of terms for applications of respiratory sounds. *European respiratory review* 597-610.

Sovijärvi, A. R., Malmberg, L. P., Charbonneau, G., Vanderschoot, J., Dalmasso, F., Sacco, C., Rossi, M. & Earis, J. E. (2000 a). Characteristics of breath sounds and adventitious respiratory sounds. *European Respiratory Review,* 10 (77)**,** 591-596.

Sovijärvi, A. R., Vanderschoot, J. & Earis, J. E. (2000 b). Standardization of computerised respiratory sound analysis. *European Respiratory Review,* 10**,** 585.

Taioli, E. & Wynder, E. L. (1991). Effect of the age at which smoking begins on frequency of smoking in adulthood. *N Engl J Med,* 325**,** 968-9.

Thacker, R. E. & Kraman, S. S. (1982). The prevalence of auscultatory crackles in subjects without lung disease. *Chest,* 81**,** 672-674.

Tollefsen, E., Langhammer, A., Romundstad, P., Bjermer, L., Johnsen, R. & Holmen, T. L. (2007). Female gender is associated with higher incidence and more stable respiratory symptoms during adolescence. *Respiratory Medicine,* 101**,** 896-902.

Vannuccini, L., Rossi, M. & Pasquali, G. (1998). A new method to detect crackles in respiratory sounds. *Technology And Health Care: Official Journal Of The European Society For Engineering And Medicine,* 6**,** 75-79.

Vyshedskiy, A., Alhashem, R. M., Paciej, R., Ebril, M., Rudman, I., Fredberg, J. J. & Murphy, R. (2009). Mechanism of Inspiratory and Expiratory Crackles. *Chest,* 135**,** 156-164.

Vyshedskiy, A., Bezares, F., Paciej, R., Ebril, M., Shane, J. & Murphy, R. (2005). Transmission of Crackles in Patients With Interstitial Pulmonary Fibrosis, Congestive Heart Failure, and Pneumonia. *Chest,* 128**,** 1468-1474.

Vyshedskiy, A., Ishikawa, S. & Murphy, R. L., Jr. (2011). Crackle pitch and rate do not vary significantly during a single automated-auscultation session in patients with

pneumonia, congestive heart failure, or interstitial pulmonary fibrosis. *Respiratory care,* 56**,** 806-17.

Walshaw, M. J., Nisar, M., Pearson, M. G., Calverley, P. M. & Earis, J. E. (1990). Expiratory lung crackles in patients with fibrosing alveolitis. *Chest,* 97**,** 407-409.

Ward, J. (2005). Physiology of breathing I. *Surgery (Oxford),* 23**,** 419-424.

Warren, C. W., Jones, N. R., Peruga, A., Chauvin, J., Baptiste, J. P., Costa De Silva, V., El Awa, F., Tsouros, A., Rahman, K., Fishburn, B., Bettcher, D. W. & Asma, S. (2008). Global youth tobacco surveillance, 2000-2007. *MMWR Surveill Summ,* 57**,** 1-28.

Workum, P., Holford, S. K., Delbono, E. A. & Murphy, R. L. (1982). The prevalence and character of crackles (rales) in young women without significant lung disease. *American Review of Respiratory Disease,* 126**,** 921-3.

Xu, X., Li, B. & Wang, L. (1994). Gender difference in smoking effects on adult pulmonary function. *European Respiratory Journal,* 7**,** 477-483.

Yadollahi, A. & Moussavi, Z. M. K. (2007). Acoustical Respiratory Flow. *Engineering in Medicine and Biology Magazine, IEEE,* 26**,** 56-61.