

**DISCORDANT LUNG FUNCTION IN
ALPHA-1-ANTITRYPSIN DEFICIENCY**

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ABSTRACT

Differing emphysema distribution in alpha-1-antitrypsin deficiency (A1AD) relates to specific lung function abnormalities. This thesis explores which factors need to be taken into account when defining A1AD phenotypes.

Miller's lung function prediction equations were the most appropriate for our A1AD population as judged from predicting their survival. A1AD phenotypes were defined by Kco and FEV₁/FVC using these equations. Those with normal lung function and those with isolated Kco abnormality had the least smoking history, least emphysema and best health status whereas those with both indices abnormal had the worst. Those with isolated FEV₁ abnormality had faster Kco decline compared to the normal and the both abnormal groups ($p=0.002$ and $p<0.001$) and were more likely to change groups over time. The best univariate predictor of survival was VA%TLC followed by TLco. Multivariate analysis found the hazard ratio (HR) for death was increased with lower TLco (lowest quartile HR 5.44) and with better Kco (highest quartile HR 2.5 compared to lowest quartile). The HR for death for the lowest VA%TLC quartile was 3.42 compared to the best quartile.

Relevant lung function equations and cut points can define meaningful distinct physiological phenotypes for A1AD. VA%TLC shows potential as a new index in this context.

DEDICATION

To all those who believed in me

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TABLE OF CONTENTS

| | |
|--|-----------|
| 1. INTRODUCTION | 1 |
| 1.1 Chronic Obstructive Pulmonary Disease | 1 |
| 1.1.1 Definition | 1 |
| 1.1.2 Prevalence and impact | 2 |
| 1.1.3 Aetiology and risk factors | 3 |
| 1.1.4 Anatomy of the lungs | 4 |
| 1.1.5 Pathology of proximal and distal bronchi in COPD | 5 |
| 1.1.6 Pathology of alveoli in COPD | 6 |
| 1.1.7 Pathogenesis of COPD | 7 |
| 1.1.8 COPD diagnosis and assessment | 9 |
| 1.1.9 Recording quality of life and respiratory symptoms | 10 |
| 1.1.10 COPD progression and prognosis | 13 |
| 1.2 Alpha-1-antitrypsin deficiency | 14 |
| 1.2.1 Pathophysiology | 15 |
| 1.2.2 Prevalence | 17 |
| 1.2.3 Pulmonary manifestations of alpha-1-antitrypsin deficiency | 18 |
| 1.2.4 Computerised Tomography | 20 |
| 1.3 Lung Function | 21 |
| 1.3.1 Uses of Lung Function | 21 |
| 1.3.2 Spirometry | 22 |
| 1.3.3 Gas transfer | 24 |
| 1.3.4 Lung volumes | 27 |
| 1.3.5 Interpretation of Pulmonary Function Tests | 29 |
| 1.3.6 The debate about the definition of COPD | 32 |
| 1.3.7 Severity | 34 |
| 1.3.8 Prediction equations | 35 |
| 1.4 Clinical phenotypes of COPD | 37 |
| 1.5 Aims | 43 |
| 2. METHODS | 44 |
| 2.1 Introduction | 44 |

| | |
|---|-----------|
| 2.2 Background about the ADAPT project | 44 |
| 2.3 Patient referrals | 45 |
| 2.4 Annual assessment | 45 |
| 2.5 Bloods tests | 46 |
| 2.6 Lung Function | 47 |
| 2.6.1 Changes in lung function equipment | 49 |
| 2.6.2 Calculating decline data: TLco, Kco, FEV ₁ | 53 |
| 2.6.3 Haemoglobin adjustments of Kco | 54 |
| 2.7 Health status | 54 |
| 2.8 CT scans | 56 |
| 2.9 Survival | 57 |
| 2.10 Data sources | 57 |
| 2.11 Defining the classification of Lung Function groups | 59 |
| 2.12 Statistical analysis | 60 |
| 3. CHOOSING THE BEST PREDICTION EQUATIONS FOR LUNG FUNCTION DATA | 61 |
| 3.1 Introduction | 61 |
| 3.2 Methods | 63 |
| 3.3 Results | 66 |
| 3.3.1 Sex differences | 73 |
| 3.3.2 Multivariate predictions | 75 |
| 3.4 Discussion | 77 |

| | |
|--|------------|
| 4. SPIROMETRIC AND GAS TRANSFER DISCORDANCE IN ALPHA-1-ANTITRYPSIN DEFICIENCY: PATIENT CHARACTERISTICS AND PROGRESSION. | 84 |
| 4.1 Introduction | 84 |
| 4.2 Methods | 86 |
| 4.3 Results | 88 |
| 4.3.1 Variables that characterise each physiological phenotype | 91 |
| 4.3.1.1 <i>Group N</i> | 91 |
| 4.3.1.2 <i>Group K</i> | 94 |
| 4.3.1.3 <i>Group F</i> | 95 |
| 4.3.1.4 <i>Group B</i> | 95 |
| 4.3.2 Longitudinal change | 96 |
| 4.4 Discussion | 99 |
| 5. IMPROVING THE DEFINITION OF LUNG FUNCTION COPD PHENOTYPES IN ALPHA-1-ANTITRYPSIN DEFICIENCY | 105 |
| 5.1 Introduction | 105 |
| 5.2 Methods | 106 |
| 5.3 Results | 108 |
| 5.4 Discussion | 119 |
| 6. GENERAL DISCUSSION | 124 |
| 6.1 Choosing the best prediction equations for lung function data | 124 |
| 6.2 Spirometric and gas transfer discordance in A1AD; patient characteristics and progression | 128 |
| 6.3 Improving the definition of lung function COPD phenotypes in A1AD | 134 |

| | |
|--|------------|
| 6.4 Limitation and generalisability | 135 |
| 6.4.1 Population | |
| 1345Methods | 136 |
| 6.4.2 Analysis | 138 |
| 6.5 Future Experiments | 139 |
| 6.6 Conclusions | 142 |
| APPENDIX 1 | 143 |
| REFERENCES | 145 |

LIST OF FIGURES

| Figure | Page |
|---|------|
| 1.1 The definitions of the subcomponents of lung volume measurements. | 28 |
| 1.2 Diagram of normal distribution with estimated lower and upper 5 th percentiles highlighted. | 32 |
| 3.1 Flow diagram of the total number of patients from the lung function dataset included in the final prediction equations study. | 67 |
| 3.2 Histograms of TLcoSR showing the spread of results for each prediction equation. | 72 |
| 3.3 Histograms of FVCSR showing the spread of results for each prediction equation. | 73 |
| 3.4 Plots of TLcoSR values from the Miller equations against those from Crap, ECCS, Roberts and Cotes for men and women. | 74 |
| 4.1 Definition of the physiological phenotypes and number of subjects in each group. | 88 |
| 4.2 Scatter graph illustrating FEV ₁ /FVCSR and KcoSR for each A1AD subject. | 90 |
| 4.3 Histograms illustrating the distribution of FEV ₁ /FVCSR and KcoSR of all subjects. | 91 |
| 4.4 Bar chart showing the movements between the physiological phenotypes groups over time. | 97 |
| 5.1 Estimated Kaplan Meier survival plots for the subjects in each of the 4 phenotypes as defined by FEV ₁ /FVCSR and KcoSR. | 110 |
| 5.2 Estimated Kaplan Meier survival plots for subjects in each of the 4 phenotypes as defined by FEV ₁ /FVCSR and TLcoSR. | 111 |
| 5.3 Estimated Kaplan Meier survival plots for subjects according to quartile of VA%TLC on entry to the study. | 114 |

5.4 Estimated Kaplan Meier survival plots for subjects in each 4 quadrants that were illustrated in Table 5.5.

LIST OF TABLES

| Table | Page |
|---|------|
| 3.1 SR criterion value thresholds for inclusion in the 10 groups including the expected proportion of a normal population in each group. | 65 |
| 3.2 Mean \pm SD demographic and survival data for men and women using ECCS equations. | 68 |
| 3.3 Results of χ^2 values for goodness of fit for Cox proportional hazard progression including age, sex, BMI, smoking status and each of the 5 lung function indices using various prediction equations. | 70 |
| 3.4 Results of the best multivariate Cox regression models using the ECCS equations and indices standardised by ht ³ . | 76 |
| 4.1 Baseline A1AD dataset demographic and lung function characteristics for men and women. | 89 |
| 4.2 Physiological indices for each of the 4 physiological groups. | 92 |
| 4.3 Demographics and other clinical indices for each of the 4 physiological groups. | 93 |
| 4.4 Median (IQR) values for total SGRQ and all its domains and mMRC dyspnoea score for all 4 groups. | 94 |
| 4.5 Demographics and lung function characteristics of group FF and FB. | 98 |
| 5.1 Mean values (SD) for baseline data in the study set out separately for men and women. | 108 |
| 5.2 Cross tabulation of subject numbers in each of the 4 phenotype groups when using TLcoSR and KcoSR in the definition. | 109 |
| 5.3 Chi-square values for the univariate cox regression for predicting the survival of the subjects from the date of the first complete lung function tests. | 113 |

5.4 Results of the best 2 multivariate cox regression models for predicting mortality using quartile of VA%TLC, TLcoSR, KcoSR, age and activity domain of SGRQ. 115

5.5 Table illustrating the number of subjects and mortality in the KcoSR and TLcoSR quartiles. 117

LIST OF ABBREVIATIONS

| | |
|------------------------|--|
| AAT | Alpha-1-antitrypsin |
| A1AD | Alpha-1-antitrypsin deficiency |
| ADAPT | A1AD and Assessment Programme for Treatment |
| ARTP | Association of Respiratory Technology and Physiology |
| ATS | American Thoracic Society |
| BMI | Body Mass Index |
| BTS | British Thoracic Society |
| CAT | COPD Assessment Test |
| CI | Confidence Intervals |
| CO | Carbon monoxide |
| COPD | Chronic Obstructive Pulmonary Disease |
| CT | Computerised Tomography |
| ECCS | European Community of Coal and Steel |
| ERS | European Respiratory Society |
| FEV₁ | Forced Expiratory Volume at 1 second |
| FVC | Forced Vital Capacity |
| GOLD | Global Initiative for Chronic Obstructive Lung Disease |
| HR | Hazard Ratio |
| HRCT | High Resolution CT |
| IQR | Inter-quartile range |
| Kco | Rate of uptake by carbon monoxide (CO) by alveoli |

| | |
|--------------------------|---|
| LLN | Lower limit of normal |
| LVRs | Lung Volume Reduction Surgery |
| LZ910 | Voxel index at -910 Hounsfield Units at level of inferior pulmonary vein (lower zone of lung) |
| MMP | Matric metalloproteinases |
| MRC | Medical Research Council |
| NE | Neutrophil elastase |
| NICE | National Institute of Clinical Excellence |
| PP | Percent of predicted |
| RV | Residual Volume |
| SGRQ | Saint George's Respiratory Questionnaire |
| SR | Standardised residual |
| TLC | Total Lung Capacity |
| TLco | Total uptake of CO by lung per unit time per unit per driving pressure |
| UHB | University Hospital Birmingham |
| UZ910 | Voxel index at -910 Hounsfield Units at level of aortic arch (upper zone of lung) |
| V_{Aeff} | Effective Alveolar Volume |
| V_A%TLC | V _{Aeff} expressed as % of TLC (measure of single breath gas mixing) |
| VI | Voxel index |

Chapter 1

INTRODUCTION

This thesis will compare different prediction equations for spirometry and gas transfer using survival to determine the most appropriate equation for a population. Then using the most appropriate prediction equations, the physiological phenotyping of alpha-1-antitrypsin deficiency (A1AD) determined by Kco and FEV₁/FVC will be explored, including the clinical features of the phenotypes, progression and their relationship to mortality. Finally the relationship between TLco, Kco and survival and how VA%TLC, a marker of gas mixing, maybe a better predictor of survival overall will be studied. This section will outline the background of COPD and A1AD and how they are diagnosed.

1.1 Chronic Obstructive Pulmonary Disease (COPD)

1.1.1 Definition

COPD is a lung disease characterised by airflow obstruction which is progressive in its nature, with minimal or no reversibility and no usual significant day to day variation in symptoms, apart from during an exacerbation. COPD is an umbrella term which encompasses different underlying patho-physiological processes that can occur in isolation or in combination including emphysema (vide infra section 1.1.6), chronic bronchitis, small airways disease, bronchospasm and bronchiectasis. Chronic bronchitis can be an important feature of COPD and in this context is defined as the presence of a

persistent cough with the production of sputum that occurs most days of the week, at least 3 months of the year and for more than 2 years consecutively in the absence of any other cause (1965).

1.1.2 Prevalence and impact

COPD is a common respiratory disease with a prevalence recently estimated to be 900,000 in England and Wales by the National Collaborating Centre for Chronic Conditions (2004). This is thought to be an underestimation of the actual number of patients, with the anticipated prevalence thought to be nearer to 3.7 million (Shahab et al., 2006). COPD is a significant cause of morbidity and mortality. According to the Healthcare Commission (2006) there are over 25,000 deaths per year from COPD in the United Kingdom and it is currently the fifth greatest cause of death claiming more lives than breast, prostate and bowel cancer.

COPD is projected to become the 3rd highest cause of ill health globally by 2020 (Lozano et al., 2012) exceeded only by cardiovascular disease and stroke. Therefore COPD has a significant financial impact on the NHS with the Health and Safety Executive estimating the annual direct healthcare costs to be £500 million (2013) with admissions to hospital as a consequence of COPD (1 in 8 of all acute hospital admissions) being a major contributor to the healthcare costs.

1.1.3 Aetiology and risk factors

The risk of developing COPD is thought to be a result of a combination of environmental and genetic risk factors. The most common established environmental cause for COPD in the Western World is smoking (Buist, 1994) which accounts for up to 95% of COPD cases. Other established causes include occupational exposure e.g. wood smoke and mineral dust (Oxman et al., 1993), biomass exposure from cooking (Liu et al., 2007) and genetic risk factors e.g. alpha-1-antitrypsin deficiency. Probable causes may include air pollution, childhood passive smoking and hyper-reactive airways (Celli, 1999). Studies in twins have shown there is a higher FEV₁ correlation in monozygotic than dizygotic twins (Redline et al., 1989) and higher rates of airflow obstruction have been reported in first degree relatives of COPD patients compared to controls (Larson et al., 1970). Both studies suggest that genetic factors may influence lung function variability. It is thought that several genetic susceptibility factors may be involved which influence the effect of the environment on the lungs which may help answer why only 10 – 20% of smokers develop clinically significant COPD (Fletcher and Peto, 1977). In genetic studies several specific single nucleotide polymorphisms (SNP) on chromosome 15q25 have been identified that are associated with nicotine addiction. The SNP's are subunits of the alpha-nicotinic acetylcholine receptor. These receptors are found on bronchial and alveolar epithelial cells and may modify the inflammatory response as a result of smoking and are associated with the development of COPD (Saccone et al., 2010). A genome wide association study (Pillai et al., 2009) identified the Hedgehog interacting protein (HIPP) locus on chromosome 4 which also contributes to the risk of developing

COPD. This interaction between genes and environment may also influence the rate of progression of the disease.

1.1.4 Anatomy of the lungs

Understanding the normal respiratory anatomy is crucial for characterising the pathological changes that occur in COPD. The thorax provides protection for the internal organs such as the heart, lungs and major blood vessels but also allows for the change in lung volumes during inspiration and expiration. There are several groups of muscles involved in the expansion of the thorax during inspiration; internal and external intercostal muscles, diaphragm, abdominal and accessory muscles. They all act in different directions to maximize lung expansion. The pleural space between the visceral and parietal pleura transmits the force during inspiration from the muscles to the lung by creating a negative pressure which aids the expansion of the lungs. Expiration does not usually require these muscles as it is a passive movement due to the elastic recoil of the lungs and chest wall. The respiratory system is divided into the upper and lower respiratory tract. The upper respiratory tract incorporates the nasal passages, pharynx and larynx. The lower respiratory tract starts with the trachea and is held patent by incomplete rings of cartilage and, therefore, can withstand the changes in intrathoracic pressures. The trachea splits into the right and left main bronchus at the main carina and many further divisions of the airways form the lobar, segmental and subsegmental bronchi and the bronchioles with each division resulting in smaller and smaller airways.

The large airways (trachea and bronchi) are not involved with gas exchange but conduct the air towards the alveoli. They are lined by ciliated columnar epithelium to aid the clearance of mucus and other particles from the airways. Beyond the bronchioles are the respiratory bronchioles, alveolar ducts, sacs and alveoli. These structures form the area of the lung where gas exchange takes place. The whole unit that is supplied by the terminal bronchiole is called the acinus. There are on average 300 million alveoli per adult and where the alveoli surface is adjacent to pulmonary capillaries allows gas exchange. This is a thin interface of single layers of endothelial and epithelial cells and basement membranes of the alveoli and capillaries fusing together so there is minimal distance for gases to diffuse. This provides a surface area of about 70m² for gas exchange (Culver, 1999). There are 2 main types of cells that form the surface of the alveoli; Type 1 which forms the main structure of the alveolar wall, Type 2 which secrete surfactant that maintains patency and aids re-inflation of the alveoli after expiration. In addition this region also contains both airway and tissue macrophages whose function is to defend against any microbes that reach the surface of the alveoli (Sibille and Reynolds, 1990).

1.1.5 Pathology of proximal and distal bronchi in COPD

There are several significant pathological changes that occur in patients with COPD. As a result of noxious stimulants (e.g. smoking) mucous gland hyperplasia (increase in the number) and hypertrophy (increase in the size) may occur (Reid, 1954). This results in a

significant increase in mucus production. Infiltration of inflammatory cells into the bronchial walls leads to oedema, damage and sometimes fibrosis. Ciliary function may be affected resulting in the airways having a reduced ability to clear the debris and bacteria from the lungs. The number of cilia can be reduced as the normal ciliated columnar epithelium is replaced by squamous metaplasia. There may be disruption of the epithelial barrier and connective tissue deposited in the airway walls (Hogg, 2004). The combination of excess mucus, bronchial wall oedema and fibrosis result in airflow obstruction which is pathognomonic of COPD (Jeffery, 1998).

Similar changes may also occur in the small airways with the addition of smooth muscle hypertrophy and development of goblet cells which are absent in the normal lung. (Cosio et al., 1980). Again these features increase the degree of airflow obstruction.

1.1.6 Pathology of alveoli in COPD

Emphysema is defined pathologically as a permanent air space enlargement distal to the terminal bronchioles (Celli et al., 1999). Disruption in the elastic fibres results in a loss in elastic recoil of the lung, distortion of the alveoli and destruction of the septa between the alveoli. There are 3 main types of emphysema that are defined by the region of the acinus that is affected by the disease; centrilobular, panlobular and paraseptal. Centrilobular emphysema is characterised by a focal destruction of the respiratory bronchioles and the central parts of the acinus. Each abnormal area is surrounded by normal lung parenchyma. This type of emphysema is typically seen in the

upper lobes and is the main type that characterises smoking induced COPD. Panlobular emphysema is characterised by a uniform destruction of the alveolar walls and involves all the areas beyond the terminal bronchioles. This type of emphysema is typically seen in the lower lobes and classically associated with alpha-1-antitrypsin deficiency (Eriksson, 1964). Paraseptal emphysema involves the alveolar ducts and sacs in the periphery of the lung and its changes are subpleural. Little is known about the associations and causes of this anatomical type of emphysema.

1.1.7 Pathogenesis of COPD

The pathogenesis of COPD is believed to be the result of several processes which are interlinked; inflammation, protease/anti-protease imbalance, apoptosis and oxidative stress. All of these are seen as a physiological response to smoking. However it is believed that this response is excessive in those smokers who develop COPD. In part the extent of the environmental exposure may play a role, for instance, there is a dose dependent relationship between the number of cigarettes smoked and the risk of developing progressive airflow obstruction (Burrows et al., 1977). Cigarettes are known to contain many harmful chemicals including free oxygen radicals, polycyclic aromatic hydrocarbons and N-nitrosaminous compounds. Studies of bronchial and lung biopsies and induced sputum from smokers have confirmed the presence of inflammation (MacNee, 2005). Biopsy and sputum samples from patients with COPD have shown an increase in T cells (predominantly CD8), macrophages, activated neutrophils (Keatings

et al., 1996) and eosinophils (Brightling et al., 2005, Saetta et al., 1994) as well as chemo-attractants e.g. interleukin-8 (IL-8) and Leukotriene B4 (Seggev et al., 1991).

Neutrophils are thought to play a central role in the pathogenesis of COPD as they release elastase and proteinase-3 which can cause the breakdown of elastin leading to emphysema (Stockley, 2002). Oxidative stress develops as a result of smoking and reactive oxygen and nitrogen species from inflammatory cells adds to this process. This in turn leads to the release of inflammatory cytokines including polymorphonuclear cell chemo-attractants. The net effect of this is to increase the number of neutrophils in the alveoli (and hence their enzymes) leading to an increase or activation of other enzymes such as elastase, cathepsins and matrix metalloproteinases (MMP). Antiproteases like alpha-1-antitrypsin and secretory leucocyte peptidase inhibitor (SLPI) may inactivate the elastase that has been released. If there is an excess of elastase then an imbalance of proteases to antiproteases may result in persistent enzyme activity and as a consequence excessive lung parenchyma damage (MacNee, 2005).

Apoptosis is a normal or regulated form of cell death and animal models have induced alveolar cell apoptosis and emphysema using Vascular Endothelial Growth Factor (VEGF) (Kasahara et al., 2000). Increased numbers of apoptotic cells have also been found in lung tissue and airways of COPD patients (Segura-Valdez et al., 2000). Factors that may stimulate apoptosis include smoking and neutrophil activity. It has also been proposed that excessive proteolysis, oxidative stress and lung cell apoptosis interact leading to the development of emphysema (Plataki et al., 2006).

Cigarette smoke induces the release of enzymes that breakdown protein and in particular elastin, from cells which are part of the innate immune system. This leads to the release of elastin fragments and in susceptible patients could lead to T and B cell-mediated immunity against elastin (Lee et al., 2007). This proposed mechanism could cause further lung destruction in emphysema (Wood et al., 2011).

1.1.8 COPD diagnosis and assessment

A diagnosis of COPD requires the demonstration of airflow obstruction by spirometry (vide infra section 1.3.2), which is not fully reversible. In addition there is usually a smoking history and appropriate symptoms. Possible symptoms include dyspnoea usually (at least initially) on exertion (hence reduced exercise tolerance), chronic cough that may be productive of sputum, history of frequent chest infections during the winter and wheeze. Other symptoms may reflect the underlying chronic systemic inflammation of COPD including fatigue, weight loss, depression and co-morbidities (Yawn and Kaplan, 2008). Methods have been developed for standardising the registration of respiratory symptoms (vide infra 1.1.9).

The signs of COPD depend on the severity of disease. In early disease there may be few but as disease progresses there may be evidence of a hyper-expanded chest as a result of chronic air trapping and hyperinflation. The patient's baseline respiratory rate may be increased, expiration may be prolonged and pursed lip breathing may be

observed together with the use of accessory muscles for respiration. On chest auscultation the breath sounds may be quiet as a result of reduced air movement during breathing and an expiratory wheeze may be audible with or without early crackles.

When the disease is severe there may be signs that are a consequence of pulmonary hypertension (cor pulmonale) including peripheral oedema, a raised jugular venous pressure and a loud pulmonary second sound. In type 2 respiratory failure there may be signs of hypercapnia including warm peripheries and a bounding pulse.

Chest radiographs of COPD patients may be normal or have flattened diaphragms as evidence of hyper-inflation with hyperlucent lungs, or bullae (>1cm in diameter) which are typically found in the upper zones of the lungs. Computerised tomography (CT) has a greater sensitivity and specificity than a chest radiograph for detecting emphysema, (Klein et al., 1992) to confirm the presence of bronchiectasis and determine whether Lung Volume Reduction Surgery (LVRS) is an treatment option by illustrating the extent and distribution of the emphysema. In addition CT scans can be used to quantify emphysema (vide infra 1.2.4).

1.1.9 Recording quality of life and respiratory symptoms

Health status in patients is measured using questionnaires that score the symptoms, physical ability, activities, emotional well-being and the impact of these on their quality of life. The questionnaires are tailored and validated for specific diseases, such as the Saint George's Respiratory Questionnaire (SGRQ) (Jones et al., 1992) which is a self-

completed questionnaire for patients with chronic airflow obstruction. A clinically significant change has been determined (Jones, 2002) which enables its use for monitoring patients over time and determining the efficacy of interventions. COPD is known to negatively impact quality of life but the degree seems to vary between patients with a similar severity of disease (at least as defined spirometrically). Airflow obstruction is known to correlate only weakly with SGRQ scores (Dowson et al., 2001).

The modified Medical Research Council dyspnoea score was initially developed in 1959 (Fletcher et al., 1959) to characterise the degree of dyspnoea perceived by a subject during daily activities. It is a 5 point scale starting at 0 which is the lowest level of shortness of breath up to 4 which is the greatest. The MRC dyspnoea score is not related to disability as measured by FEV₁ (Bestall et al., 1999) but a good relationship has been shown between the MRC and walking test performance (McGavin et al., 1978). So as a measure of respiratory disability NICE (2004) suggest patients with MRC ≥ 3 would gain most benefit from pulmonary rehabilitation.

There are other questionnaires available for measuring impact of disease on quality of life of which some are generic and others specific for COPD. The Medical Outcomes Study short form 36 item questionnaire (SF-36) is a generic measure of health related quality of life and was designed for use in clinical practice and general population surveys (Ware and Sherbourne, 1992). The self-completed questionnaire measures 8 health concepts including impact on physical, emotional and social activity and pain. The outcome of the SF-36 has been shown to relate to dyspnoea measured by

multidimensional baseline dyspnoea index (Mahler and Mackowiak, 1995) and has been validated as a tool to measure quality of life in symptomatic COPD.

The EQ5D is a simple self-completed questionnaire that has 2 parts: descriptive and a visual analogue scale (VAS). The descriptive section includes 5 dimensions: mobility, self-care, usual activities, pain and anxiety/depression. The subject scores each dimension as: no, some or extreme problems. The VAS allows the subject to rate their health with a score of 0 (worst) to 100 (best imaginable). The EQ5D has been shown to be valid and reliable in its use in COPD (Pickard et al., 2008).

One of the newest available questionnaires is the COPD Assessment test (CAT) which is a short, 8 question validated tool for measuring the impact of COPD on the patient's life and how it changes over time but also to initiate discussion between the health care professional and patient (Jones et al., 2009). It was developed using psychometric and statistical techniques. The patient rates how true 8 statements on cough, phlegm, chest tightness, shortness of breath, limitation on activities, confidence, sleep and energy are on a scale of 0 to 5 with a final score of between 0 to 40 (40 being the worst) (Jones et al., 2009). The CAT has been shown to be sensitive to changes in quality of life after exacerbations (Jones et al., 2012) and also following pulmonary rehabilitation (Dodd et al., 2011).

1.1.10 COPD progression and prognosis

Several factors have been shown to contribute to a faster physiological decline in COPD patients. Patients with COPD who stop smoking on average have a slower FEV₁ decline compared to those who continue smoking. This was illustrated best in the Lung Health Study which was a randomized controlled trial comparing smoking cessation and regular inhaled ipratropium bromide in a group of 6,000 middle aged smokers with airflow obstruction (Anthonisen et al., 1994). The cohort was followed up 11 years later and observed those who continued to smoke had a faster FEV₁ decline than those who had stopped at the beginning of the original study (Anthonisen et al., 2002).

Bronchodilator reversibility has also been shown in COPD to correlate with FEV₁ decline (Vestbo et al., 2011). This group found that those with significant reversibility (as defined by a 12% FEV₁ increase above baseline value and ≥ 200 ml increase post bronchodilator) had 17 ± 4 mls per year greater FEV₁ decline than those without reversibility. Donaldson et al (2002) found that COPD patients with 2 or more exacerbations per year (average 2.92) had a 40.1ml/year decline in their FEV₁ compared to those with less than 2 exacerbations per year (32.1ml/year). The exact mechanism behind exacerbations and lung function decline is unknown but such episodes are generally associated with an increase in inflammation (Bhowmik et al., 2000) and since inflammation is believed to underlie the pathophysiology such episodes might be predicted to cause short periods of greater lung damage. The Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) observational study explored the role of exacerbations in COPD (Hurst et al., 2010). The study confirmed that moderate and severe

exacerbations had a significant negative impact on lung function. Also the best predictor of a patient having an exacerbation in the first year of the study was a treated exacerbation in the year before study entry (OR 4.30). The frequency of exacerbations during the first year of the study had a sensitivity of 60% and specificity of 83% for predicting the frequency in the 2nd year and the number of exacerbations were stable over the 3 year study. The number of exacerbations also increased with the severity of airflow obstruction as defined by GOLD (Global Initiative for Chronic Obstructive Lung Disease). COPD patients with frequent exacerbations were found to have a worse quality of life, history of gastro-oesophageal reflux disease and increased white cell count.

1.2 Alpha-1-antitrypsin deficiency

Alpha-1-antitrypsin deficiency (A1AD) is the most recognised genetic cause of COPD with an estimated prevalence of up to 1 to 2% in COPD patients depending on the cohort being studied (DeMeo and Silverman, 2004). It is an autosomal co-dominant genetic disorder and is one of the most common genetic disorders in Caucasians. Its incidence has been shown to be comparable to cystic fibrosis and sickle cell disease (Wilson-Cox, 1989).

A1AD was first described by Laurell and Eriksson (1963) when they observed the absence of an α_1 band on protein electrophoresis. Alpha-1-antitrypsin deficient patients have an increase predisposition to develop lung and liver disorders and more rarely,

panniculitis and vasculitis. A1AD patients have been divided into 3 categories depending on the A1AD plasma level: normal, deficiency and null. Those with a normal level do not have an increased risk for lung or liver problems but those with low or non-detectable levels do have the predisposition to develop early onset emphysema in the 3rd and 4th decade (Eriksson, 1965).

1.2.1 Pathophysiology

Alpha-1-antitrypsin (AAT) is a 52kD glycoprotein and is made mainly by hepatocytes and to a lesser extent by lung epithelial cells and macrophages. AAT is an acute phase protein but its main role is to inhibit serine proteinases, which are enzymes with serine at the active site that cleave specific peptide bonds in other proteins (especially matrix proteins). Neutrophil elastase is one of these enzymes and is thought to be the most critical in the development of emphysema in A1AD. Neutrophil elastase is made during cell differentiation and stored in the azurophil granules of polymorphonuclear neutrophils. It is released on degranulation and can degrade many different proteins including elastin which is believed to be the central process to the pathophysiology of emphysema (Stockley, 2002).

The AAT protein is encoded by the Protease Inhibitor (Pi) locus on the chromosome 14q32.1 (Billingsley et al., 1993) and is a highly pleomorphic protein with many different alleles. These alleles were initially defined by their migration velocity in starch-gel

electrophoresis (Fagerhol and Laurell, 1970). The alleles had differing velocities as a result of the protein charges from the different amino acid variants (Fagerhol and Laurell, 1967). The most common alleles separated by their movement in an electrical field are M (medium), S (slow), F (fast) and Z (very slow). There have been more than 100 genetic variants identified which are associated with differing AAT plasma levels. The normal phenotype MM has an average AAT serum level of approximately 30µm. The most common clinically relevant form of the deficiency is the homozygote Z variety referred to as PiZZ. PiZZ has a critically low serum concentration of AAT, usually about 5µm. Plasma levels in patients with PiZZ are thus approximately 10% of the normal for the MM allele and the heterozygote MZ are approximately 60% of the normal levels.

These low levels for PiZZ are as a consequence of a replacement of a single amino acid (glutamic acid at position 349 for lysine) within the alpha-1-antitrypsin protein which leads to spontaneous polymerisation and therefore accumulation within the hepatocytes resulting in low circulating levels. In PiZZ only 15% of the AAT produced is released into the circulation leaving 85% blocked in hepatocytes. These are observed as large intracellular inclusions in the hepatocyte endoplasmic reticulum (Lomas et al., 1992).

The development of emphysema in these patients is due to an imbalance between harmful serine proteinases such as neutrophil elastase (NE) and the protective effect of AAT. AAT is one of the major anti-elastase defences within the lung including the interstitium and alveolar region so when AAT is absent or lower than normal within the alveoli, as in A1AD, the patient is at increased risk of developing emphysema due to the unopposed action of any locally released NE (Gadek et al., 1981). Cigarette smoking

and the uninhibited action of elastase contributes to this protease/anti-protease imbalance as increased neutrophils have been found to be present in bronchoalveolar lavage (Morrison et al., 1987) and in lung parenchyma (Lomas and Mahadeva, 2002) in patients with low AAT levels compared to those with normal levels thereby leading to both an increased NE and reduced anti-elastase load (Morrison et al., 1987). Recent data show neutrophils accumulating in the interstitium where AAT polymers are present (Parmar et al., 2002).

1.2.2 Prevalence

The normal MM genotype is present in 94 to 96% of Caucasians (Kamboh, 1985) and heterozygote for the Z deficiency (MZ) occurs in 2 to 3% (ATS/ERS, 2003). Several studies have been undertaken to determine the prevalence of A1AD. One of the largest screening programmes was undertaken between 1972 and 1974 in Sweden of newborn babies (Sveger, 1976). One hundred and twenty seven PiZZ patients were identified from a population of 200,000 which translated into a prevalence of 1 in 1600 newborns. The incidence of A1AD however varies between countries with the highest prevalence of the homozygous Z deficiency being observed within North and Western European countries especially around the Baltic where it is widely accepted to have arisen (Denden et al., 2012). The World Health Organisation estimates the prevalence in the UK is 1 in 2000 (1997a). In the United States of America (USA) a population based study including 20,000 blood donors in St Louis calculated the prevalence at 1 in 2800

(Silverman et al., 1989). The prevalence in Australia and New Zealand is thought to be similar to the USA (Luisetti and Seersholm, 2004). There are limited cohorts reported from Japan, China and South Korea where the incidence is much lower.

1.2.3 Pulmonary manifestations of A1AD

Most subjects with A1AD are identified either by the onset of respiratory symptoms in their 3rd or 4th decade or through family screening, with just a small number being identified following investigation of liver disease. In the UK registry 76% of PiZZ patients presented with symptoms and 19% were identified through family screening (Needham and Stockley, 2004). A significant delay has been shown to occur between the onset of symptoms and subsequent diagnosis and this may be up to 7 years (Stoller et al., 1994) which is thought, in part, to be due to A1AD being under-recognised by health care professionals and erroneous diagnoses of asthma. Common symptoms at presentation are similar to those of patients with usual COPD, including dyspnoea on exertion, cough, phlegm, wheeze and recurrent chest infections. In A1AD emphysema with its symptoms have been shown to develop at an earlier age than the spirometric evidence of COPD and also seem to be out of proportion to the smoking history (Needham and Stockley, 2004). The age of onset of airflow obstruction is highly variable for subjects with A1AD. The age when symptomatic obstructive lung disease presents in A1AD has been found to be between 32 – 41 years in patients with a smoking history (Larsson, 1978, Eriksson, 1965). Rarely patients present with respiratory symptoms before the age of 25

years. Never smokers with homozygous α_1 deficiency have been found to have a delayed onset of symptoms and often have an almost normal lifespan (Tanash et al., 2008). Other studies have shown that some smokers as well as non-smokers fail to complain of symptoms (Stoller et al., 1994, Wall et al., 1990) and less than 60% of A1AD patients develop significant airflow obstruction (Larsson, 1978). Patients also vary in the relationship between airflow obstruction and gas transfer abnormality (which is a more direct marker of emphysema) suggesting that some have a predominantly airways phenotype rather than an emphysema phenotype (Needham and Stockley, 2004).

Bronchodilator response in A1AD is also variable (Eden et al., 2003) and may support an initial diagnosis of asthma especially as patients present at a younger age when asthma is more prevalent than COPD. Up to 43% of A1AD patients have a history of chronic bronchitis (Needham and Stockley, 2004) and 26% of patients have also been shown to have bronchiectasis on High Resolution CT (HRCT) scans (Dowson et al., 2002) which is similar to the incidence in usual COPD (O'Brien et al., 2000).

There are also risk factors that affect the rate of decline in the lung function in A1AD which are similar to those seen in usual COPD. The most important is smoking and current smokers have been found to have a faster decline in FEV₁ compared to ex- and never smokers with mean values of 70ml/year (confidence interval (CI) 58 – 82), 41ml/year (CI 36 – 48), and 47ml/year (CI 41 – 53) respectively (Piitulainen and Eriksson, 1999). In addition there is a dose response between smoking history and rate of decline.

Several factors have been shown to have a negative impact on lung function decline in A1AD including exacerbation frequency (Dowson et al., 2001), bronchodilator reversibility (Dawkins et al., 2009) and air pollution in particular PM10 (particles with a diameter of 10 micrometres or less) and ozone (Wood et al., 2010).

1.2.4 Computerised Tomography (CT)

As previously described, patients with A1AD develop early onset emphysema which is classically a panlobular type with a basal predominance. CT has been shown to be more sensitive than chest radiography in detecting emphysema (Sanders et al., 1988) and HRCT which is performed using 1 – 2 mm sections is particularly good in this respect. CT scanning has been validated as a measure of emphysema in vivo and correlates with exercise capacity (Dowson et al., 2001), health status and lung function (Parr et al., 2004, Knudson et al., 1991). CT density of the lungs has also been validated against pathology and has been shown to be a sensitive and specific measure of emphysema in A1AD (Parr et al., 2006), a better predictor of mortality than FEV₁ and Kco (Dawkins et al., 2003) and relates to the progressive decline in FEV₁ (Parr et al., 2006).

The basis of CT lung densitometry is to measure the attenuation of x-rays through lung tissue and express this relative to their attenuation through water where water is scored as 0 and air as -1000 Hounsfield Units (HU). Hayhurst et al (1984) showed that patients with centrilobular emphysema had more voxels (3-dimensional pixels) in the range of -900 to -1000 HU than non-emphysematous patients. The Voxel Index (VI) is the

percentage of low density lung voxels below a specified threshold (Parr et al., 2004). The higher the VI the more emphysema present on the CT. An alternative method for quantifying CT densitometry is the 15th percentile point. This is the value in Hounsfield Units below which 15% of voxels with the lowest density are distributed. Parr et al (2006) showed there is good correlation between the 15th percentile point and voxel index techniques when assessing lung densitometry in different pulmonary diseases. The density threshold is set in this thesis as $\leq -910\text{HU}$ and has been described by others and validated against pathological assessment of emphysema (Muller et al., 1988).

1.3 Lung function

1.3.1 Uses of lung function

There are many reasons for requesting pulmonary function tests for a patient including diagnosis, monitoring, response to treatment and for public health purposes (Miller et al., 2005b). The tests are commonly used to confirm the presence of lung pathology related to structural abnormalities, to monitor disease progression over time, to assess response to a specific medication or therapy and for pre-operative assessment, (as FEV_1 is an indicator of a patient's post-operative risk (Ferguson and Durkin, 2002)). Lung function can be used to assess disability prior to rehabilitation and as confirmation for insurance purposes. From a public health perspective lung function is an essential measurement performed during occupational and epidemiological surveys, for example the post 9/11 surveillance of people exposed at the World Trade centre disaster in New

York (Friedman et al., 2011) and respiratory symptoms were associated with lower spirometry results during the first examination of World Trade Centre responders (Udasin et al., 2011).

1.3.2 Spirometry

Spirometry is used to measure the volume of air exhaled over time during a maximum forced expiratory manoeuvre. This entails the subject inhaling until their lungs are maximally full and then exhaling as fast and for as long as possible in one continuous blow until no more air can be expelled. Many different types of equipment have been used to record spirometry. Historically water sealed and dry sealed spirometers have been commonly used and all the tests in this thesis were undertaken using dry rolling seal spirometers (Morgan, Sensor Medics). Now the most commonly used devices are flow meters such as the Fleisch pneumotachograph which has fine capillaries through which the air passes. There is a linear relationship between the flow of air and the pressure drop due to the viscous resistance of the gas. Other flow meters such as stator/rotor devices or ultrasonic devices are also now more commonly used.

The main measurements performed during spirometry are Forced Vital Capacity (FVC) and Forced Expiratory Volume in 1 second (FEV_1). Using both these measurements the ratio of FEV_1 to FVC can then be calculated. The FVC is the maximum volume of air that is exhaled from maximum inspiration (Total Lung Capacity (TLC)) using a maximal forced effort and the FEV_1 is the volume of air exhaled in the first second of a maximal

forced effort from maximum inspiration. These measurements are dependent on several factors: the equipment, the patient and the laboratory staff who are leading the measurements. Patient cooperation is one of the key elements to achieving as accurate measurements as possible. Errors in the measurement may be as a consequence of coughing during expiration, early stopping of the test, air leak around the mouthpiece, failure to inspire to maximum volume and inadequate effort. Errors due to cooling need to be considered as temperature variation may lead to an under-reading in the peak expiratory flow and an over-reading in the latter part of the blow (Miller and Pincock, 1986). There are several measures that are undertaken within each laboratory to ensure the errors are as minimal as possible including calibration, checking for leaks and biological controls. It is essential to ensure quality control is undertaken in each laboratory so that the measurements are comparable between each patient and each laboratory. A minimum of 3 FVC manoeuvres are advised (Miller et al., 2005b) and once they are deemed appropriate i.e. no errors, then they are checked to confirm they are repeatable. The difference between the largest FVC and the next largest should be <0.15 L and the same for FEV_1 .

The spirometry measurements enable us to distinguish between obstructive and restrictive respiratory diseases. Obstructive spirometry is present when the FEV_1 is reduced disproportionately to the FVC due to the obstruction of the airways thereby leading to a lower flow. The plateau usually seen at the end of a normal expiration may not be present or may occur much later in patients with airflow obstruction as the air takes longer to be expired. In COPD usually the FVC is normal however late in the

disease process premature airway closure will reduce FVC as well. Other diseases which may have an obstructive spirometry include asthma and bronchiectasis. Spirometry is able to suggest a restrictive defect but lung volumes and gas transfer measurements are needed to clarify the situation in more detail. A restrictive spirometry shows FEV_1 and FVC are reduced proportionately and the FEV_1/FVC ratio is usually normal or raised.

1.3.3. Gas transfer

Measuring gas transfer determines the ability of the lungs to exchange gas across the membrane between the alveoli and the pulmonary capillaries. This measurement is affected by the structural and functional properties of the lungs and conducting airways (Macintyre et al., 2005). The structural properties include the size of the alveolar surface area, the thickness of the alveolar membrane, any airway closure and the volume of blood in the capillaries that supply the alveoli. The functional properties include composition of the gas in the alveoli, diffusion characteristics of the alveolar membrane, the level of haemoglobin in the capillaries, the carbon monoxide gas tension within the blood and the distribution of ventilation and perfusion to the alveoli.

Gas transfer measurement is usually performed using the single breath method originally described by Marie Krogh in 1915 (Krogh, 1915) and then modified to include helium as a reference gas by Blakemore et al (1957). The manoeuvre starts with slow exhalation to residual volume and then rapid inhalation to TLC of a mixture of carbon

monoxide (CO) 0.3%, helium, oxygen and nitrogen. This is followed by a breath hold for a pre-set time (9 to 11 seconds) followed by slow expiration. During the expiration a sample of alveolar gas is collected (the first 500 to 1000ml is disregarded as the initial sample comes from the large airways). The CO₂ and water vapour are absorbed and helium and carbon monoxide concentrations quantified. CO is used as it binds tightly and quickly to the haemoglobin molecules and as a result the partial pressures of CO are minimal. Helium is used as a tracer gas which distributes rapidly within the airways and alveoli but is unable to cross the alveolar-capillary membrane and so its dilution provides a means to measure the volume of lungs into which the gases were inhaled.

This manoeuvre measures 3 indices: TLco, Kco and the alveolar volume (V_A) accessed by the single breath which is termed the effective alveolar volume (V_{Aeff}). Kco is the rate of uptake of CO by the alveoli and V_{Aeff} is the gas volume in the lung which contains the inhaled CO. TLco is derived by multiplying these 2 measurements:

$$K_{co} \times V_{Aeff} = TL_{co}$$

TLco is the total uptake of the CO by the lung per unit time per unit driving pressure and reflects the surface area that is available within the lung for gas exchange. The driving pressure is influenced by any CO in the blood prior to the gas transfer measurement (which may be seen in heavy smokers) as this would exert a back pressure thus reducing the transfer of CO into the blood. The ERS/ATS have also developed standards for gas transfer measurements (Macintyre et al., 2005) including acceptable measurement limits. Importantly patients need to inhale to within 85% of their largest

known vital capacity otherwise the V_A is underestimated which impacts the gas transfer measurements. At least 3 minutes is allowed between each test to enable the test gases to be cleared and there should be 2 acceptable tests with the TLco measurements within 1 mmol/min/kPa of each other to ensure a valid result. No more than 5 tests are advised as the CO level within the blood does not return to baseline and is likely to affect the later results by back pressure.

Gas transfer within the lungs involves multiple steps: firstly the delivery of CO to the airways and the alveoli, then the mixing and diffusion of the CO within the alveoli and alveolar ducts. CO is then transferred across the alveolar membrane and mixes and diffuses within the lung parenchyma and alveolar capillaries and finally diffuses across the red blood cell membrane and reacts with the haemoglobin. Interpreting the gas transfer results involves understanding the influence of all these factors especially whether the result reflects the alveolar membrane (D_m) or the capillary blood volume (V_c). If the V_c is reduced following pulmonary embolism, in the presence of vasculitis or haemoglobin is reduced then the TLco will also reduce. If the D_m is affected (e.g. emphysema, interstitial lung disease and pulmonary oedema) or inspired air cannot access the alveoli correctly (airways disease) then the TLco may also be reduced. Conversely an increased TLco may reflect an increase in V_c , an increased haemoglobin concentration (polycythaemia) or the presence of free blood within the airways such as in pulmonary haemorrhage.

The interpretation of the K_{co} needs to be taken into account with the TLco and V_A as they are critically dependent on each other and therefore should not be interpreted

separately. Kco decreases if the haemoglobin decreases or the alveolar/capillary diffusion is reduced. This occurs in many pathological processes including primary pulmonary hypertension, vasculitis, loss of surface area in emphysema and thickened interstitium in pulmonary fibrosis. Kco increases with increasing pulmonary blood flow per alveolus which is seen following a pneumonectomy but can also be increased when the TLco is measured at lung volumes below the predicted total lung capacity e.g. chest wall restriction, neuromuscular disease or poor technique.

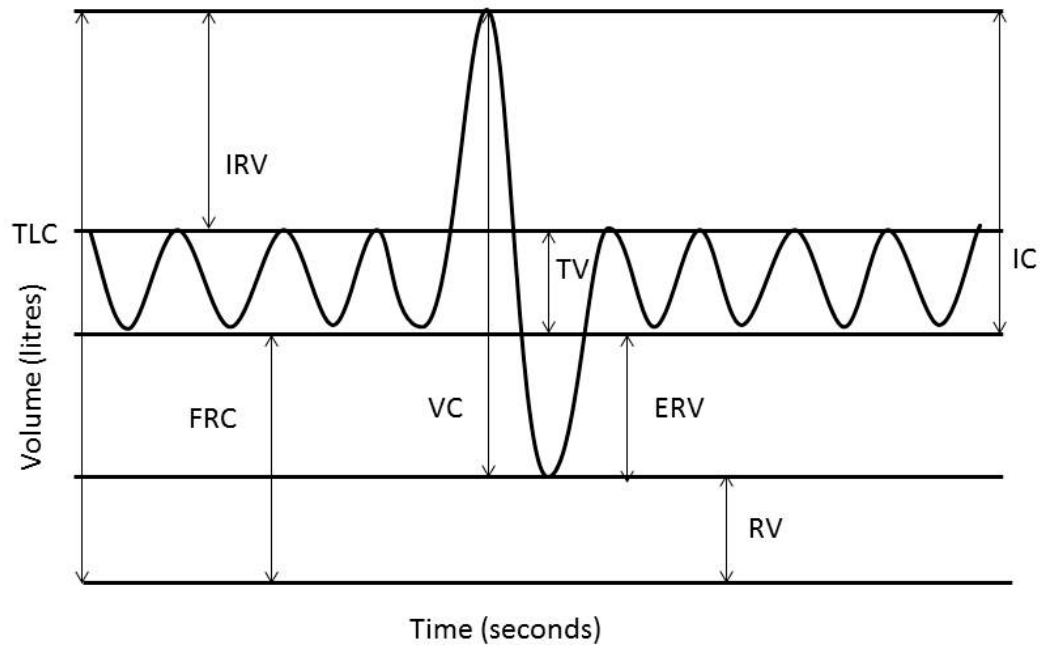
TLco is nearly always decreased in patients with COPD and is particularly reduced if emphysema is present because of the alveolar-capillary membrane destruction. A reduced Kco has been found to relate to both the severity of emphysema assessed by CT (Gould et al., 1991) and the degree of emphysema found on autopsy (Burrows et al., 1966) indicating that it is a valid measure of the emphysema process.

1.3.4 Lung volumes

The 'lung volume' is taken as the volume of gas in the lungs (Wanger et al., 2005). The definitions of the sub components of lung volume are illustrated in figure 1.1. Measuring absolute lung volumes is a technical challenge which can restrict their use within clinical practice. On the other hand they can provide helpful information in some settings for instance, if spirometry has a mixed picture with some obstructive features and restrictive features (vide supra 1.3.2). Lung volumes can help differentiate between, or attribute proportion to, the restrictive or obstructive component.

Figure 1.1

The definitions of the sub-components of lung volume measurements.



TLC: Total lung capacity, IRV: Inspiratory Reserve Volume, VC: Vital Capacity, FRC: Functional Residual Capacity, TV: Tidal Volume, RV: Residual Volume, ERV: Expiratory Reserve Volume, IC: Inspiratory capacity

A variety of techniques have been developed to measure lung volumes including body plethysmography, nitrogen washout and gas dilution. Body plethysmography involves the patient sitting in an airtight cabinet breathing through a shuttered mouthpiece. Measurement employs the principle of Boyle's law where changes in pressure and volume are inversely proportional at a constant temperature within an airtight space but the product remains constant. Thus assessing the pressure change in the box can determine lung volume changes during respiration by interpolation (Coates et al., 1997,

Dubois et al., 1956). The helium dilution method has been previously described in Section 1.3.3. In healthy subjects both methods are comparable but in COPD they may differ as the helium may not reach equilibrium during the single breath method due to airflow obstruction and therefore may result in an underestimation.

In COPD hyperinflation and air trapping are a significant cause of morbidity and poor quality of life due to worsening dyspnoea (O'Donnell, 2006). LVRS has been found in a select group of emphysematous patients to improve static lung elastic recoil, dynamic hyperinflation and improve respiratory muscle function and as a result improve dyspnoea and exercise capacity (Martinez et al., 1997).

1.3.5 Interpretation of Pulmonary Function Tests

Determining whether results are in the “normal range” presents some problems as the range is wide. The average value for a presumed healthy population is taken as 100% predicted. Percent of predicted for an individual result is calculated from the actual value divided by the average value for a similar healthy subject from the normal population and then multiplied by 100. This method was first suggested as a “rule of thumb” by Bates, Macklem and Christie (1971). It is currently used widely as the basis for determining severity and confirming a diagnosis of COPD as GOLD (Pauwels et al., 2001) and National Institute of Clinical Excellence (NICE) (2004). It has been traditionally accepted that the limits of normal have been the predicted value $\pm 20\%$ (Sobol and Sobol, 1979) as most of the tests outside this range are abnormal. However

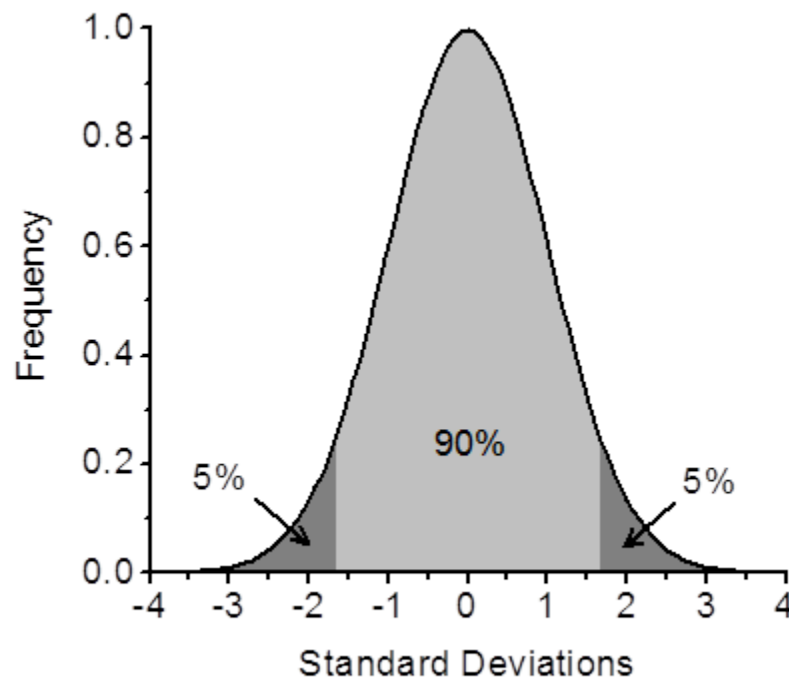
the reason for 80% to be adopted was that it is approximated to the true 90% confidence limits for FEV₁ (Bates et al., 1971). Using % of predicted (PP) for interpreting results may aid understanding of respiratory indices across all medical and surgical specialities but it is statistically and physiological invalid (Sobol and Sobol, 1979, Miller and Pincock, 1988). Using the PP approach assumes that the scatter of results in a normal population is proportional to the predicted value so as the value is smaller the scatter around it is also smaller. Using the PP method also assumes that different indices can be compared in terms of deviation and % of predicted and for each index the PP is the same for all patients however this is not the case for lung function indices (Quanjer et al., 2012a). For these reasons the PP approach has been challenged by Miller et al and the assumptions have been found to have no statistical validity and in addition the PP approach introduces an age, height and sex bias (Miller et al., 2009).

There are several other methods used to illustrate whether an observation is outside what would be normally expected. Z-scores are one of these methods and provide a way for comparing results from a test to a normal population. The score is the number of standard deviations, which is the measure of how the data are spread around the mean, from the mean value of the reference population and illustrates where the measure lies within a normal distribution curve. A Z score of 1 means the observation is 1 standard deviation above the mean. An example of how Z scores are used is in interpreting bone densitometry results. The results indicate whether someone has evidence of osteoporosis or osteopenia by comparing their measurements against patients of the same age, sex, weight and ethnicity. Standard residuals (SR) are similar to Z scores and

are calculated by dividing the residual (difference between the actual value and the estimated or expected value) by the standard deviation of the residuals. Results illustrated using % of predicted do not take into account that standard deviation varies with age (Stanojevic et al., 2008, Quanjer et al., 2012b). So unlike SR, comparisons using % of predicted across different groups in term of age, sex, weight and ethnicity are inaccurate. The Lower limit of normal (LLN) method uses standardised residuals to determine if a lung function result is outside the major part of the normal range (Miller et al., 1985, Quanjer et al., 1993). LLN is defined as -1.645 SR which is an estimate of the lower 5th percentile within a normal population. LLN removes the bias of height, age and sex but will clearly introduce a false positive rate of 5%. The 90% confidence limits have been used for defining limits of normal in lung function tests because these tests are not used indiscriminately on the general population but are targeted to patients. Hence the *a priori* probability of the test being positive is already high and so some specificity in the result is traded for increased sensitivity. If lung function tests were applied to a general population (as for epidemiological reasons) the 95% confidence limits should be applied which would give only a 2.5% false positive abnormality for results below this level (see Figure 1.2).

Figure 1.2

Diagram of normal distribution with estimated lower and upper 5th percentiles highlighted.



1.3.6 The debate about the definition of COPD.

There remains a vigorous international debate about the method to determine if an FEV_1/FVC ratio result is unusual or abnormal and this has some impact on the diagnosis of COPD. The American Thoracic Society (ATS) in 1987 (ATS, 1987) classified airflow obstruction as a fixed ratio of $FEV_1/FVC < 0.75$. In 1997 the British Thoracic Society (BTS) advised using < 0.70 (1997b) and NICE (2004) followed on from this using the same fixed ratio of < 0.7 but also requiring the FEV_1 to be $< 80\%$ predicted. The GOLD group (2010) first published their strategy in 2001 and also recommended a post bronchodilator $FEV_1/FVC < 0.7$ but did not require an abnormal FEV_1 .

In 2005 the ATS/ERS joint task force advised the use of lower limit of normal for FEV₁/FVC rather than the fixed ratio to help reduce the risk of false positives (Pellegrino et al., 2005). NICE has since updated its stance in 2010 to be in keeping with GOLD, so the defined FEV₁/FVC for COPD remains fixed at <0.70 and the FEV₁ level is not a prerequisite for the diagnosis of airflow obstruction.

Much of the controversy around using the FEV₁/FVC fixed ratio is because it is known to be inversely related to age (Swanney et al., 2008) and it differs between the sexes. The ratio reduces by 2% per decade of life (Hughes, 2009) and at any age the ratio is 2% higher in women than men. The following studies have indicated that using a fixed ratio increases the over-diagnosis of airflow obstruction in the elderly and under-diagnosis in the young; Hardie et al (2002) studied 71 healthy never smokers who were over the age of 70 years and found that 35% had a FEV₁/FVC below 0.7 and 50% of those over 80 years had a ratio <0.70 and a third had a FEV₁ <80% of predicted. Hansen et al (2007) found about 20% of older subjects were misclassified as having COPD using the fixed ratio and around 50% of younger subjects who were abnormal were classified as normal.

Several studies have been performed comparing the prevalence of COPD within a population when defined by LLN compared to the fixed ratio <0.70. Shirtcliffe et al (2007) studied 749 randomly selected subjects and compared the age-adjusted prevalence of COPD when classified by GOLD or LLN. The prevalence defined by GOLD was 14.2% (11.0 – 17.6 95% confidence intervals) and 9.5% (7.1 – 11.8) by LLN. They found the diagnosis of COPD by GOLD increased in males and in those who were

older than 40. However one limitation of this study was that the mean age of the population was 54.9 (± 12.8) which is young for a usual COPD population. Miller et al (2011) assessed 11,413 patients from UK, New Zealand and United States and found that when using the fixed ratio of $FEV_1/FVC < 0.7$ and 80% predicted limits for other lung function tests, 24% of patients were misclassified with regard to disease process and the number of false positives was higher in men and older subjects and many were falsely labelled as having emphysema.

GOLD updated the strategy document in 2011 and have recognised that there are false positives and negatives from using the fixed ratio in older adults and patients under 45 years but suggested that without longitudinal studies validating LLN, it remains inappropriate to adjust the recommendation of using the fixed ratio at present. Thus the spirometric definition of airflow obstruction remains unresolved.

1.3.7 Severity

The severity of airflow obstruction is determined by the FEV_1 as a % of predicted and is the same for both the NICE guidelines and GOLD strategy: namely mild (Stage 1) $FEV_1 \geq 80\%$, moderate (Stage 2) $FEV_1 \geq 50\%$ and $< 80\%$, severe (Stage 3) $FEV_1 \geq 30\%$ and $< 50\%$ and very severe (Stage 4) $FEV_1 \leq 30\%$. However FEV_1 correlates weakly with the degree of dyspnoea in COPD patients (Mahler et al., 1984) and observational studies have shown quality of life scores (Domingo-Salvany et al., 2002) and the degree of dyspnoea (Nishimura et al., 2002) are more accurate predictors of risk of death than

FEV₁ alone. COPD is widely recognised as a multi-system disease and models have been created and validated to enable better prediction of prognosis in COPD. One example is the BODE index (Celli et al., 2004) which incorporates 4 factors to determine the risk of death: namely Body Mass Index (BMI), degree of airflow Obstruction, Dyspnoea and Exercise capacity (6 minute walk test) all of which independently predict risk. The additional benefit is likely to be the result of small but distinct additional risk for each of the components.

GOLD have more recently suggested a new combined assessment method that includes 3 variables: symptoms (mMRC dyspnoea score or CAT score), airflow limitation and history of exacerbations (Vestbo et al., 2013). This approach acknowledges that spirometry is not the only variable that determines treatment. Patients are placed in 1 of 4 groups depending on whether they are classified as low or high risk for each of the variables. The group then helps guide appropriate pharmacological treatment.

In summary spirometry is crucial for the diagnosis of COPD but caution is needed with regard to how spirometry results are used as the method affects probability of diagnosis at different ages and between the sexes. Factors other than spirometry also need to be taken into account when predicting prognosis and is the subject of ongoing research.

1.3.8 Prediction equations

As has been discussed above the determination of whether a test result is normal or abnormal for an individual subject is complex. Since spirometric lung function first

emerged for assessment in the mid 1800's (Thackrah, 1831, Hutchinson, 1846) it has been shown that lung function is dependent on sex, age and height of the patient. Lung function laboratories now use prediction equations for lung function that have been determined from studies performed on patients thought to be normal and free of any disease (Becklake, 1986, Quanjer et al., 1993). The predicted values for this population are then compared to the patient's value to determine whether it is normal or not. There are several issues concerning which prediction equations to use that affect the conclusions and management decisions made for a patient. Errors in defining predicted lung function can have an impact by under or over diagnosing disease and estimating disease severity and sex bias (Miller and Pedersen, 2010).

Studies that are undertaken to determine prediction equations should ideally use the same equipment and protocol for their measurements and the population of subjects should be matched for the patients who are being assessed. It is unlikely that measurements performed on older instruments are comparable to those on more modern equipment. Previously the protocol and methods for lung function measurements were wide and diverse but the ATS/ERS standardisation document (Miller et al., 2005a) has helped to reduce these differences and thereby improve the reliability of the results. Deriving new equations for new equipment is difficult and expensive since a large number of healthy individuals need to be tested. The international respiratory societies do not agree on consistent recommendations for prediction equations across the world (Pellegrino et al., 2005). The ATS have recommended NHANES III for spirometry but ERS has not made any specific spirometry

recommendations. For gas transfer none of the international respiratory societies currently recommend a particular equation.

Several factors need to be taken into consideration when choosing an appropriate prediction equation for a specific population and these include the age range of the population, ethnicity, the equipment and protocol used for the measurements, socio-economic factors and environmental effects (e.g. altitude). But several studies have shown that many users are not aware of the validity of equations they use and rely on the default values set by the manufacturer of the lung function equipment (Dowson et al., 1998, Stanojevic et al., 2010).

1.4 Clinical phenotypes of COPD

The word 'phenotype' originates from the Greek, *phainein* meaning 'to show' and *typos* 'type'. The phenotype of an organism is the set of characteristics or traits that are observable, in terms of function and structure, and are the result of the underlying genes of that organism and their interaction with environmental factors. For instance eye colour is determined by the underlying genetics but the phenotype is the colour of the eyes that we observe externally. Phenotypes have been described in many different medical diseases and have been utilised to identify subgroups within a specific disease that have differing degrees of progression and clinical outcomes e.g. chronic hepatitis B viral infection (Kao et al., 2002).

There has been increasing interest worldwide in COPD phenotypes over recent years because for a disease that is heterogeneous, individual phenotypes make prognosis and therapeutic intervention potentially more accurate and effective. Phenotypes in COPD should ideally provide clear information on prognosis and identify subgroups that respond best to specific therapies enabling clinically meaningful outcomes to be assessed (Han et al., 2010). For research purposes phenotypes would also allow health care workers to select more homogenous groups of patients to be recruited into clinical trials for specific therapies as has emerged from the phosphodiesterase 4 inhibitor clinical trials (Calverley et al., 2009). COPD phenotypes should have important predictive value (Han et al., 2010) and be carefully validated. Phenotypes can be identified in a number of ways including clinical symptoms, signs and tests as well as outcomes from clinical trials and more recent statistical methods such as cluster analysis.

Cluster analysis uses a variety of different statistical methods to classify individuals (depending on chosen variables) into relatively homogenous groups (Wardlaw et al., 2005). Three important considerations need to be made before performing cluster analysis; firstly to ensure the individuals are not too similar thereby producing potentially misleading results. Secondly, care needs to be taken in selecting appropriate variables to determine the groups and finally deciding how many variables to use in the analysis (Weatherall et al., 2010). Burgel et al (2010) used principal component analysis to identify clusters of COPD patients. The variables they included were age, BMI, dyspnoea score, exacerbation rate, FEV₁ % of predicted, pack year history and SGRQ.

Four groups were identified which were defined by age and severity of disease including comorbidity and could not have been identified by the GOLD classification alone. COPD is known to be a complex and heterogeneous disease and cluster analysis enables us to explore this complexity in more detail.

Two of the first phenotypes described in COPD nearly 60 years ago were the distinct: 'pink puffers' and 'blue bloaters' and were attributed to Dornhorst (1955). The 'pink puffer' was so described because of the characteristic laboured breathing with pursed lips and the patient's had relatively normal arterial oxygen and carbon dioxide levels. It was proposed that the predominant underlying pathology was mainly emphysema. 'Blue bloaters' had significant hypoxia, carbon dioxide retention and cor pulmonale and were thought to be predominantly chronic bronchitis patients. The existence of these 2 phenotypes had been mainly based on a small study which compared clinical data and autopsy findings (Burrows et al., 1966) but other studies have not confirmed their results (Cullen et al., 1970, Mitchell et al., 1976). Clinically there is a significant overlap between these phenotypes but they are still recognised as polarised variants. There are also pathological phenotypes including those with centrilobular emphysema, panacinar emphysema, paraseptal emphysema and small airways disease but these are more difficult to distinguish without CT.

Acute exacerbations of COPD have a detrimental effect on the patient and exacerbation-susceptible phenotypes have been described (Hurst et al., 2010). Exacerbations have been shown to relate to a poorer quality of life (Seemungal et al., 1998), increased risk of death (Soler-Cataluna et al., 2005) and accelerated lung

function decline in COPD (Kanner et al., 2001, Donaldson et al., 2002). It is vital to identify the patients who have frequent exacerbations in order to target appropriate preventative treatment. The most reliable predictor has shown to be the patient's past history of exacerbations (Donaldson and Wedzicha, 2006). As this phenotype appears to be easily recognised it should enable preventative treatments and management to be targeted. A good example is Roflumilast, a phosphodiesterase-4 (PDE4) inhibitor with anti-inflammatory properties. Calverley et al (2009) found a significant reduction in exacerbations in COPD patients who took roflumilast who had a history of exacerbations, stable state cough and sputum production (taken to be indicative of chronic inflammation) and GOLD stage III and IV airflow obstruction which indicates a specific phenotype to benefit (as per the labelled indication).

CT become a major tool for phenotyping patients with COPD (Parr, 2011) because of its capacity to characterise the main morphological components of the disease, namely, airways disease, bronchiectasis and emphysema. The National Emphysema Treatment Trial (NETT) (Fishman et al., 2003) was a randomised controlled trial performed to determine the benefits of LVRS. An important subgroup was identified that benefitted from LVRS with predominantly upper lobe emphysema and low exercise capacity and subsequently a lower mortality and morbidity post-surgery than the comparable group who received usual medical management. The NETT study therefore identified a radiological COPD phenotype with a positive outcome to a specific intervention.

Physiological indices like FEV₁, FVC and their ratio are used to confirm the presence of COPD and determine its severity. Unfortunately these indices used alone explain less

than 10 – 25% of the disease impact on exercise capacity, quality of life and symptoms (Brown et al., 2008, Jones, 2001, Mahler et al., 2004) and as a defining feature of the disease it does not provide a phenotype. The rate of decline in FEV₁, on the other hand, may identify a phenotype that characterises the ‘fast decliners’. An increased rate of FEV₁ decline in COPD has been shown to be associated with increased frequency of exacerbations (Celli et al., 2008) in the TOwards a Revolution of COPD Health (TORCH) trial. *Post-hoc* analyses of the Understanding Potential Long Term Impacts on Function with Tiotropium (UPLIFT) trial categorised patients into quartiles dependant on their FEV₁ rate of decline over the 4 years of the study (Tashkin, 2010). Those with the least impaired FEV₁ at the beginning of the study had the most rapid decline in FEV₁ over the course of the study. The quartile with the faster FEV₁ decline had more frequent exacerbations, worse quality of life, increased risk of being hospitalised during an exacerbation and increased risk of death. Rapid decline in FEV₁ has previously been shown to predict morbidity, mortality and risk of hospitalisation (Wise, 2006) in COPD. If a COPD phenotype can be identified in which subsequent FEV₁ decline is more than expected, then patients with that characteristic could be included in clinical trials exploring potential therapies specifically aimed at slowing the rate of FEV₁ decline. A consensus definition of the rapid decliner phenotype would facilitate such a study.

A1AD is a recognised genetic risk factor for COPD (Bachmann and Laurell, 1963). But even for those with the most severe form of A1AD deficiency (PiZZ) there is variable clinical presentation and rate of development of COPD (DeMeo and Silverman, 2004) or CT abnormality. Parr et al (2004) showed that a low FEV₁ in A1AD related more closely

to lower zone emphysema defined by CT scan densitometry analysis whereas a low Kco related better to upper zone emphysema. Holme et al (2007) further explored these findings by describing four discrete groups with PiZZ A1AD using a combination of spirometry and gas transfer. The groups were recognised as normal, those with an isolated Kco or isolated FEV₁ defect or those with a combined abnormality of both FEV₁ and Kco. Lung densitometry, as assessed by computerised tomography, health status and some physiological parameters were compared between the groups (n=10 to 15 in each group). Patients with an isolated abnormality in FEV₁ or Kco had a different distribution of emphysema, with a more basal distribution in those with an isolated abnormality in FEV₁ and more apical distribution in those with isolated gas transfer defect. However the factors associated with these variations in physiological phenotypes or their effects were unknown, although an isolated defect in Kco was found to be associated with a significant reduction in health status. The numbers in this study were small and importantly there was no longitudinal data to observe the rate of decline in lung function, movement between the physiological groups or associations between demographics and physiological patterns. However this pulmonary data does indicate that even with a known single gene defect, the nature of the pathology and physiology can be diverse suggesting other factors play a role.

1.5 Aims

The most appropriate prediction equations to be used for the subsequent thesis work were determined using a large tertiary lung function database and survival as a novel and clinically relevant end point.

Descriptive studies were then undertaken to understand physiological phenotypes of A1AD patients defined using FEV_1/FVC and K_{co} by:

1. Defining the clinical, physiological and radiological characteristics of the phenotypes including demographics.
2. Exploring the mortality rates and progression between and within each of the phenotypes.

Finally further work was undertaken to explore whether TL_{co} or $VA\%TLC$, a measure of gas mixing, would be more clinically appropriate than K_{co} in defining the phenotypes.

Chapter 2

METHODS

2.1 Introduction

The work in this thesis was based on detailed analysis of data obtained from a large cohort of patients with A1AD. These data were obtained over a prolonged period of time, starting from before my involvement in this research project. This section describes how these data were obtained. The collection of the data for this thesis was approved by the South Birmingham Ethics Committee (LREC3359) and written informed consent was obtained from participating subjects.

2.2 Background about the ADAPT project

The Alpha-1-antitrypsin Deficiency and Assessment Programme for Treatment (ADAPT) program was launched in December 1996 with the aim to develop a UK registry for A1AD patients. The purpose of the programme was to establish a more detailed understanding of A1AD by recruiting patients, collecting extensive demographic data, obtaining accurate respiratory physiological measurements and radiological data to assess patients and develop methodology for interventional therapeutic trials and ways to monitor progression.

2.3 Patient Referrals

When the ADAPT project was originally launched UK respiratory clinicians were made aware of the programme through several different pathways: (i) the British Thoracic Society, (ii) through personal contacts of Professor Stockley, (iii) presentations across the country, and (iv) through the testing laboratory in Sheffield. When a patient's sample was diagnosed as having a deficiency by the Protein Reference Unit, Immunology Department, Sheffield, the patient was sent an information leaflet about the ADAPT program. Most initial referrals for patients came from secondary care because clinicians were aware of the program through the routes above. Subsequently patients have been seen from both primary and secondary care physician referrals as well as patient self-referral.

2.4 Annual assessment

At the patient's initial visit they were reviewed by Prof Stockley. For the patients who provided written consent to be part of the ADAPT programme, they subsequently attended for a baseline assessment including detailed data of the patient's symptoms, medical history and diagnosis. A full clinical examination was performed as well as full lung function including dual reversibility, capillary ear lobe blood gases, measurement of health status including the SGRQ, and a quantitative CT scan was done unless a routine scan had been undertaken within 12 months at the patient's local hospital. If a recent CT was available then a copy of the report and CT were acquired for the patient's notes.

The patients then attended annually, where possible, for at least the first 3 years thereby ensuring 4 consecutive lung function tests. Thereafter patients were reviewed every 1 – 2 years depending on the patient's stability. Each visit had a similar structure to the baseline although the frequency of CT imaging has been reduced in recent years and repeat reversibility testing is not usually undertaken although all physiological measurements and any CT scanning is undertaken post bronchodilator.

2.5 Blood Tests

Patient's serum alpha-1-antitrypsin levels were determined using an immunoassay and phenotyping by isoelectric focusing usually confirmed by genotyping in a central U.S. laboratory (Heredilab, Salt Lake City, UT). At each assessment visit the patient had routine bloods tests performed including full blood count, renal and liver function and gamma-GT. Three additional samples were taken for research purposes; 1 EDTA tube was stored at -20°C for later DNA extraction; 1 red serum tube and 1 further EDTA sample were centrifuged at 4°C 500rcf for 8 minutes then aliquoted and stored in 3 cryotubes at -70°C for later research studies.

2.6 Lung Function

All lung function testing was performed when the patient was clinically stable (at least 6 weeks post exacerbation) by a fully trained Band 6 or 7 Respiratory Physiology Technician in the Lung Investigation Unit at the University Hospitals Birmingham (UHB) NHS Trust.

At the baseline review the patient underwent full lung function testing which included pre and post dual bronchodilator measurements (post nebulised 5mg salbutamol and 500mcg ipratropium bromide). The lung function testing was performed according to the guidelines published jointly by the British Thoracic Society and the Association of Respiratory Technicians and Physiologists (1994). The absolute values in the ADAPT database included pre and post bronchodilator FEV₁, FVC and the ratio of FEV₁ to FVC (FEV₁/FVC), absolute FEV₁ change between pre and post bronchodilator, measure of carbon monoxide transfer (TLco), alveolar volume (VA), TLco/VA (i.e. Kco), Residual Volume (RV) and TLC. Spirometry was measured using wedge bellows (CareFusion, San Diego, California, USA), lung volumes by helium dilution and gas transfer by the single breath carbon monoxide method (Blakemore et al., 1957).

An arterial ear lobe capillary blood test was also performed to obtain pH, PaO₂, PaCO₂, base excess (BE) and bicarbonate.

The predicted lung function values for all lung function measurements were initially calculated in the same way as the UHB lung function laboratory using European

Community of Coal and Steel (ECCS) regression equations (Quanjer et al., 1993) except for Kco which was calculated using Cotes equation (Cotes, 1970). The Standardized Residuals (SR) values were derived using the equation:

$$SR = (\text{observed} - \text{predicted})/RSD$$

RSD = residual standard deviation taken from the regression equation.

In light of finding discrepancies in the way these equations affected the distribution of results with regard to sex further work was undertaken in Chapter 3 using other equations to determine which were best for the data.

Having acquired the baseline data for the PiZZ patients the longitudinal lung function data for the current study was collated for all patients up until October 1st 2009 including post dual bronchodilator FEV₁, TLco and Kco absolute values. This involved performing an Access search for all previous lung function data. Where the search of the ADAPT database was not complete the Queen Elizabeth Lung Function (QELF) database was used to identify any missing data for each patient. The QELF database started from 2000 so where the patient's baseline data started from 1996 the patient's notes were reviewed in order to obtain missing data. Patients who had had a lung transplant had post-transplant data removed. During the ADAPT programme 2 clinical intervention trials had taken place: REPAIR (Stolk et al., 2012), which was using a novel retinoid receptor agonist in the treatment of emphysema in A1AD and EXACTLE (Dirksen et al., 2009) which was using intra-venous Prolastin (alpha-1-antitrypsin replacement) to

evaluate the frequency and progression of emphysema using CT in A1AD. Patients who had been randomised to receive the active drug during either of these trials had their data which was obtained during and after the study, removed from any analysis.

2.6.1 Change in lung function equipment

During the ADAPT programme there had been several changes of equipment for the measurement of gas transfer. Initially (from 1996) all the lung function investigations were performed using Benchmark equipment (P.K.Morgan, Chatham, Kent, UK). On 23rd April 2002 a replacement lung function kit was installed; Jaeger (Jaeger UK) referred to as “Old Jaeger”. Therefore from April 2002 until 22nd September 2006 all baseline ADAPT lung function investigations were performed on the “Old Jaeger” kit. In September 2006 new software was installed onto the “Old Jaeger” and the Benchmark equipment was discontinued so all the baseline investigations were performed on kit referred to as “New Jaeger”. For all patients studied with the original Benchmark kit annual lung function was continued on the same equipment, until 22nd September 2006. From 2002 all new patients were studied on the Jaeger equipment. However the software upgrade raised the possibility that the change in equipment or software may alter the Kco or TLco absolute values from 2006. BIOqc data of 3 healthy physiologists (2 males and 1 female) showed a slight dip in the Kco and TLco measurements after the change to the “New Jaeger”. In view of this I reviewed patient’s longitudinal lung function

for Kco and TLco and to assess whether there was a significant change in the measurements before and after the equipment or software change.

As described above there were two changes in the lung function equipment and software between 2002 and 2006. Data were reviewed to determine whether these changes altered the absolute values for Kco and TLco.

In order to determine this, 2 groups of patients were examined in more detail:

1. Those who had had measurements made on the original Benchmark and then on “New Jaeger” equipment.
2. Those who had had measurements performed on “Old” and then “New Jaeger” equipment.

On an excel spreadsheet several calculations were performed which are described below:

$$y = mx + c$$

This is the equation for a linear regression line; ‘x’ and ‘y’ represent the coordinates of the points that lie on the line, ‘m’ is the gradient and ‘c’ is where the line crosses the y axis (y-intercept).

1. SLOPE – calculates the slope of the linear regression line (m in the above linear equation).
2. Relative Standard Deviation (RSD) STEYX – calculates the amount of error of the linear regression line.
3. INTERCEPT – calculates where the linear regression line crosses the y axis (c in the above linear equation).
4. R value (CORREL) – measure of the reliability of the line or relationship between the x and y values.
5. r-square (CORREL x CORREL) – see R value.
6. Predval – predictive value of the next lung function measurement after the equipment/software change taken from the predictive slope of lung function change calculated above.
7. Difference = actual measurements – predicted value
8. Standardized Residual (SR) – Difference/RSD of the slope
9. Mean predicted and recorded measurements

Those with r -squared >0.7 and only those with a negative slope before equipment change were analysed further. Positive slopes may suggest that the patients were either not optimally treated at baseline or the data represented a technical problem.

For the change from Benchmark to “New Jaeger” 24 patients were analysed for Kco and 27 for TLco. Unfortunately there were insufficient patients in the A1AD database with 4 consecutive annual data points to analyse any effect of changing from “Old” to “New Jaeger”. Therefore the QEPFT database was used to find patients with at least 3 TLco and Kco measurements prior to the change of 22/9/06 and hence included a broader patient set.

In the equipment change of Benchmark to “New Jaeger” there was no significant step up or down in the absolute values for TLco and Kco. The change of “Old” to “New Jaeger” for TLco was insignificant. Twenty one patients were initially included in the analysis for the change of “Old” to “New” Jaeger. There was a trend towards an over-recording by the “New” Jaeger of the Kco measurement compared to the predicted, taken from the linear regression equation (mean difference between recorded-predicted=0.06 mmol/kPa/min/L) but it didn’t reach significance ($p=0.08$). This may have been due to a Type 2 error i.e. too small patient numbers. When the data was analysed for those with a decline and r -squared >0.5 was performed, the mean difference was still 0.06 mmol/kPa/min/L ($n=58$) but it was now significant ($p=0.0003$). This significant trend for the over-recording of the Kco by the “New” Jaeger was seen in those patients with r -squared >0.6 and >0.7 . Therefore 0.06mmol/kPa/min/L was subtracted from the absolute Kco for the patients who had had measurements performed both before and

after changing to the Jaeger with the new software ("New Jaeger") to provide comparable results.

Benchmark to New Jaeger:

TLco Mean difference 0.05 mmol/min/kPa (SD 1.71) n=27

Kco Mean difference 0.04 mmol/min/kPa/L (SD 1.78) n=24

Old to New Jaeger:

TLco Mean difference 0.02 mmol/min/kPa (SD=0.73) n=19

Kco Mean difference 0.06 mmol/min/kPa/L (SD=0.16) n=58

2.6.2 Calculating decline data: TLco, Kco, FEV₁

The rate of change in TLco, Kco and FEV₁ were calculated using a SLOPE equation in Microsoft Excel. Only the patients who had at least 4 data points were included in the analysis.

2.6.3 Haemoglobin adjustments of Kco.

The level of haemoglobin can affect the results obtained from TLco measurements because the rate of carbon monoxide uptake declines as the haemoglobin concentration decreases due to the reduction in available binding sites for the carbon monoxide (Macintyre et al., 2005). One study showed in patients with anaemia TLco decreased by 7% for a 10g/L decrease in haemoglobin (Dinakara et al., 1970) but the effect in non-anaemic asymptomatic never smokers was less (2% for every 10g/L) (Gulsvik et al., 1992). This effect can be taken into account using equations that adjust for deviations in haemoglobin from standard (14.6 g/dL in men and 13.4 g/dL in women and children under 15 years of age (Macintyre et al., 2005)). Unfortunately only 118 out of the 530 (22%) A1AD patients had haemoglobin levels available at baseline so the unadjusted TLco measurements were included in the final dataset. Of the available haemoglobin measurements, the mean for males (n=49) was $15.4 \pm \text{SD } 0.92$ g/dl and for females (n=69) $13.8 \pm \text{SD } 1.17$ which were comparable to the standard as defined by MacIntyre et al (2005). Only 2 of the 118 subjects with a haemoglobin value available at baseline would have needed their TLco adjusting by more than 5%.

2.7 Health status

Several different health status measurements were collected when the patient attended for their annual review. These were completed before the lung function measurements were performed or any other procedures undertaken. One of these health status

questionnaires was the SGRQ (Jones et al., 1992). The SGRQ was designed to measure health impairment in patients with respiratory diseases including COPD. The scores are divided into 3 subsections; symptoms (assessing chest specific questions), activity (limitations brought about by chest symptoms especially dyspnoea) and impact (influence of their respiratory illness on employment, panic, medication and expectation). Each subsection is scored from 0 – 100 with 0 indicating no impairment as thought by the patient. A weighted total score, also 0 – 100 is calculated from the 3 individual subsections.

Until 2005 the SGRQ was given to the patient to complete before each annual review. From 2005 the SGRQ was completed only at baseline.

When the patients were clinically reviewed they were asked particularly about any chest symptoms including dyspnoea and chronic bronchitis using the MRC definition (Fletcher and Peto, 1977). From the answers given at the baseline interview and for some patients in subsequent visits, the modified MRC dyspnoea score (Fletcher, 1960) was also calculated:

Modified Medical Research Council (MRC) Dyspnoea Scale Grade

- 0 “I only get breathless with strenuous exercise”
- 1 “I get short of breath when hurrying on the level or walking up a slight hill”

2 “I walk slower than people of the same age on the level because of breathlessness or have to stop for breath when walking at my own pace on the level”

3 “I stop for breath after walking about 100 yards or after a few minutes on the level”

4 “I am too breathless to leave the house” or “I am breathless when dressing”

2.8 CT scans

The HRCT scans were performed using a General Electric Prospects Scanner (General Electrical Medical Systems, Milwaukee, WI). CT scan 1mm slices were taken at 10mm intervals across the thoracic cavity during full inspiration. The CT scan densitometry was performed using a propriety density mask program (General Electrical Medical Systems) which enabled the Voxel Index (VI) to be determined. The VI results that have been included matched as closely as possible to the baseline investigations.

At baseline CT scans were obtained to determine the presence, extent and distribution of emphysema and/or bronchiectasis. In the beginning of the ADAPT program all the scans were done at UHB. As the study progressed more CT scan assessments had already been undertaken at the patient’s local hospital and so these were requested and reviewed rather than repeated. A verbal description of the scan was filed in the notes because these scans obtained locally were unsuitable for quantitative analysis. The

presence or absence of lung pathology was routinely entered into the database and where there were some gaps individual patient's notes were reviewed to obtain these data.

2.9 Survival

Assessing mortality was not an integral part of the ADAPT programme and there was no formal mechanism for reporting these events. Patients attend from the whole of the UK and as there was no IT system that enabled a review of patient's details and clinical activity outside the local area the programme was reliant on relatives or local physicians providing the information. In order to capture the survival of these subjects I utilised the NHS central database with the help of the UHB Information Department using the subjects' available demographic data. For two subjects a match was not found on the censor date of 1/7/2012 and these were assumed to be still alive.

2.10 Data sources

The patient data were collected from two ADAPT Access databases; "Sitebase" and "Regbase". Sitebase was the original database from 1996 when the ADAPT project was launched and was subsequently recreated due to a Trust change in software in

2000/2001 to Regbase. The two databases do not link with each other so two separate patient searches were performed.

Using the patient's unique registry number ID and ADAPT number, all PiZZ patients with complete baseline data were combined from the two ADAPT databases and the patients' details and demographic data were saved to an Excel file along with additional information that is described in more detail below:

a). Whether the patient was an index patient, i.e. was the first medically identified patient in a family, or non-index if they were identified through family screening. Any patients who presented because of liver abnormalities were excluded.

b). Smoking history including age at which the habit started or stopped and, if relevant, average cigarettes smoked and current smoking status. The pack year value was calculated:

Pack year history = number of cigarettes per day × no of years smoked/20

c). Height in metres and weight in kilograms at baseline were measured and BMI calculated:

Body Mass index = (weight (kg)) / (height (metres))².

The BMI was then used to classify the patients into 4 groups

0 = BMI <20

1 = BMI 20 – 24.9

2 = BMI 25 – 29.9

3 = BMI ≥ 30

d). Date of birth and the date of the baseline lung function tests were used to calculate the age of the patient at the time of the lung function tests.

2.11 Defining the classification of lung function groups

After the dataset was complete the patients were classified into groups defined by their FEV₁/FVC ratio and Kco expressed as standardised residuals in keeping with the recommendations of the recent ATS/ERS statement (Pellegrino et al., 2005). Abnormal was defined as below the lower limit of normal set at -1.645 SR, which defined the lower 5th percentile. The phenotypes are defined as:

Group N FEV₁/FVC SR ≥ -1.645 SR

Kco SR ≥ -1.645 SR

Group K FEV₁/FVC SR ≥ -1.645 SR

Kco SR < -1.645 SR

Group F $FEV_1/FVC \text{ SR} < -1.645 \text{ SR}$

$Kco \text{ SR} \geq -1.645 \text{ SR}$

Group B $FEV_1/FVC \text{ SR} < -1.645 \text{ SR}$

$Kco \text{ SR} < -1.645 \text{ SR}$

2.12 Statistical analysis

Statistical analyses were performed using PASW Statistics v.18, IBM SPSS. Non-parametric data were compared using Mann-Whitney U test and a t-test for parametric data. The Pearson's chi-squared test was used to compare distribution of data between groups. A p value of ≤ 0.05 was accepted as significant with Bonferroni correction for multiple comparisons. Change in FEV_1 , Kco and TLco was calculated for the patients with ≥ 3 years annual follow-up using linear regression.

Chapter 3

CHOOSING THE BEST PREDICTION EQUATIONS FOR LUNG FUNCTION DATA

3.1 Introduction

Managing patients with respiratory disease usually requires the measurement of the patient's lung function for diagnosis, monitoring and in determining severity. Since spirometric lung function began being recorded in the mid 1800's (Thackrah, 1831, Hutchinson, 1846) it has been found that lung function is dependent on sex, age and height of the patient. Lung function laboratories now use prediction equations for lung function which have been determined from studies performed on patients who are thought to be normal and free of any disease (Becklake, 1986, Quanjer et al., 1993). The predicted values are then compared to the patient's value to determine whether the patient is normal or not. There are several issues involved in making the decision about which prediction equations to use that can affect the conclusions and management decisions made for a patient. Errors in defining predicted lung function can have an impact in terms of under or over diagnosing disease, mistakes when estimating disease severity and sex bias (Miller and Pedersen, 2010).

Studies that are undertaken to determine prediction equations should ideally use the same equipment and protocol for their measurements and the population of subjects should be matched for the patients that are being considered. It is unlikely that measurements performed on older instruments are comparable to the more modern

equipment. Previously the protocol and methods for the lung function measurements were wide and diverse but the ATS/ERS standardisation document (Miller et al., 2005b) has helped to reduce these differences and thereby improve the reliability of the results. Deriving new equations for new equipment is difficult and expensive since large surveys are needed of healthy individuals. The international respiratory societies did not agree on consistent recommendations for prediction equations across the world (Pellegrino et al., 2005). The ATS have recommended NHANES III for spirometry but ERS has not made any specific spirometry recommendations. For gas transfer none of the international respiratory societies recommend a particular equation.

Several factors need to be taken into consideration when choosing an appropriate prediction equation for a specific population and these include the age range of the population, ethnicity, the equipment and protocol used for the measurements, socio-economic factors and environmental effects e.g. altitude. But several studies have shown that many users are not aware of the equations that they are using and are relying on the default values set by the manufacturer of their lung function equipment (Dowson et al., 1998, Stanojevic et al., 2010).

Interest in this topic initially was triggered from observations made when defining the physiological phenotypes of A1AD where the initial analysis returned results showing unexpected sex differences. The aim of this study was to use survival data from a large

number of NHS patients who had had lung function measured in our laboratory to see whether this outcome could help determine which prediction equations were most appropriate for UK patients, with the null hypothesis that survival prediction when using different prediction equations would be the same.

3.2 Methods

We included the lung function data of patients who had attended the University Hospital Birmingham (UHB) for their investigations which had been collated onto a database that was first set up in December 1996. Only patients with full lung function tests were included in the study and for those with multiple lung function tests only the first complete within day set of tests was included in the analysis. All of the lung function measurements were performed using the ARTP standards which are based on the 1993 standards (Quanjer et al., 1993) published by the ERS. Spirometry was measured using a Vitalograph wedge bellows spirometer initially then latterly using a Jaegar pneumotachograph. Gas transfer was measured using a Morgan Model C (Morgan Medical, Chatham, Kent, UK) or by a Jaeger Masterscreen system (Erich Jaeger, Hoechberg, Germany). Quality control was completed using appropriate guidelines and the instruments were calibrated prior to each patient and weekly biological control testing was also completed.

The initial search involved exporting all the data from a Microsoft Access database and transferring it to Excel. The subjects were sorted first by their hospital unit number

looking for any duplicates and secondly by the date of test. Only the first complete lung function measurements were included in the final dataset. Subjects were excluded if they did not have a hospital number as we would not have been able to establish survival using the NHS central database. Subjects who were actively involved with any clinical trials were also excluded. After this initial process there were a total of 24,605 lung function records. The lung function measurements collected included FEV₁, FVC, TLC, RV, TLco and Kco. The FEV₁/FVC ratio was calculated.

Data from 2123 non Caucasian patients were excluded from further analysis as the number was too small to be able to investigate ethnicity correction factors and their validity. This meant that we had data for a total of 8340 patients. Using the NHS central database the survival status of these patients was determined. The NHS number, name, sex and date of birth were entered on this database and for 8139 of the initial 8340 patients a match was found and survival determined. Survival was calculated as the time that had elapsed from the date of their first complete lung function measurements to either March 5th 2011 if they were alive or until their date of death.

Then several different prediction equations were applied to the 8139 patients to determine the SR for each of the lung function indices including FEV₁, FVC, TLco, Kco, TLC and RV. The method for calculating SR values has previously been described in the 1993 ERS statement on lung function (Quanjer et al., 1993). The prediction equations that were chosen are commonly used in the United States of America and Europe and also several equations were included that have only recently been published and therefore are not yet in widespread use. For spirometry the equations

included were ECCS (Quanjer et al., 1993), Knudson et al. (1976), Roberts et al. (1991), the Lambda-Mu-Sigma (LMS) equations (Stanojevic et al., 2008, Manocha, 2003), Miller et al. (1986), NHANES III (Hankinson et al., 1999), Crapo et al. (1981) and Kuster et al. (2008). For gas transfer I used ECCS (Quanjer et al., 1993), Roberts et al. (1991), Miller et al. (1983), Crapo and Morris (1981) and Cotes (1970). The LMS equations differ from most of the other equations in that they encompass a broader range of ages from 4 to 80 and also the LMS, NHANES III and Kuster equations use power functions, that is, height and/or age are included in the equation as a power i.e. squared or cubed. This usually reflects that these factors have a non-linear relationship within the dataset.

Each lung function index was then divided into 10 groups determined by the SR value as shown in Table 3.1.

Table 3.1

| Group | Criterion | % of Normal Pop. |
|-------|-----------------------------|------------------|
| 1 | SR ≥ 1.0 | 15.9 |
| 2 | SR ≥ 0.5 and < 1.0 | 15.0 |
| 3 | SR ≥ 0.0 and < 0.5 | 19.1 |
| 4 | SR ≥ -0.5 and < 0.0 | 19.1 |
| 5 | SR ≥ -1.0 and < -0.5 | 15.0 |
| 6 | SR ≥ -1.5 and < -1.0 | 9.2 |
| 7 | SR ≥ -2.0 and < -1.5 | 4.4 |
| 8 | SR ≥ -2.5 and < -2.0 | 1.7 |
| 9 | SR ≥ -3.0 and < -2.5 | 0.5 |
| 10 | SR < -3.0 | 0.1 |

SR criterion value thresholds for inclusion in the groups are shown together with what proportion of a normal population would be included in each group.

This way of determining the groups was used to observe how each of the equations pitched the patient data with respect to a normal distribution. We also undertook analysis using deciles of FVC, FEV₁ and TLco when divided by height cubed and deciles for age were also included in the analysis as age relates to survival when considering lung function (Chinn et al., 2007, Miller et al., 2009).

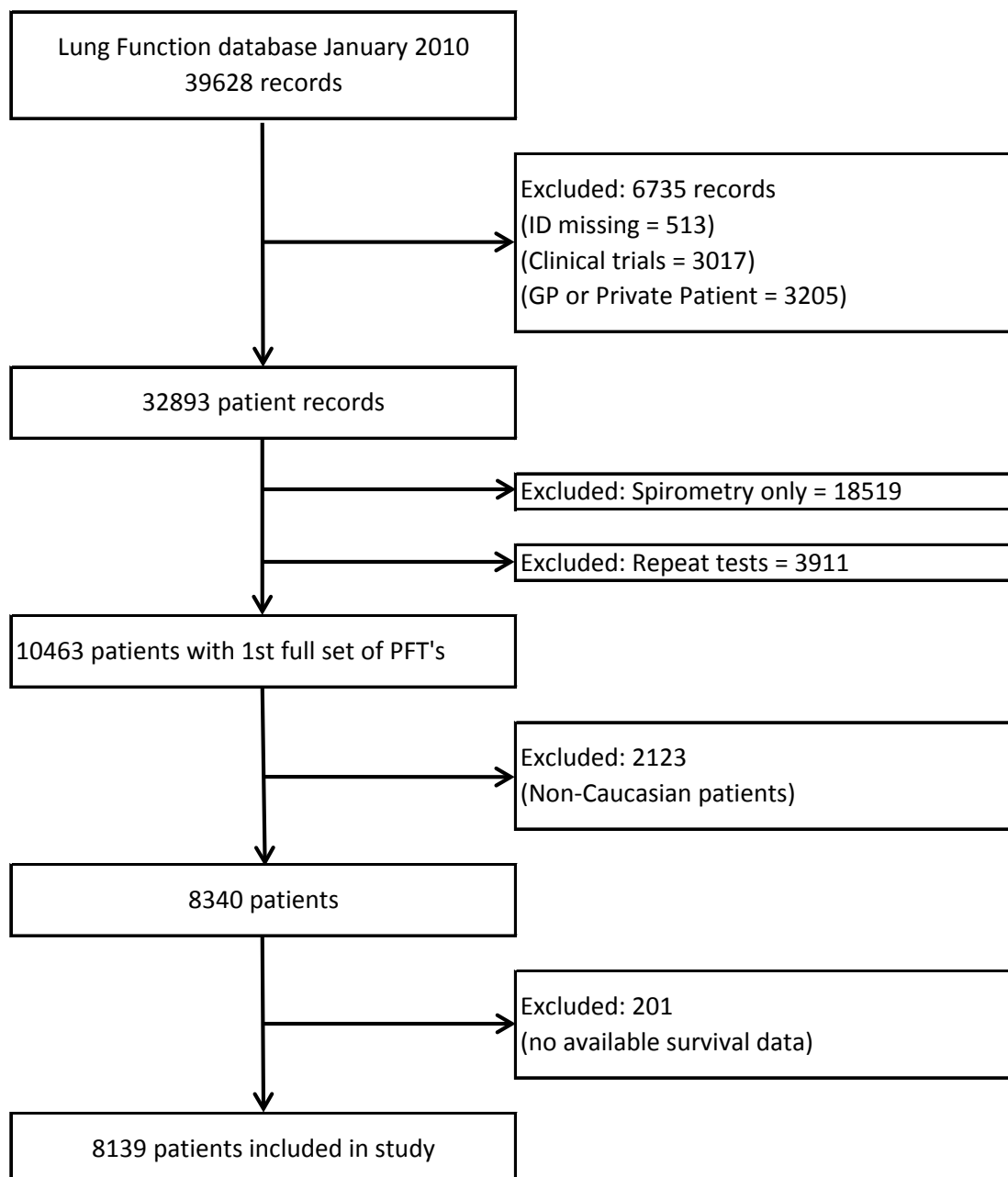
Cox proportional hazard regression models were determined to predict the hazard ratio (HR) for all-cause mortality using IBM SPSS version 19 with all the data inputted as categorical variables. Using categorical variables from the groups described above reduced possible bias from extreme outliers.

3.3 Results

Figure 3.1 illustrates the flow of patients from the total lung function dataset to those included in the final study, with the number of patients excluded at each step and the reasons why. There were 201 suitable patients that we were unable to establish survival. This group was not different in terms of age but had slightly more females (51% compared to 47%) than the matched group. This may be as a result of females changing their surnames when they marry.

Figure 3.1

Flow diagram of the total number of patients from the lung function dataset to those included in the final study.



Of the 8139 subjects that were included in the analysis 52.6% were male, 31.5% had never smoked, 49.4% were ex-smokers and 19.2% were current smokers. These smoking data are comparable to the UK population (Robinson and Harris, 2011). Table 3.2 outlines the demographics and survival for each sex with mean and standard deviation (SD) for lung function data represented as SR for each sex, using the ECCS equations. The males were significantly older, taller, smoked more, had worse spirometry indices (absolute and SR), worse KcoSR but comparable TLcoSR. The men also had a worse survival.

Table 3.2

| | Women | | Men | | |
|-------------------------|-------|-------|-------|-------|---------|
| | Mean | ±SD | Mean | ±SD | |
| Age Yrs | 58.2 | ±15.2 | 59.8 | ±14.5 | p<0.001 |
| Survival Yrs | 4.9 | ±3.3 | 4.3 | ±3.1 | p<0.001 |
| Ht m | 1.6 | ±0.10 | 1.7 | ±0.1 | p<0.001 |
| BMI Kg·m ⁻² | 28.2 | ±6.7 | 27.9 | ±7.4 | ns |
| Pkyrs | 18.3 | ±25.0 | 31.30 | ±37.4 | p<0.001 |
| FVC L | 2.69 | ±0.80 | 3.75 | ±1.11 | p<0.001 |
| FEV ₁ L | 1.88 | ±0.77 | 2.45 | ±1.03 | p<0.001 |
| FEV ₁ /FVC | 0.69 | ±0.16 | 0.65 | ±0.17 | p<0.001 |
| TLco mmol/min/kPa | 5.48 | ±1.86 | 6.85 | ±2.66 | p<0.001 |
| Kco mmol/min/kPa/L | 1.41 | ±0.39 | 1.31 | ±0.43 | p<0.001 |
| FVCSR | 0.10 | ±1.41 | -0.41 | ±1.46 | p<0.001 |
| FEV ₁ SR | -0.91 | ±1.58 | -1.40 | ±1.64 | p<0.001 |
| FEV ₁ /FVCSR | -1.39 | ±2.29 | -1.67 | ±2.27 | p<0.001 |
| TLcoSR | -1.66 | ±1.34 | -1.60 | ±1.60 | ns |
| KcoSR | -1.22 | ±0.77 | -1.72 | ±1.49 | p<0.001 |

Mean ± SD data for the 4282 men and 3857 women patients separately using ECCS equations. Significance level for comparison of results between the two sexes is shown.

The suggested but unconfirmed diagnosis entered on the lung function request forms included 14.7% COPD, 9.3% Asthma, 7.7% Pulmonary fibrosis and 68.3% unclear, with a mortality of 26%, 26%, 30% and 27% respectively for each of these diagnostic groups. The mortality for the whole dataset was 27% (2209 deaths, 61.6% male, Male: Female ratio 3:2).

We also reviewed the correlations between the lung function indices. They were all significantly different from zero correlation but this is because the dataset is so large. We used an arbitrary threshold of 0.4 for the Spearman correlation coefficient as being potentially clinically meaningful (16% of variance explained). When this threshold was applied then the important correlations included FVCSR with FEV₁SR, FEV₁/FVCSR and TLcoSR (0.75, 0.45 and 0.43 respectively). FEV₁SR correlated with FEV₁/FVCSR and TLcoSR (0.62 and 0.46 respectively) and TLcoSR correlated with KcoSR (0.75). These correlations are as one might expect which helps to support the validity of the dataset and subjects that were used.

Multivariate Cox regression models were derived using age in deciles, BMI, smoking status and sex as predictors of survival along with the grouping lung function values for FEV₁, FVC, FEV₁/FVC, TLco and Kco. The models were also derived using lung function in deciles instead of our groupings. This did not alter the pattern of results so we continued to use our defined lung function groups as they are clinically more applicable and relevant. The survival of the best group (best group = highest Chi squared) was used to compare the hazard of death across the other groups. Also the hazard ratio of death for the older deciles was compared to those in the youngest decile

(age <37.8 years) and the hazard ratio of death for men compared to women was also compared across our population. Cox regression models were determined for the deciles for FVC/ht³, FEV₁/ht³ and TLco/ht³ which did not require the use of prediction equations. Table 3.3 illustrates the chi-squared values for each of the models where the larger values represent a better fit, the HR for sex and the HR for the group with SR<-3.0 for the different prediction equations (entered into the models as 10 groupings illustrated in Table 3.1).

Table 3.3

| | FEV ₁ SR | | | FVCSR | | | FEV ₁ /FVCSR | | | TLcoSR | | | KcoSR | | |
|----------------------------|---------------------|------|-------|----------------|------|-------|-------------------------|------|-------|----------------|------|-------|----------------|------|-------|
| | X ² | HR | | X ² | HR | | X ² | HR | | X ² | HR | | X ² | HR | |
| | | Sex | Worst | | Sex | Worst | | Sex | Worst | | Sex | Worst | | Sex | Worst |
| Miller | 907 | 1.60 | 1.83 | 1043 | 1.51 | 2.87 | 886 | 1.64 | 0.64 | 1468 | 1.54 | 8.53 | 1220 | 1.54 | 3.76 |
| ECCS | 901 | 1.56 | 1.62 | 1038 | 1.46 | 2.73 | 889 | 1.66 | 0.65 | 1418 | 1.67 | 6.68 | 1207 | 1.31 | 2.77 |
| Roberts | 899 | 1.61 | 1.97 | 1030 | 1.63 | 3.55 | 898 | 1.62 | 0.65 | 1398 | 2.06 | 8.43 | 1203 | 1.69 | 4.33 |
| Crapo | 902 | 1.55 | 1.72 | 1035 | 1.57 | 2.91 | 880 | 1.67 | 0.65 | 1341 | 1.51 | 4.94 | 1201 | 1.57 | 3.59 |
| LMS | 912 | 1.64 | 1.98 | 1037 | 1.58 | 3.20 | 898 | 1.65 | 0.88 | - | - | | - | - | |
| Knudson | 907 | 1.60 | 1.83 | 1031 | 1.56 | 2.74 | 900 | 1.61 | 0.55 | - | - | | - | - | |
| NHANES | 911 | 1.60 | 1.88 | 1016 | 1.59 | 2.78 | 888 | 1.63 | 0.67 | - | - | | - | - | |
| Kuster | 907 | 1.60 | 1.83 | 1028 | 1.60 | 3.00 | 886 | 1.64 | 0.64 | - | - | - | - | - | - |
| Test/ht³ | 913 | 1.67 | 1.74 | 1049 | 1.86 | 2.64 | - | - | | 1526 | 1.64 | 7.69 | 1162 | 1.17 | 3.47 |
| Cotes | - | - | | - | - | | - | - | | 1348 | 2.96 | 10.05 | 1159 | 2.21 | 4.34 |

Results for χ^2 values for goodness of fit from Cox proportional hazard regression using age, sex, BMI, smoking status (all known to influence survival) and each of 5 lung function indices using various prediction equations. Hazard ratio (HR) for being male and HR for the group with SR values <-3.0 (worst) are also shown, which were all significantly different from unitary hazard.

All the TLco models were significantly better than the Kco and FVC models. The FVC models were better than the FEV₁ and FEV₁/FVC models. The models that included the Miller equations had the best fit for FVC, TLco and Kco as judged by the sum of the chi-squared values of the indices. NHANES and LMS prediction equations for FEV₁ were the only 2 better than Miller but overall there was less difference between the equations for spirometry alone. So Miller's prediction equations for gas transfer and spirometry were used for coherence in our population. For the models which included the index/height-cubed the chi-squared results were either comparable to the best fit prediction equations or in the case of TLco, the best compared to all the other equations.

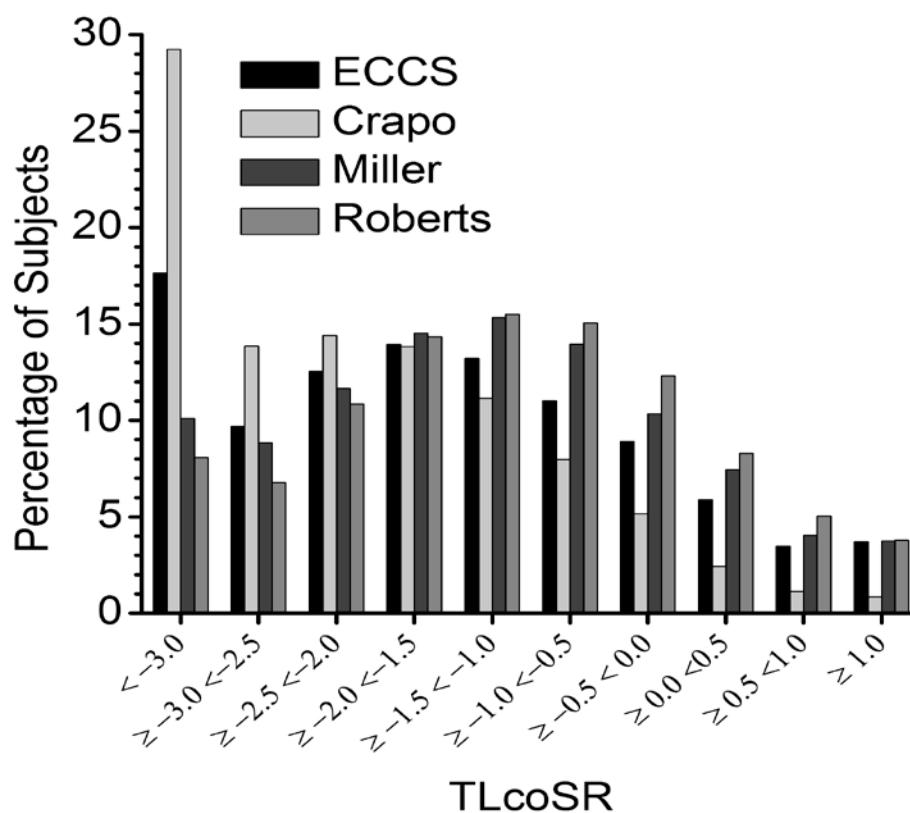
There were differences in the HR for sex between the equations for models including gas transfer. There were no major differences in sex HR between the spirometry regression models. When TLcoSR was defined by Cotes and Roberts there was a significantly worse HR for men compared to women, than the other equations (2.96 and 2.06 respectively). The overall ratio of deaths for men compared to women in our dataset was 1.6. This difference suggests that there is an imbalance in the way the equations are accounting for men and women.

The HR for the group with SR values <-3.0 were also derived (see Table 3.3). The subjects in the FEV₁/FVCSR group <-3.0 seemed to have a survival advantage over those with the highest SR values. This is as a result of FEV₁/FVCSR increasing as some respiratory diseases progress, i.e. interstitial lung disease, but also decreasing in other respiratory disease.

The spread of the data between the equations did vary. Figure 3.2 illustrates the spread of data when using TLco.

Figure 3.2

Histograms of TLcoSR showing the spread of results for each of the prediction equations. The worst lung function groups are to the left and best to the right.

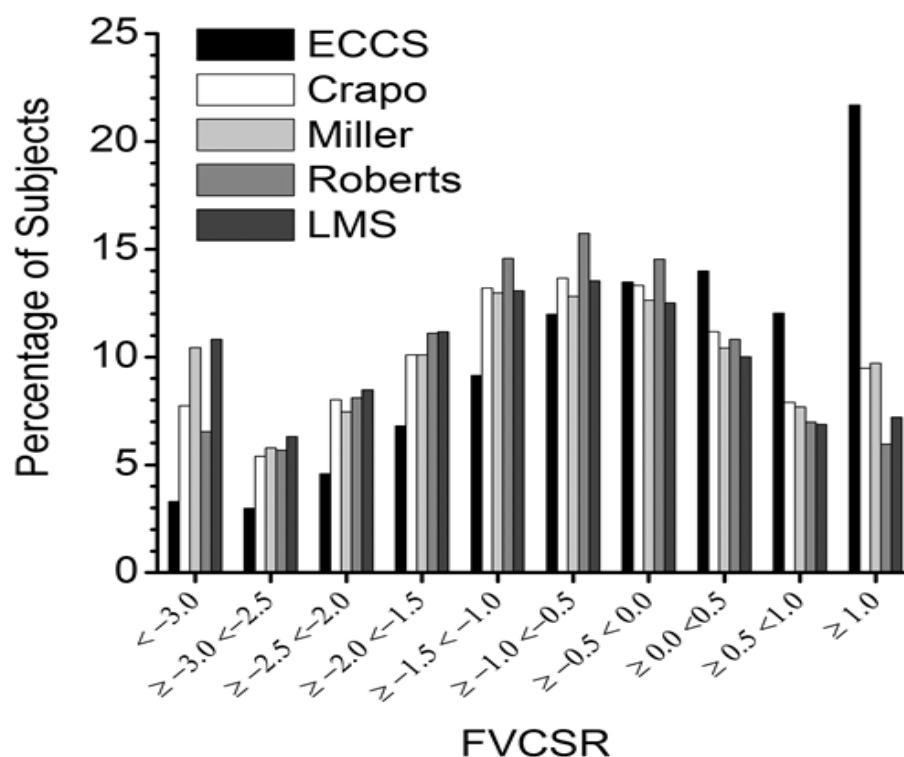


When using the ECCS, Miller and Roberts equations the spread of the data was mostly comparable except in the most severe category (SR <-3.0) where ECCS had more subjects. Crapo had a slightly different distribution compared to the other equations. Crapo had the least number of subjects with normal SR (SR >0.0) but more than any of the others in the worse group (SR <-3.0). Therefore Crapo shifted all the subjects'

results to the left (more abnormal) this is because their predicted values were higher. ECCS had disproportionately more in the supranormal range ($SR > 1.0$) and less in the abnormal range when comparing the spread of data using FVCSR (Figure 3.3). The other equations seemed to be comparable in their distribution.

Figure 3.3

Histograms of FVCSR showing the spread of results for each of the prediction equations. The worst lung function groups are to the left and best to the right.



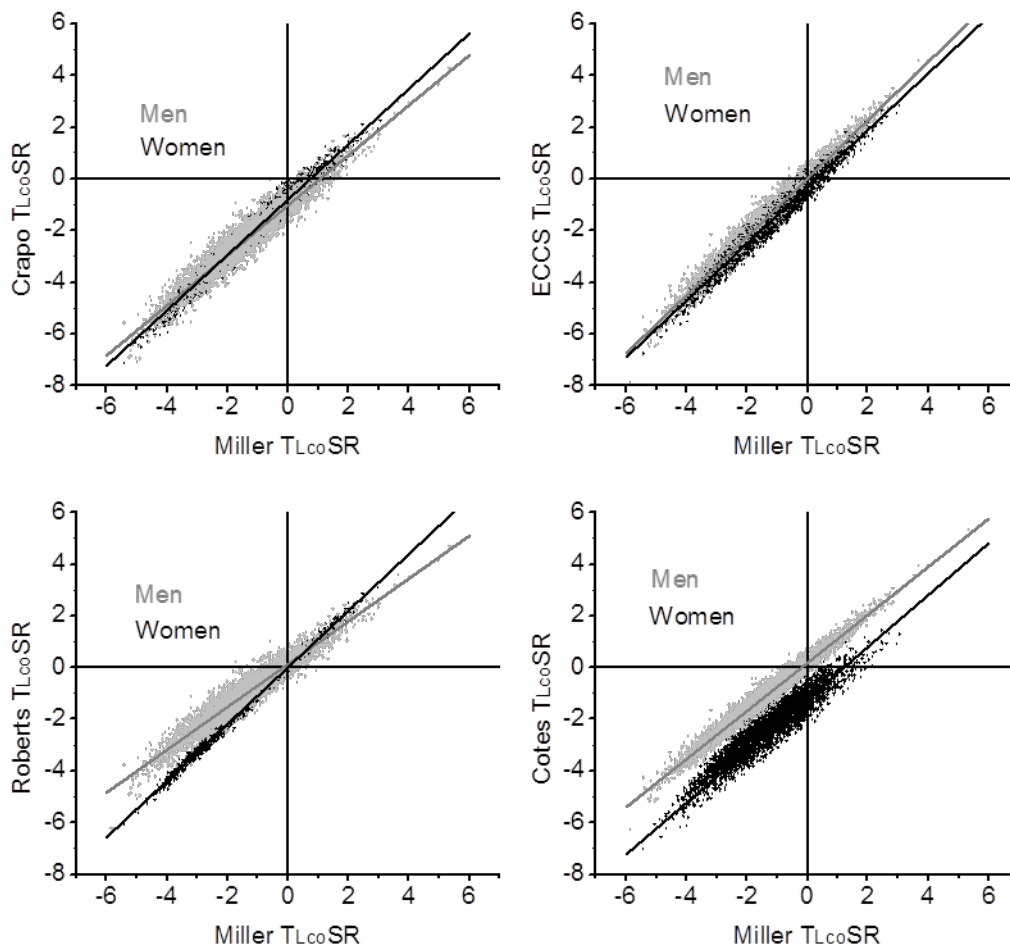
3.3.1 Sex differences

Figure 3.4 illustrates the plots of TLco SR for Miller against Crapo, ECCS, Roberts and Cotes. Crapo has the best overlay of the values for females and males but overall Crapo

equations shifted all the values to the left thereby making them more negative compared to the other equations. Roberts and Cotes, in particular, had a separation of the values between the sexes which indicates that these equations introduce a sex difference which could be seen by the worse HR for men compared to women compared to the other equations. The plot of TL_{co}/ht^3 compared to TL_{co} Miller was similar to that of Crapo with overlay of the sexes.

Figure 3.4

Plots of $TL_{co}SR$ values from the Miller equations against those from Crapo (top left), ECCS (top right), Roberts (bottom left) and Cotes (bottom right). Results for men are in grey and women in black. Regression lines for each sex are shown with separation of the lines reflecting sex differences between the equations.



3.3.2 Multivariate predictions

Multivariate predictions were undertaken of all the lung function indices, age, sex, smoking (pack year and status) and BMI using step multivariate analysis. The lung function indices were determined using ECCS equations as it has a complete set of equations for all the lung function indices. When the model was determined using bins dependent on the SR values (Table 3.4) then the best model included age, sex, BMI, TLcoSR, FVCSR, FEV₁SR, TLC₁SR and KcoSR with an overall chi-squared value of 1643. Indices including smoking, RVSR and FEV₁/FVCSR did not add significant value to the model. The best model using height-cubed lung function indices included age, sex, BMI, TLco/ht³, FVC/ht³ and FEV₁/ht³. Lung volumes (RV and TLC) and smoking were not included in the model as the confidence intervals for HR were not significantly different from unity and impaired the overall model. In both cases TLco entered the model first as the most important lung function index within the model. FVC entered the models before FEV₁ which is likely to be as a result of FVC better reflecting the size of the lungs. In both models FEV₁ either as SR or height-cubed appeared to have a protective effect. A lower FEV₁ had a better hazard ratio than those with a normal FEV₁.

Table 3.4

| $\chi^2 = 1643$ | | | $\chi^2 = 1626$ | | |
|--------------------------|-----------------|---------------------|---------------------------------------|--------------|---------------------|
| | | HR (95% CL) | | | HR (95% CL) |
| Age | ≤ 38 | 1 | Age | ≤ 38 | 1 |
| | 72 to 77 | 4.42 (3.55 to 5.51) | | 72 to 77 | 2.59 (2.05 to 3.27) |
| | >77 | 5.65 (4.54 to 7.03) | | >77 | 2.92 (2.32 to 3.68) |
| Sex | Female | 1 | Sex | Female | 1 |
| | Male | 1.23 (1.09 to 1.38) | | Male | 1.72 (1.57 to 1.88) |
| BMI | >35.8 | 1 | BMI | >35.8 | 1 |
| | 20.9 to 22.8 | 1.39 (1.13 to 1.71) | | 20.9 to 22.8 | 1.44 (1.17 to 1.77) |
| | <20.9 | 1.53 (1.24 to 1.87) | | <20.9 | 1.55 (1.27 to 1.90) |
| TLcoSR | >1.0 SR | 1 | TLco/ht³ | ≥ 1.92 | 1 |
| | -3.0 to 2.5 SR | 1.69 (1.09 to 2.61) | | 0.75 to 0.93 | 5.50 (4.27 to 7.08) |
| | <-3.0 SR | 2.23 (1.40 to 3.54) | | <0.75 | 7.65 (5.92 to 9.88) |
| FVCSR | >1.0 SR | 1 | FVC/ht³ | >0.93 | 1 |
| | -3.0 to -2.5 SR | 2.84 (2.05 to 3.93) | | 0.46 to 0.54 | 1.89 (1.44 to 2.49) |
| | <-3.0 SR | 2.70 (1.90 to 3.84) | | <0.46 | 2.35 (1.79 to 3.09) |
| FEV₁SR | >1.0 SR | 1 | FEV₁/ht³ | >0.70 | 1 |
| | -3.0 to -2.5 SR | 0.62 (0.46 to 0.83) | | 0.23 to 0.31 | 0.45 (0.33 to 0.60) |
| | <-3.0 SR | 0.47 (0.35 to 0.64) | | <0.23 | 0.37 (0.27 to 0.50) |
| TLCSR | >1.0 SR | 1 | | | |
| | -3.0 to -2.5 SR | 1.55 (1.20 to 1.99) | | | |
| | <-3.0 SR | 1.64 (1.28 to 2.09) | | | |
| KcoSR | >1.0 SR | 1 | | | |
| | -3.0 to -2.5 SR | 2.02 (1.29 to 3.18) | | | |
| | <-3.0 SR | 2.42 (1.50 to 3.89) | | | |

Results of the best multivariate Cox regression models using the ECCS equations and indices standardised by ht³. Chi-square (χ^2) values for each model and the hazard ratios (HR) for lung function index groupings with their 95% confidence limits are shown for the worst two groupings of each index included in the model.

3.4 Discussion

This study has shown when using a large UK based dataset of patients that there are differences between prediction equations in terms of how they spread lung function data and this has a significant impact on the ability to predict survival from lung function. This approach to choosing the most appropriate lung function prediction equations for a dataset by using survival analysis has not been used before.

The results of the study showed that Miller's equations, based on an American population, were overall the most appropriate for our UK patients. There have been several studies that have explored differences between prediction equations in terms of spirometry but not for gas transfer. Collen et al (2008) compared NHANES III to Crapo, Knudson and Morris prediction equations. Their aim was to gain an idea of the impact of implementing the ATS/ERS guidelines which recommend using NHANES III. In non-Hispanic white patients the authors explored the differences in classification i.e. obstructive, normal and restrictive by FVC. They found a significant discordance between all the equations and NHANES III more so for Knudson (59.6% concordant). They also observed that age >50 years and obesity were factors associated with increased likelihood of discordance. Lebowitz and Holberg (1990) compared the distribution using 12 prediction equations and the spread of FEV₁ data. He found that most had fairly similar means and standard deviations with the exception for older (in time not age) and more complex equations. Potential limitations of these studies include the use of percent of predicted to compare the groups which may introduce an age, sex and height bias (Miller and Pedersen, 2010) but also the end points that were being

measured were relatively subjective. By using survival to compare prediction equations we are using a clinically relevant end point that is clearly defined.

In this study TLco was the best predictor of survival in our patients with FVC being the second best. A TLco <85% predicted has previously been shown to be a significant predictor of all-cause mortality in a general US population (Neas and Schwartz, 1998) which was independent from both spirometry and any apparent respiratory disease. Measuring TLco provides a measure of the size of the lungs as well as a measure of their gas exchange and this is likely to explain why it is such a good predictor of survival. FVC which was the second best predictor, only provides a measure of the size of the lungs. RV, TLC, FEV₁/FVC and Kco were not as good predictors compared to TLco. All of these 4 indices can be either high or low in disease. For instance FEV₁/FVC is low in obstructive disease and high in restrictive disease. So these indices may not have a linear relationship between their value and their ability to predict survival. FEV₁, FVC and TLco all decrease with disease progression and therefore it would be expected that they would be able to predict survival. FVC is usually preserved in patients with mild obstructive disease therefore mortality may be low but in more severe obstruction the FEV₁ and FVC become low so FVC is likely to be better at predicting survival in these patients.

The populations that were used to derive the prediction equations had some similarities, in that all were based on a Caucasian population, but had many more differences.

Wanger (2005) outlined factors that need to be taken into consideration when choosing an appropriate prediction equation for a given population; including age, instrumentation, recording protocol, socio-economic, and environmental e.g. altitude. He also recommended that all the lung function indices are derived from the same source or population. There were differences in the equipment used to obtain the lung function measurements for the equations that we used for spirometry and gas transfer. The data collection for these equations ranged from between the 1950s to the 1990s so it is also likely that the recording protocols were varied and diverse because the agreed standards were changing up until the most recent ATS and ERS standardised protocols in 2005. Some populations had subjects included with a smoking history and some included subjects with respiratory symptoms, thus the study population may not have been completely healthy. Gas transfer measurement can be affected by both the altitude that a lung function test is performed at and the altitude that a person lives at (Macintyre et al., 2005, Remmers and Mithoefer, 1969). Crapo et al (Crapo and Morris, 1981, Crapo et al., 1981) based his prediction equations on a highly selective population in Salt Lake City which is located at 1200m above sea level and this may account for why his equations yield some of the unusual results. The age and height ranges of the subjects varied between the different prediction equations along with the sex distribution and whether haemoglobin was considered and corrected for in the gas transfer measurement.

We found the equation that was a best fit for our data was based on a US population from Michigan. Michigan is known to have a significant immigrant population particularly from Germany, Ireland, England and France which may partly explain why this population proved to be the best match for our UK population. All of the above differences help us understand why there were significant differences between the equations in terms of the spread of data particularly at the extremes of the normal distribution.

As has been described, some of the prediction equations for TLco had large sex differences in terms of the HR for sex and also how the equations dealt with sex. These apparent differences can show a perceived sex difference in disease prevalence that is, in fact, spurious. Roberts and Cotes in particular had higher HR for male sex compared to the other equations. As already illustrated when TLco by Miller was compared to that by Cotes the sexes were dealt with in different ways. The TLco of females by Cotes were pitched lower than Miller which would explain the worse HR as the females were being dealt with in an inappropriately more severe group therefore making it appear that their survival was better than the males. It is not entirely clear what population Cotes (1970) derived his female prediction equations from, but we do know that the number of subjects in each of the sexes was below 150 which has recently suggested to be the minimum needed in each sex group to obtain valid prediction equations (Quanjer et al., 2011).

TLco/ht³ was the best predictor of survival compared to all the other lung function indices in our study. Previous work has shown FEV₁ divided by a power of height can provide a good prediction for long term survival, as was shown in the Framingham study (Gordon et al., 1977), the Reykjavik study (Chinn et al., 2007) and the Copenhagen City Heart Study data (Miller et al., 2009). It was also found to be a good method for exploring lung function decline in COPD (Fletcher and Peto, 1977). Using FEV₁ divided by a power of height is trying to standardise FEV₁ by taking into account some sex and size differences between subjects. Using raw FEV₁ is problematic because of obvious sex and size differences in FEV₁. In our dataset LMS was one of the best spirometry equations for survival and this equation uses a power relationship to height.

There are several potential biases or influences within our dataset and our approach in this study. Firstly there were 201 subjects whose survival data were not available for the study and therefore not included within the analysis. Their results were not skewed in any way or different from the rest of the group and therefore we do not think that this has affected the result of our study. The subjects in our dataset have all had lung function measured at the University Hospital Birmingham which is a tertiary centre for heart, lung or liver transplant as well as some specialist neurophysiological and cancer services but these accounted for under 10% of the referrals so the effect of possible unusual diseases on survival is likely to be minimal. Despite this the population had a high mortality rate of 27% with a mean survival of only 4.9±3.3 years in women and 4.3±3.1 years in men despite being a relatively young population (58.2±15.2 years in women and

59.8±14.5 years in men) so the prediction equations that were most appropriate for our population may not be applicable in a general population. Most of the prediction equations have not included the very elderly within their reference population therefore affecting the ability of the equations to predict for this age group. With 25% of our population over 70 years old, in whom you would expect the survival to be worse, poorer lung function predictions may have affected the result. The LMS equations have the best cover for older subjects and did perform well. There are currently plans to improve prediction equations by ensuring the extremes of ages are included and therefore different conclusions may be reached.

Unfortunately we did not have any accurate data available for the diagnosis of our subjects so we were not able to look specifically at different disease categories. For instance it would have been of interest to have been able to explore FEV₁/FVC in more detail and its use within subjects known to have airflow obstruction. However, our findings are not dependent on knowing the exact diagnosis and the referral diagnosis and mortality within our dataset were not skewed. The mortality of the subjects was analysed as all-cause mortality as the exact cause of death was not available to us. It would clearly have been of interest to analyse the data for respiratory mortality as was undertaken for the Copenhagen City Heart Study data (Miller et al., 2009) where the conclusions were the same as for all-cause mortality but the HR ratios for association between lung function and respiratory mortality were all very much higher. Ideally it would be easier and more consistent to use one set of prediction equations across all the lung function indices. Unfortunately, in our study, there were only 3 sets of equations

where this was possible. Miller, for instance, used chest X-rays (CXR) to determine his TLC and RV prediction equations (Kilburn et al., 1992). This approach involves considerable potential error as TLC and RV are 3 dimensional measurements whilst the CXR is a 2-dimensional picture.

So our study explored a different approach to choosing an appropriate prediction equation by using survival which is a well-defined and clinically meaningful outcome.

In conclusion we have used a novel approach using survival data of patients to guide lung function laboratories in being able to choose the most appropriate prediction equations for their patients. Some equations had a sex bias which may impact on the prevalence of disease. We found that TLco was the best single predictor of survival in our patients followed by FVC. Miller TLco prediction equations were the best for our population and for spirometry Miller and LMS were as good as each other.

Chapter 4

SPIROMETRIC AND GAS TRANSFER DISCORDANCE IN ALPHA-1- ANTITRYPSIN DEFICIENCY; PATIENT CHARACTERISTICS AND PROGRESSION

Using the most appropriate prediction equations as determined from the work in Chapter 3 this Chapter describes the A1AD physiological phenotypes and the patient's characteristics including progression.

4.1 Introduction

COPD is a group of conditions characterised by irreversible airflow obstruction. There is increasing awareness of phenotypic differences in physiological, radiological and clinical characteristics that occur in varying proportions in this heterogeneous disease. These phenotypes may reflect different underlying pathological processes, contrasting prognoses and management strategies. Identifying phenotypes will improve the understanding of COPD and may facilitate specific management regimens. Valid phenotypes should provide prognostic information and have predictive value for the patient (Han et al., 2010) with support from mortality analysis.

A1AD is the most recognised genetic susceptibility factor for COPD. It is an autosomal co-dominant disorder which affects around 1 in 2000 Caucasians in Northern Europe and predisposes the development of early onset emphysema (Eriksson, 1965). The most common clinically relevant deficiency type is the homozygote Z variant (PiZZ), associated with a critically low serum concentration of α -1-antitrypsin and (classically) basal pan-acinar emphysema.

Previous studies from our group have related A1AD phenotypes to emphysema distribution and physiology. Parr et al (2004) showed Kco related better to upper zone emphysema and FEV₁ to lower zone emphysema. Holme and Stockley (2007) explored this by assessing a small number of age-matched patients with isolated FEV₁ or Kco abnormality defined as <80% predicted for age and sex. The data confirmed that isolated FEV₁ abnormality linked with a more basal emphysema distribution and isolated Kco abnormality with a more apical distribution. However, the small number of subjects precluded determining whether the patterns change with time and any demographic association.

The aim of the current study was to explore factors that might predict or be associated with physiological phenotypes and to assess progression over time to determine whether or how these phenotypes might change.

4.2 Methods

The first 530 PiZZ patients with complete baseline data to 2008 who attended the Antitrypsin Deficiency Assessment and Programme for Treatment (ADAPT) were included in a cross-sectional study. Some of these data have contributed to previous work. The longitudinal study included 255 with at least 3 years follow up (4 data points). Patients were reviewed at least 6 weeks after any exacerbation and underwent full pulmonary function tests using BTS/ARTP guidelines (1994), blood gas sampling, quantitative CT imaging (where possible), health status assessment (SGRQ) and breathlessness assessment (modified MRC dyspnoea score). For a sub-group of patients mean exacerbation frequency per year was defined using the Anthonisen criteria (Anthonisen et al., 1987). Although subjects were entered into the ADAPT project at differing stages of their disease their survival was defined from the date of their first lung function tests until the censor date of 1/7/2012 or date of death if that occurred earlier. South Birmingham Ethics Committee approved this study (LREC number 3359) and written informed consent was obtained from all patients.

Spirometry was measured pre and post nebulised salbutamol (5mg) and ipratropium bromide (500mcg) using a wedge bellows spirometer (CareFusion, San Diego, California, USA), lung volumes by helium dilution and gas transfer using the single breath carbon monoxide method (Blakemore et al., 1957). Predicted values were determined using Miller regression equations (Miller et al., 1983, Miller et al., 1986)

except for lung volumes where the European Community of Coal and Steel (ECCS) equations (Quanjer et al., 1993) were used. Abnormality of physiological data was determined using the standardised residual (SR) to define the lower limit of normal

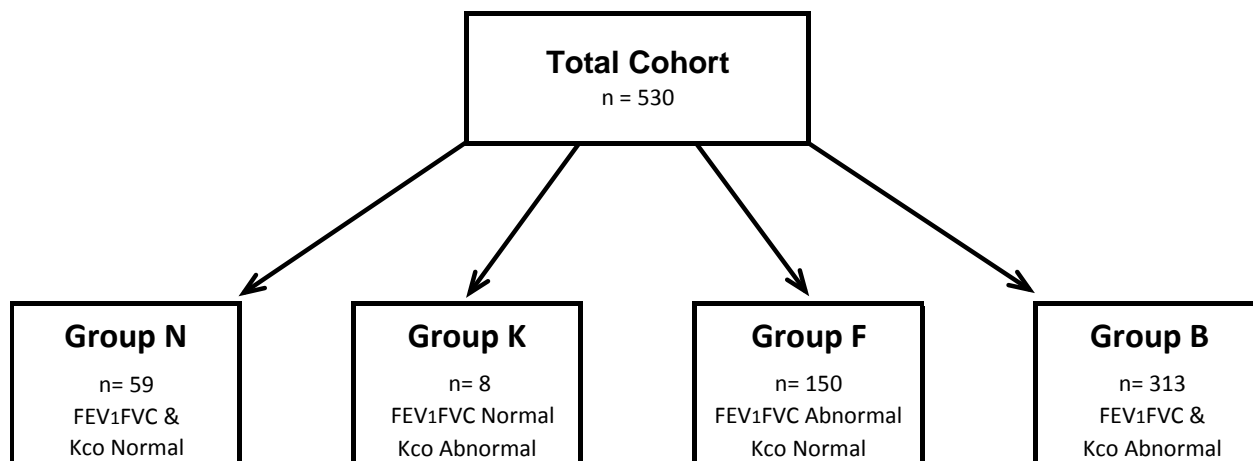
(-1.645 SR) as recommended by ATS and ERS (Pellegrino et al., 2005). SR values = (observed result – predicted result)/RSD where RSD is the residual standard deviation for the prediction equation (Miller and Pincock, 1988). Reversibility was defined as >200ml and >12% increase defined from the predicted FEV₁ (Pauwels et al., 2001, 1995).

HRCT was available for 92% (n=489) of the patients. All scans were assessed by experienced radiologists and emphysema was noted using recognised criteria (Naidich, 1991). For 300 of the patients this was undertaken in our department using a protocol to determine the voxel index (VI) at a density threshold of -910 Hounsfield Units (HU) at the level of the inferior pulmonary veins for the lower zones (LZ910), and the level of the aortic arch for upper zones (UZ910) as described previously (Parr et al., 2004).

Subjects were classified into physiological groups using lower limit of normal (LLN) for post-bronchodilator FEV₁/FVC and Kco (Figure 4.1). The group with **N**ormal FEV₁/FVC and Kco were labelled **N**, Both tests abnormal **B**, abnormal FEV₁/FVC alone **F** and abnormal Kco alone **K**. Index cases refer to patients identified following presentation with symptoms and non-index to individuals identified through family screening.

Figure 4.1

Definition of the physiological groups and the number of subjects in each group.



Statistical analysis was performed using PASW Statistics v.18, IBM SPSS. Non-parametric data were compared using Mann-Whitney U test and a t-test for parametric data. Pearson's chi-squared test was used to compare distribution of data between groups. A p value of ≤ 0.05 was accepted as significant with Bonferroni correction for multiple comparisons. Change in FEV₁ and Kco was calculated for the patients with ≥ 3 yearly annual follow-up using linear regression. Multivariate logistic regression analysis was performed to identify variables that predicted movement between subgroups.

4.3 Results

Demographic details of the 530 subjects are shown in Table 4.1 and the distribution of baseline FEV₁/FVCSR and KcoSR for each subject is illustrated in Figure 4.2.

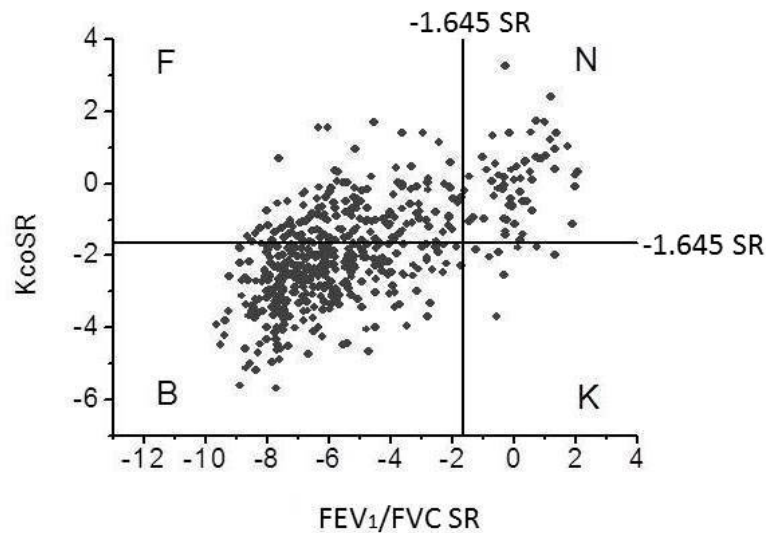
Table 4.1

| | Males (SEM) | | Females (SEM) | |
|--------------------------|--------------|--------------|---------------|--------------|
| n (%) | 316 (60) | | 215 (40) | |
| Age (yrs) | 51.0 (0.57) | | 50.4 (0.76) | |
| Height (m) | 1.76 (0.004) | | 1.62 (0.004) | |
| Weight (kg) | 78.9 (0.86) | | 68.2 (1.08) | |
| BMI (kg/m ²) | 25.3 (0.24) | | 25.9 (0.37) | |
| Pack year history | 20.19 (0.94) | | 13.47 (0.89) | |
| | Absolute | SR | Absolute | SR |
| FEV ₁ Litres | 1.85 (0.06) | -4.98 (0.14) | 1.59 (0.06) | -3.37 (0.17) |
| FVC Litres | 4.64 (0.06) | 0.34 (0.10) | 3.22 (0.06) | -0.30 (0.11) |
| FEV ₁ /FVC % | 38.8 (0.96) | -7.29 (0.17) | 48.2 (1.36) | -5.03 (0.20) |
| TLC Litres | 8.21 (0.07) | 1.74 (0.09) | 5.69 (0.06) | 1.30 (0.09) |
| RV Litres | 3.03 (0.06) | 2.04 (0.13) | 2.18 (0.05) | 1.24 (0.15) |
| RV/TLC % | 36.8 (0.54) | 0.54 (0.10) | 37.9 (0.74) | 0.31 (0.12) |
| TLco mmol/min/kPa | 6.58 (0.15) | -2.17 (0.08) | 5.27 (0.14) | -1.73 (0.09) |
| Kco mmol/min/kPa/L | 1.01 (0.02) | -2.13 (0.08) | 1.15 (0.03) | -1.45 (0.09) |

Baseline dataset demographic and lung function characteristics for males and females. Values are given as mean with standard error in parentheses. Physiological values are presented as absolute or standardised residuals (SR). Note all female demographic data are lower than for males ($p < 0.001$) (except for BMI and age) and all female physiological data expressed as SR are lower ($p \leq 0.001$) except for FVC, RV/TLC and Kco.

Figure 4.2

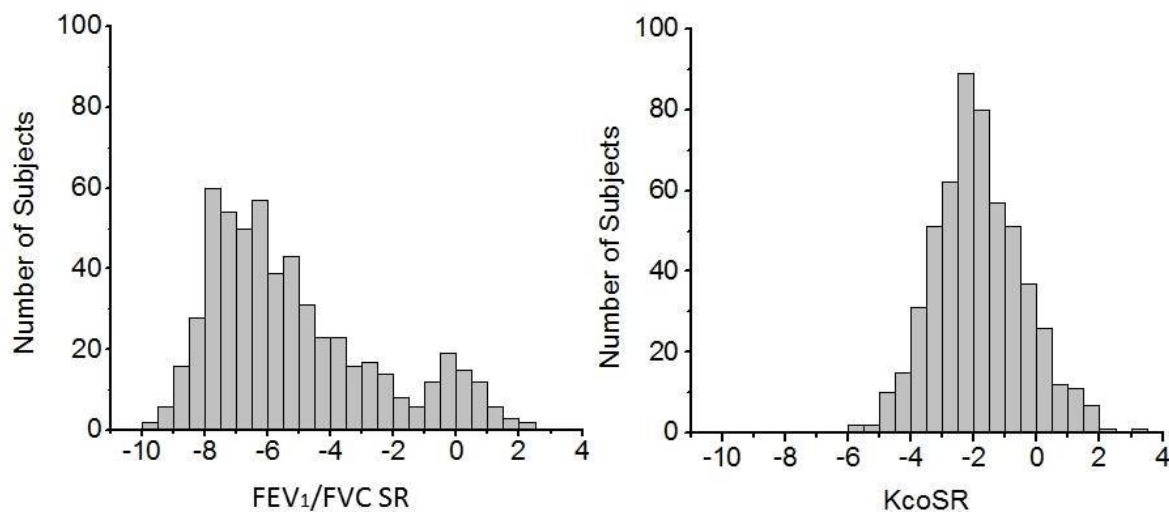
Scatter graph illustrating FEV₁/FVCSR and KcoSR for each subject.



The FEV₁/FVCSR for the cohort had a bimodal distribution (Figure 4.3) whilst Kco was normally distributed. Groups N and K (normal FEV₁/FVC) were grouped together and compared to Groups F and B (FEV₁/FVC abnormality). Those with abnormal FEV₁/FVC had a greater smoking history than those where it was normal (median pack years 18.00; IQR 7.20 - 27.00 and 0.00; 0.00 - 8.00 respectively; $p < 0.001$), included a higher proportion of ever smokers and fewer never smokers (86% vs. 46% and 14% vs. 54% respectively; $p < 0.001$), consisted of more men (63% vs. 37%; $p < 0.001$), more index cases (88% vs. 31%; $p < 0.001$), were older (mean 51.7 years SEM ± 0.46 vs. 44.1 ± 1.59 ; $p < 0.001$) and had more basal emphysema assessed as the ratio of LZ910 to UZ910 voxel index (13.88 SEM ± 0.86 vs. 2.25 ± 1.08 respectively, $p < 0.001$).

Figure 4.3

Histograms illustrating the distribution (n=530) of FEV₁/FVC SR (median -7.10; IQR -8.7 to -4.6) and KcoSR (mean -1.85±SEM 0.06) for all the subjects.



4.3.1 Variables that characterise each physiological phenotype.

4.3.1.1 Group N

These patients had lung function tests in the normal range (Table 4.2) including arterialised pO₂.

Table 4.2

| | Group N | Group K | Group F | Group B |
|-------------------------------------|-----------------------|-----------------------|------------------------|------------------------|
| FEV ₁ (L) | 3.41 (2.58 to 4.37) | 3.55 (2.78 to 4.51) | 1.52 (1.07 to 2.06) | 1.29 (0.91 to 1.75) |
| FEV ₁ SR | 0.62 (-0.37 to 1.40) | 0.58 (-1.08 to 1.96) | -4.40 (-5.75 to -3.25) | -5.51 (-6.58 to -4.18) |
| Kco (mmol/min/kPa/L) | 1.63 (0.04) | 0.98 (0.10) | 1.33 (0.02) | 0.83 (0.01) |
| Kco SR | 0.19 (0.13) | -2.20 (0.23) | -0.72 (0.06) | -2.77 (0.05) |
| FVC (L) | 4.25 (0.17) | 4.70 (0.50) | 3.71 (0.10) | 4.19 (0.07) |
| FVC SR | 0.29 (0.19) | 0.91 (0.75) | -0.84 (0.14) | -0.22 (0.10) |
| FEV ₁ /FVC (%) | 0.81 (0.77 to 0.86) | 0.78 (0.73 to 0.84) | 0.43 (0.33 to 0.53) | 0.32 (0.25 to 0.40) |
| FEV ₁ /FVC SR | -0.12 (-0.71 to 0.57) | -0.65 (-1.16 to 0.27) | -6.16 (-7.80 to -4.33) | -8.10 (-9.42 to -6.56) |
| TLC (L) | 6.14 (0.19) | 6.78 (0.60) | 6.61 (0.12) | 7.68 (0.09) |
| TLC SR | 0.60 (0.15) | 1.00 (0.61) | 0.93 (0.11) | 2.06 (0.08) |
| RV (L) | 1.64 (0.06) | 1.94 (0.25) | 2.49 (0.06) | 3.00 (0.06) |
| RV SR | -0.41 (0.14) | -0.04 (0.61) | 1.25 (0.14) | 2.39 (0.14) |
| RV/TLC (%) | 27.36 (1.08) | 28.87 (3.18) | 37.90 (0.73) | 38.99 (0.56) |
| TLco (mmol/min/kPa) | 9.19 (0.38) | 6.00 (0.73) | 7.10 (0.16) | 4.97 (0.10) |
| TLco SR | 0.13 (0.14) | -2.09 (0.30) | -1.13 (0.07) | -2.78 (0.05) |
| Arterial oxygen (kPa) | 11.52 (0.17) | 10.81 (0.52) | 9.23 (0.09) | 8.87 (0.09) |
| Reversibility (%) | 15 | 50 | 27 | 22 |
| FEV ₁ decline (mls/year) | -53.24 (14.6) | -124.3 (22.9) | -31.09 (8.4) | -46.29 (5.2) |
| Kco decline (mmol/min/kPa/L/yr) | -0.030 (0.006) | -0.034 (0.012) | -0.054 (0.004) | -0.032 (0.003) |

Physiological indices for each of the 4 physiological groups.

Values are mean with standard error in parentheses or median with interquartile ranges for non-parametric data and percentages (%) rounded up to the nearest whole number. Kco and FEV₁ decline is represented in subjects only with longitudinal lung function data available for ≥3 years.

The majority (64.4%) were female which is an over representation of the UK population (Office of National Statistics 51% female) and also higher than in groups F (p=0.046) and B (p<0.001) (Table 4.3).

Table 4.3

| | Group N n=59 | Group K n=8 | Group F n=150 | Group B n=313 |
|--------------------------|---------------------|----------------------|-----------------------|-----------------------|
| Age (yrs) | 43.6 (1.64) | 47.4 (5.66) | 52.3 (0.81) | 51.4 (0.55) |
| Males (%) | 36 | 50 | 51 | 68 |
| Index (%) | 25 | 63 | 84 | 90 |
| Pack year history | 0.00 (0.00 to 9.50) | 0.00 (0.00 to 7.05) | 14.50 (1.00 to 26.25) | 19.00 (9.00 to 27.00) |
| Never smokers (%) | 53 | 63 | 24 | 10 |
| Ex-smokers (%) | 42 | 25 | 69 | 82 |
| Current smokers (%) | 5 | 12 | 7 | 8 |
| BMI | 27.3 (0.75) | 23.3 (1.21) | 27.1 (0.39) | 24.5 (0.24) |
| BMI >30 (%) | 29 | 0 | 21 | 11 |
| BMI >25 (%) | 56 | 38 | 65 | 43 |
| BMI <20 (%) | 3 | 0 | 3 | 14 |
| Lower Zone VI (<-910 HU) | 11.4 (7.2 to 23.8) | 16.5 (15.2 to 39.3) | 42.9 (28.7 to 59.2) | 55.4 (46.9 to 65.0) |
| Upper Zone VI (<-910 HU) | 11.4 (4.9 to 17.8) | 12.5 (10.9 to 35.3) | 25.0 (13.3 to 33.1) | 40.8 (29.5 to 49.3) |
| Total SGRQ | 19.59 (5.8 to 36.7) | 31.09 (27.7 to 46.7) | 45.24 (29.9 to 59.3) | 53.67 (39.6 to 64.7) |

Demographics and other clinical indices for each of the 4 groups. Values are mean with standard error in parentheses, median with interquartile ranges for non-parametric data or as a proportion of the group (%) rounded up to the nearest whole number.

Group N patients were younger (on average) than groups F and B ($p<0.001$) and the majority were non-index. There was a minimal smoking history and less than patients in groups B and F ($p<0.001$) and the UK in general. The mean BMI of group N was comparable to F, a third being obese (BMI >30) as in the UK population (ONS 2010 26% adults over 16). Group N had the least quantified emphysema ($p<0.001$) and prevalence of visible emphysema (17.6%) compared to the other groups and a lower UZ910 voxel index than groups F and B ($p\leq 0.004$)

Group N also had a worse health status than a healthy population (Ferrer et al., 2002) although better than groups F and B for the total SGRQ score ($p<0.001$) including the activity, impacts and symptoms domains ($p<0.001$). Group N also reported less symptoms of shortness of breath than groups F and B ($p<0.001$). See Table 4.4.

Table 4.4

| | Group N | Group K | Group F | Group B |
|---------------------|----------------------|----------------------|----------------------|----------------------|
| Total SGRQ | 18.50 (5.8 to 36.7) | 31.09 (27.7 to 46.7) | 45.24 (29.9 to 59.3) | 53.67 (39.6 to 64.7) |
| SGRQ symptoms | 36.47 (17.3 to 61.8) | 60.20 (40.4 to 70.8) | 61.41 (45.3 to 79.4) | 67.06 (51.0 to 78.8) |
| SGRQ activity | 18.45 (0.0 to 44.5) | 44.50 (20.9 to 63.2) | 60.38 (41.4 to 79.7) | 72.82 (53.6 to 92.5) |
| SGRQ impacts | 10.80 (3.6 to 27.5) | 18.80 (14.2 to 37.1) | 30.20 (18.2 to 45.8) | 39.05 (24.9 to 50.0) |
| mMRC dyspnoea score | 1.00 (0 to 2) | 2.00 (1 to 2) | 2.00 (2 to 3) | 3.00 (2 to 4) |

Median (interquartile range) values for total SGRQ, all its domains and modified Medical Research Council Dyspnoea score for all 4 groups.

4.3.1.2 Group K

These patients had low Kco and TLco (Table 4.2) and 50.0% were female (Table 4.3). The majority (62.5%) were index cases with minimal smoking history (like group N). The average BMI was 23.3 ± 1.21 with none defined as obese.

The prevalence of visible emphysema (50.0%) and amount was comparable to group N and the group also had a worse health status than a healthy population (Ferrer et al., 2002).

4.3.1.3 Group F

Group F had airflow obstruction with gas transfer in the normal range though lower than Group N ($p < 0.001$) (Table 4.2). The mean RV/TLC was within the normal range but was above the normal range in 20.7% indicating significant gas trapping. The average pO_2 was just below the normal range and lower than group N ($p < 0.001$). All lung function was however better than for group B ($p \leq 0.001$), half of the patients were female and most (84.0%) were index cases (Table 4.3). Smoking history was greater than for group N ($p < 0.001$) but less than for group B ($p < 0.007$) and 21.3% were obese (comparable to Group N).

Group F had more emphysema than group N ($p < 0.001$) but less than group B ($p < 0.001$). Health status was also worse than N ($p < 0.001$) though better than B ($p = 0.003$) for total SGRQ and all 3 domains. Group F reported more symptoms of shortness of breath than group N ($p < 0.001$) but less than group B ($p < 0.001$).

4.3.1.4 Group B

Group B had the worst physiology, lower pO_2 than groups N and K ($p < 0.001$) and respiratory failure was present in 9.6%. Group B had the lowest proportion of females, were mainly index patients (Table 4.3), had the greatest smoking history ($p \leq 0.042$ vs. other groups) and more underweight patients than group F ($p = 0.009$).

The group had the highest visual evidence of emphysema (80.3%) and LZ910 voxel indices compared to all other groups ($p \leq 0.001$ respectively), a higher UZ910 voxel index than groups N and F ($p < 0.001$) and a worse total SGRQ score and all its domains than

groups N and F ($p \leq 0.003$) but not K ($p = 0.055$). Dyspnoea score was also greater than for all other groups ($p \leq 0.001$).

4.3.2 Longitudinal change

There were 255 subjects with ≥ 4 complete lung function results over at least 3 years (mean 5.37 ± 0.13 years). The average change in FEV_1 (mls/year) and Kco (mmol/min/kPa/L/year) are summarised in Table 4.2. There were no significant differences between the groups for FEV_1 decline although group F had a faster Kco decline than either group B ($p < 0.001$) or N ($p = 0.002$). There was no relationship between age and decline in FEV_1 or Kco. The average decline in lung function was greater than expected for age indicated by a mean change of $FEV_1\%$ predicted per year (-0.56 ± 0.25 , -2.34 ± 0.58 , -0.61 ± 0.17 and -0.89 ± 0.18) and Kco% predicted per year (-2.04 ± 0.34 , -1.58 ± 0.69 , -3.65 ± 0.26 and -1.85 ± 0.25) in groups N, K, F and B respectively.

During the follow-up 140 patients died including 6 from group N, 1 from group K, 34 from group F and 99 from group B (equivalent to 2.0%, 3.0%, 5.7% and 8.6% of the respective groups/year).

Figure 4.4 summarises movement between physiological groups during follow-up. The majority of group N stayed in N but 30.0% moved (4 to F, 6 to K and 2 to B). The group with the largest movement was F where 61.6% moved to B ($n=45$).

The 28 remaining in group F were compared to the 45 who moved to B (Table 4.5).

Figure 4.4

Bar chart showing for each of the patient groups at baseline (horizontal axis) the percentage of subjects who were in each group at the end of the follow up (mean 3.87 ± 2.47 years). The number within the bars indicates the % of patients who remained in their baseline group.

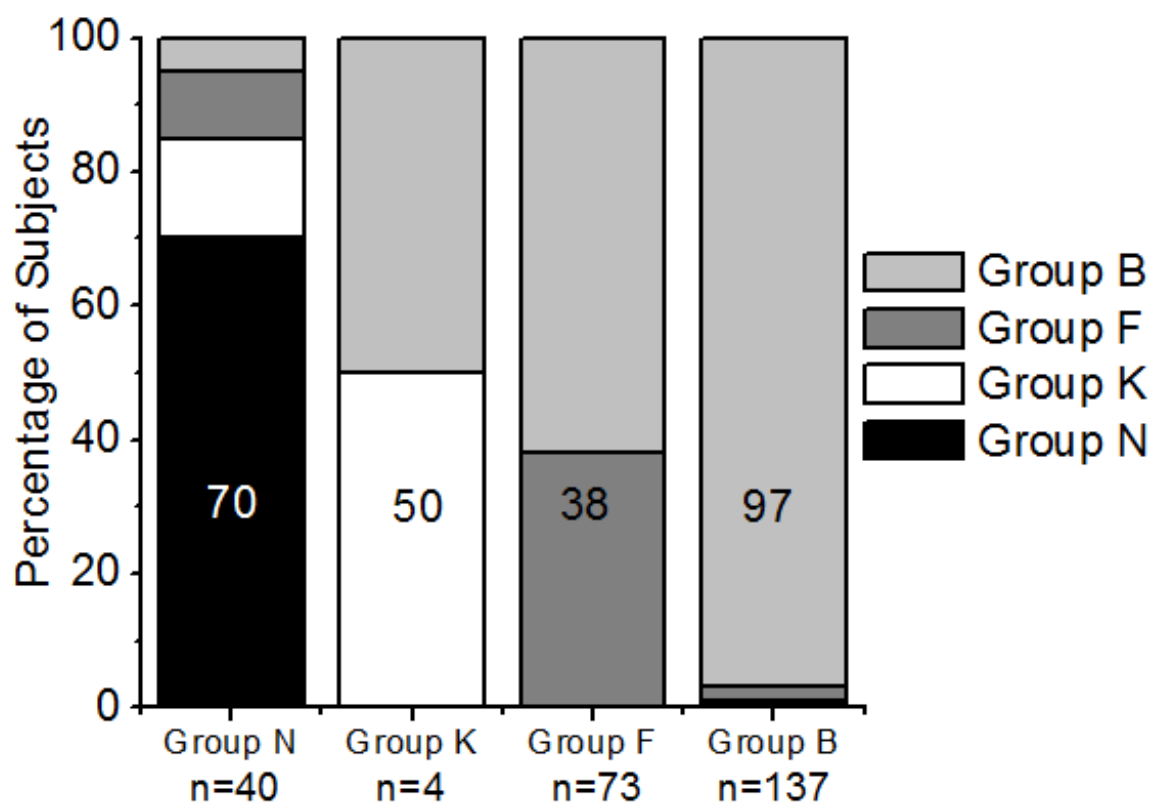


Table 4.5

| | Group FF n=28 | Group FB n=45 |
|-----------------------------------|------------------------|---------------------------|
| Age (yrs) | 53.7 (1.6) | 51.9 (1.4) |
| Males (%) | 39 | 64* |
| Pack year history | 9.8 (0.0 to 21.0) | 22.0 (7.0 to 27.8) |
| Ever smokers (%) | 64 | 76 |
| Total SGRQ | 53.4 (35.4 to 66.2) | 52.0 (34.0 to 59.4) |
| mMRC dyspnoea score | 2 (2 to 3) | 3 (2 to 3) |
| BMI | 27.6 (0.9) | 26.3 (0.7) |
| Lower Zone VI (<-910 HU) | 35.6 (27.6 to 46.9) | 47.8 (31.8 to 59.8) |
| Upper Zone VI (<-910 HU) | 18.3 (10.3 to 26.0) | 29.6 (21.6 to 35.9)** |
| Follow-up, years | 5 (4 to 5) | 5 (4 to 7) |
| Baseline FEV ₁ SR | -3.00 (-3.88 to -1.84) | -3.52 (-4.59 to -2.94)* |
| Last FEV ₁ SR | -2.97 (-3.83 to -2.09) | -3.83 (-4.83 to -2.76)** |
| Baseline FEV ₁ /FVC | 0.50 (0.37 to 0.57) | 0.40 (0.32 to 0.48)** |
| Baseline FEV ₁ /FVC SR | -4.25 (-5.89 to -3.17) | -5.51 (-6.55 to -4.76)* |
| Last FEV ₁ /FVC | 0.47 (0.35 to 0.52) | 0.32 (0.28 to 0.42)**** |
| Last FEV ₁ /FVC SR | -4.82 (-6.13 to -3.74) | -6.33 (-6.93 to -5.50)*** |
| Baseline Kco SR | -0.16 (0.11) | -0.98 (0.08)**** |
| Last Kco SR | -0.84 (0.12) | -2.34 (0.08)**** |
| Baseline TLC SR | 0.81 (0.28) | 0.97 (0.17) |
| Baseline RV SR | 1.07 (0.29) | 1.56 (0.28) |
| Arterial oxygen (kPa) | 9.14 (0.16) | 9.11 (0.19) |
| Kco decline (mmol/min/kPa/L/yr) | -0.037 (0.005) | -0.064 (0.005)**** |
| Exacerbation rate/year | 2.00 (0.37) | 2.12 (0.27) |

*p<0.05 **p<0.01 ***p<0.005 ****p≤0.001

Demographics and physiology of group FF (those in group F at baseline and follow-up) and FB (those in group F at baseline and group B at follow-up). Percentages (%) are rounded up to the nearest whole number.

There were no differences in baseline or follow up demographics except those who moved from F to B who were predominantly male, with more upper zone emphysema,

lower baseline Kco, faster Kco decline, and more airflow obstruction at baseline and follow up than those who stayed in group F (Table 4.5).

Females who moved from F to B had a lower baseline Kco than males (-1.29 ± 0.08 and -0.81 ± 0.09 respectively; $p=0.002$) although subsequent decline was greater in males (mean -0.071 ± 0.006 SEM mmol/min/kPa/L/year and -0.051 ± 0.007 respectively; $p=0.047$).

Logistic regression showed the independent predictors of movement from F to B were baseline Kco and male sex, which together predicted 89.0% of movement. No other variable including exacerbations independently predicted movement from F to B.

4.4 Discussion

These results are from the largest cohort of untreated A1AD patients with extensive demographic and follow-up data and demonstrate four distinct physiological groups based on Kco and FEV₁/FVC.

The data show a modal reduction in Kco but a bimodal distribution of airflow obstruction. This suggests that the protease/anti-protease imbalance caused by antitrypsin deficiency leads to general parenchymal disease whilst determinants of airflow obstruction require additional factors accounting for the bimodal distribution of FEV₁/FVC. We have shown that if the emphysema is distributed apically rather than basally the FEV₁ is more likely to be normal (Parr et al., 2004). Alternatively it may

represent a difference in the time course as Kco is an earlier marker of parenchymal damage whereas spirometry is a later marker of airways physiology (Holme et al., 2013). This discordance requires further epidemiological and genetic study.

Patients were classified into 4 distinct groups: N (data in the normal range); K (abnormal Kco); F (abnormal FEV₁/FVC) and B (abnormal FEV₁/FVC and Kco). It is unlikely these 4 groups represent a single process of progression for two reasons. Firstly (except group N) the groups have the same average age and secondly there are different demographic features associated with each group. It is possible that other exposures such as pollution and/or smoking habit may play some role. For instance the greater smoking history of group B compared to group F may reflect the balance of parenchymal versus airways disease and length of exposure may influence those who subsequently move from group F to group B. However this latter possibility seems unlikely since almost exclusively the patients recruited to the longitudinal data have stopped smoking by their first visit.

Group N had physiological features consistent with a normal population. The majority were non index patients with minimal smoking history consistent with previous literature indicating better physiology in non-index patients (Silverman et al., 1989) and benefits of not smoking (Larsson, 1978). However the group had symptoms and impaired health status which may reflect those who were obese and, as most were female (64%), the tendency to be more aware of symptoms (Dales et al., 2006). Alternatively this may also

reflect the presence of very early disease whilst in the normal ranges as the spirometry was greater than for a healthy population whilst Kco was lower.

Group K patients were slightly older, 50% female and had impaired Kco despite normal post bronchodilator spirometry. Smoking history was minimal although most (63%) were index patients having presented with symptoms. Health status was worse than for group N consistent with increased symptoms including cough, phlegm, wheeze and dyspnoea recorded in the SGRQ symptom domain. The older age but minimal smoking history suggests this could be the usual progression for some patients (never smokers) from group N.

Group F had impaired spirometry and air trapping but normal Kco although the mean value of -0.73 SR SEM ± 0.06 suggests some reduction. The group had an equal sex distribution, a tendency to be overweight and greater smoking history (than groups N and K). These features suggest smoking has an important effect on spirometry and this, with a significant proportion of overweight subjects (22%) and reduced arterial oxygen tension, significantly affects health status. Despite the lower than average but “normal” Kco, lung densitometry indicated significant lower zone emphysema and lesser upper zone change consistent with previous data for this physiological group (Holme and Stockley, 2007).

Group B had the worst health status, mortality, spirometry, air trapping and Kco impairment. The group was predominantly male and had the greatest smoking history although the average age was similar to group F. Group B also had the greatest

proportion of underweight subjects (often associated with an emphysema phenotype) and emphysema both in the lower zone and, importantly, in the upper zone (Parr et al., 2004). The combination of all these factors is likely to be the reason for the greater mortality of this group.

This data on A1AD patients have for the first time allowed comprehensive assessment of longitudinal change up to 11 years. The average decline in FEV₁ was not different between groups although Kco decline was greatest in group F. Changes were greater than expected for age indicating a real pathological change especially for Kco in group F with a significant proportion moving to B.

Patients moving to B were predominantly male, had slightly worse spirometry at baseline and follow-up and more upper zone emphysema consistent with a lower baseline Kco (Parr et al., 2004). Importantly the subsequent decline in Kco was more rapid in those whose Kco became abnormal (as would be predicted) than those whose Kco remained in the normal range.

Brantly et al (1988) in a study of rapid A1AD decliners over a shorter period, identified 24 similar patients. They reported the average FEV₁ decline was 1.5 times normal, (51±82ml/year) similar to our groups. However they noted a 10 times greater than normal decline in TLco. The reasons for this differing progression of spirometry and TLco were unknown but our current and previous data (Parr et al., 2004) suggest it reflects an increasing distribution of the emphysema to the upper zone, a feature of males and time.

The importance of this observation is that spirometry and Kco reflect 2 different, at least in part, interdependent physiological processes of emphysema and airflow obstruction. Previous work in A1AD has shown FEV₁ decline is dependent on baseline FEV₁ with greatest changes seen in patients with moderate airflow obstruction (FEV₁ 50 – 80% of predicted). Kco decline was greatest in patients with more severe airflow obstruction thought to be a later phenomenon, relating to the extension of emphysema to the upper zones (Dawkins et al., 2009). Parr et al (2009) reported that augmentation therapy in A1AD showed more treatment effects in the basal than the middle and apical regions hypothesising that differing pathology types and processes of emphysema are important determinants. For instance a polymorphism of MMP-3 has been implicated in gas transfer values in A1AD (McAloon et al., 2009) which preferentially mark apical emphysema (Parr et al., 2004) and may explain the lesser effect of alpha-1-antitrypsin augmentation in this region. Studies of a MMP-9 SNP shown to influence emphysema distribution in usual COPD (Ito et al., 2005) showed it may have a similar influence in A1AD but the rate of progression of upper zone emphysema was too low to have adequate power (Wood et al., 2007). It is important that this issue is resolved since it may impact on the indications or expectations for augmentation therapy in A1AD. Current indications for treatment are based on the presence of moderate disease where FEV₁ decline is greatest. Our study shows that using phenotypes determined by spirometry and Kco may identify candidates for augmentation therapy more clearly. For instance a subgroup with abnormal FEV₁/FVC and faster Kco decline was identified indicating disease progression independent of FEV₁ decline and a different focus for treatment. If emphysema at the apex arises from a distinct, pathological process related

to MMPs, augmentation therapy may be less effective in such patients. This possibility clearly warrants further exploration to determine whether the apical distribution of emphysema represents a natural progression related to severity and/or a distinct pathophysiological phenotype. Prospective studies including quantitative progression in all regions of the lung matched with epidemiological and genetic data are clearly indicated.

A potential limitation to our study is that patients enter the program at different disease stages. This varies due to the effects of occasional late diagnosis, differences in referral patterns to the UK registry and the approach to family screening. This could influence the baseline demographics, physiological characteristics and observed disease progression. Nevertheless we found distinct phenotypes from which general conclusions can be drawn especially emphasising the importance of Kco in assessing A1AD.

In summary we identified distinct physiological phenotypes in patients with A1AD with differing demographic background that likely influence the progression of the disease suggesting underlying epigenetic influences.

Chapter 5

IMPROVING THE DEFINITION OF LUNG FUNCTION COPD PHENOTYPES IN ALPHA-1-ANTITRYPSIN DEFICIENCY

Chapter 4 described the patient's characteristics within the physiological phenotypes defined using KcoSR and FEV₁/FVCSR. This Chapter will explore other lung function indices that are readily available that may be more appropriate when defining these phenotypes.

5.1 Introduction

When defining phenotypes the most appropriate determinants need to be applied thereby ensuring the phenotypes are valid and reliable. Chapter 4 described physiological phenotypes in patients with A1AD defined using Kco and FEV₁/FVC. Kco, TLco and alveolar volume in a single breath (V_{Aeff}) are closely inter-related and all have been found to be reduced in severe emphysema (Hughes and Pride, 2012) and therefore consideration needs to be given to determine to whether Kco is the better index to be used in this context or whether either TLco or V_{Aeff} are more appropriate. In A1AD Kco has previously been shown to have the fastest decline in subjects with severe and very severe airways obstruction and so seems to be a relatively late phenomenon (Dawkins et al., 2009). Kco also had a predictive ability for all cause and

respiratory mortality which exceeded that of the FEV₁ (Dawkins et al., 2003). Neither of these studies considered V_{Aeff} or TLco. Dowson et al (2001) studied 43 PiZZ patients over a 2 year period and found that the number of exacerbations related to TLco (r=-0.31 p=0.02). The same study showed that TLco, rather than FEV₁ and vital capacity (VC), was an independent predictor in the decline of the SGRQ activity domain.

Little work has been done to explore the relationship between A1AD and TLco so we used a large A1AD cohort to define physiological COPD phenotypes using lower limit of normal of FEV₁/FVC, Kco and TLco. We then explored these phenotypes further taking into consideration changes in V_{Aeff} to see how they relate to patient characteristics and survival.

5.2 Methods

The survival status of the A1AD subjects was determined by matching the National Health Service (NHS) number, address, name and postcode with the NHS care records service. In the case of 2 subjects a match between any of these indices was not found and so they were assumed to be alive. Subjects entered the ADAPT project at different stages in their disease so survival was defined from the date of their first (baseline) lung function tests until the censor date of 1st July 2012 or the date of their death if it occurred before the censor date.

The methods for lung function tests including spirometry, lung volumes and gas transfer, choice of predictive equations, calculations for lower limit of normal have previously

been described in Chapter 2. The effective alveolar volume measurement determined from the gas transfer test was expressed as a percentage of the TLC and was used as a measure of single breath gas mixing ($V_A\%TLC$).

HRCT was performed in 92% of the subjects (n=489). All the scans were assessed by an experienced radiologist with the presence of emphysema noted. The voxel index (VI) was determined from the HRCT of the thorax and a density threshold of -910 Hounsfield Units (HU) was set for the upper zones (UZ910) at the level of the aortic arch and for the lower zones (LZ910) at the level of the inferior pulmonary veins.

Subjects were classified into physiological phenotypes using lower limit of normal. The groups were defined using FEV_1/FVC and either Kco or TLco. Group **N** had both normal tests, Group **B** had both abnormal tests, Group **K/T** had abnormal Kco or TLco alone respectively depending on which index was used and Group **F** had abnormal FEV_1/FVC .

Statistical analysis was performed using Stata/SE Version 11.0 (StataCorp LP, Texas, USA). Parametric data was compared using t-test and non-parametric using the Mann Whitney U test. The comparison between the data distribution between the groups was performed using Pearson's chi-squared test. Bonferroni correction for multiple comparisons was accepted as significant with a probability of ≤ 0.05 . Changes in FEV_1 , Kco and TLco were calculated for subjects with at least 3 years of annual follow-up data using linear regression. The predictive factors that were used to determine Cox proportional hazards regression included survival, if the subject had ever smoked, the sex of the patient and their physiological phenotype.

5.3 Results

530 PiZZ patients were included in this study of which 60% were male with a mean age of 50.7 (10.6) years of age. Table 5.1 illustrates the mean values (SD) for the baseline data in women and men.

Table 5.1

| | Women n=214 | | Men n=316 | | |
|-------------------------|-------------|--------|-----------|---------|----------|
| | Mean | (SD) | Mean | (SD) | |
| Age yrs | 50.4 | (11.2) | 50.9 | (10.2) | ns |
| Follow up yrs | 8.6 | (3.2) | 9.1 | (3.6) | ns |
| Deaths % | 24.8 | | 27.5 | | ns |
| Ht m | 1.62 | (0.07) | 1.76 | (0.07) | |
| PkYrs Smoking | 13.5 | (13.0) | 20.2 | (16.67) | p<0.0001 |
| BMI Kg·m ⁻² | 25.9 | (5.4) | 25.3 | (4.2) | ns |
| FEV ₁ L | 1.59 | (0.85) | 1.85 | (1.08) | |
| FVC L | 3.22 | (0.83) | 4.64 | (1.12) | |
| FEV ₁ /FVC | 48.2 | (20.0) | 38.8 | (17.1) | p<0.0001 |
| TLco mmol/min/kPa | 5.29 | (1.98) | 6.62 | (2.58) | |
| Kco mmol/min/kPa/L | 1.15 | (0.37) | 1.02 | (0.41) | |
| FEV ₁ SR | -3.37 | (2.47) | -4.98 | (2.41) | p<0.0001 |
| FVCSR | -0.29 | (1.63) | -0.34 | (1.84) | ns |
| FEV ₁ /FVCSR | -5.03 | (2.96) | -7.29 | (3.04) | p<0.0001 |
| TLcoSR | -1.72 | (1.37) | -2.14 | (1.38) | p<0.001 |
| KcoSR | -1.45 | (1.34) | -2.08 | (1.62) | p<0.0001 |
| VA%TLC | 81.2 | (11.1) | 80.5 | (11.0) | ns |
| UZ910 | 17 | (0.5) | 35.4 | (17.0) | p<0.0001 |
| LZ910 | 22.3 | (0.9) | 48.5 | (17.8) | p<0.05 |
| SGRQ Activity | 62.3 | (26.9) | 61.6 | (27.6) | ns |
| SGRQ Symptoms | 58.7 | (22.1) | 61.8 | (23.8) | ns |
| SGRQ Impacts | 33.1 | (18.3) | 35.2 | (20.3) | ns |
| SGRQ Total | 46.1 | (19.5) | 47.8 | (21.0) | ns |

Mean values (SD) for baseline data set out separately for all the women and men in the study. UZ910 refers to the emphysema score of upper zones using 910 Hounsfield unit cut off.

Men had a greater mean pack year history than females ($p<0.0001$), worse absolute FEV₁/FVC ($p<0.0001$), FEV₁SR ($p<0.0001$), FEV₁/FVCSR ($p<0.0001$), KcoSR ($p<0.0001$), TLcoSR ($p<0.001$) and more evidence of emphysema in the lower and upper zones ($p<0.05$ and $p<0.0001$ respectively). There were no differences in age, mortality, BMI, or any of the SGRQ domains. When the phenotypes were defined using TLcoSR (Table 5.2) there were 58 subjects in Group N, 9 in Group K, 135 in Group F and 328 in Group B. When defined using KcoSR there were similar numbers in Group N and K (59 and 8 respectively) compared to groups defined by TLcoSR. There were more patients in Group F when defined by KcoSR and slightly less in Group B.

Table 5.2

| KcoSR | TLcoSR | | | | Total |
|-------|--------|---|-----|-----|-------|
| | N | T | F | B | |
| N | 56 | 3 | 0 | 0 | 59 |
| K | 2 | 6 | 0 | 0 | 8 |
| F | 0 | 0 | 104 | 47 | 151 |
| B | 0 | 0 | 31 | 281 | 312 |
| Total | 58 | 9 | 135 | 328 | 530 |

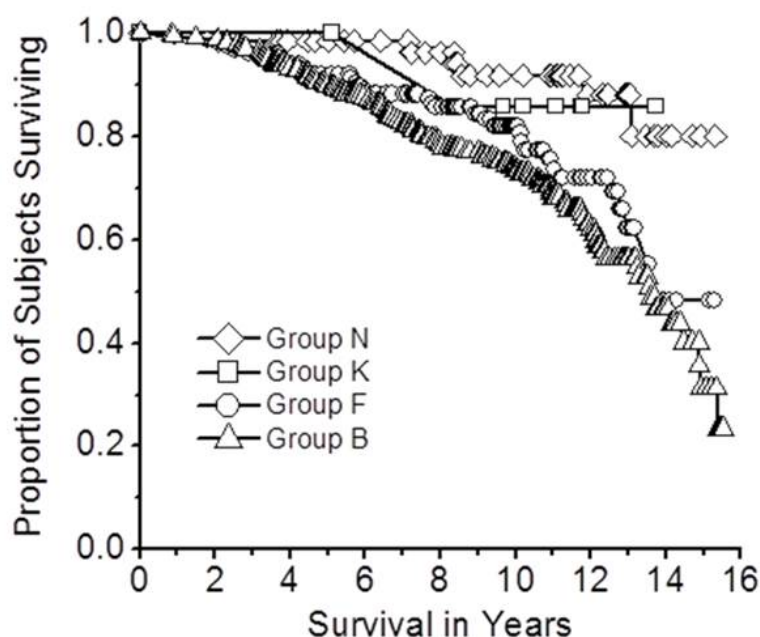
Cross tabulation of subject numbers in each of the four phenotype groupings when using TLco or Kco in the definition. The numbers in the grey squares show those in the same groups when using Kco and TLco.

The duration of follow-up for the subjects ranged from 0.8 to 15.5 years and by the censor date (1/7/2012) a total of 123 had died (23.2% of the whole dataset) of which 65.0% of them were male with a mean (SD) survival of 7.0 (3.5 years). Of the 407 who

survived, 58.0% were male with a mean (SD) survival of 9.5 (3.3) years. Figure 5.1 illustrates the Kaplan Meier survival plots for the subjects in each of the 4 phenotypes as defined using KcoSR and FEV₁/FVCSR.

Figure 5.1

Estimated Kaplan Meier survival plots for the subjects in the four groupings using KcoSR and FEV₁/FVCSR. Group N had both KcoSR and FEV₁/FVCSR normal, Group K only KcoSR was abnormal, Group F only FEV₁/FVCSR was abnormal and in Group B both were abnormal. The absolute patient numbers surviving at each 2 year interval are shown at the bottom of the figure.



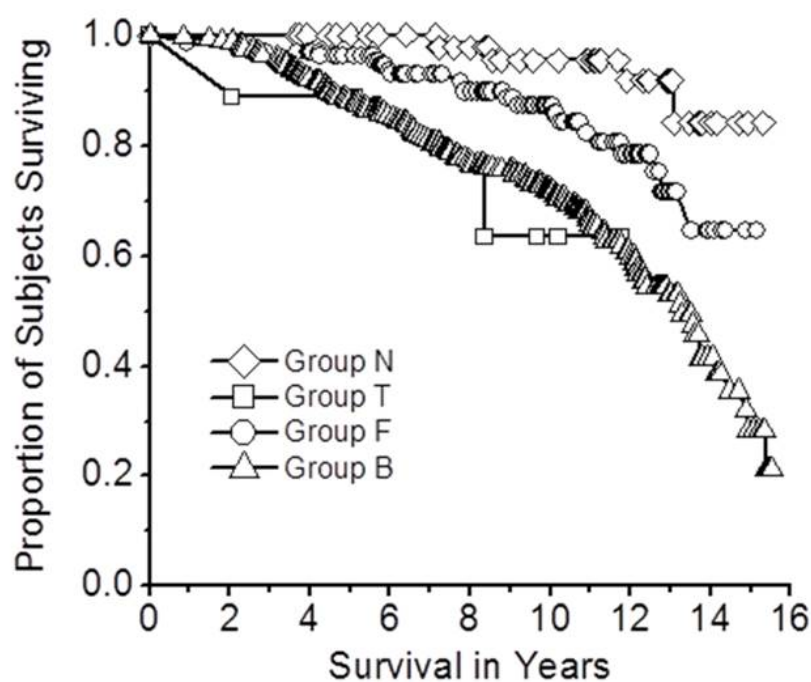
| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|----|---|
| Group N | 60 | 59 | 59 | 59 | 55 | 55 | 53 | 48 | - |
| Group K | 9 | 9 | 9 | 9 | 8 | 8 | 8 | 8 | - |
| Group F | 140 | 134 | 129 | 123 | 118 | 106 | 87 | 68 | - |
| Group B | 295 | 286 | 265 | 244 | 227 | 201 | 166 | 92 | - |

Eighty percent of Group N were still alive after 15 years follow up and a similar proportion (85%) was seen in Group K. Groups B and F had a worse survival with 50%

of Group F surviving at 15 years and 25% of Group B and both groups converged over time. Figure 5.2 shows the Kaplan Meier survival curves for the groups using TLcoSR and FEV₁/FVCSR.

Figure 5.2

Estimated Kaplan Meier survival plots for the subjects in the four groupings using TLcoSR and FEV₁/FVCSR. Group N had both TLcoSR and FEV₁/FVCSR normal, Group T only TLcoSR was abnormal, Group F only FEV₁/FVCSR was abnormal and in Group B both were abnormal. The absolute patient numbers surviving at 2 year intervals are shown at the bottom of the figure.



| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|----|---|
| Group N | 59 | 59 | 59 | 59 | 56 | 56 | 54 | 50 | - |
| Group T | 10 | 9 | 9 | 9 | 6 | 6 | - | - | - |
| Group F | 127 | 123 | 122 | 118 | 112 | 105 | 91 | 82 | - |
| Group B | 301 | 290 | 265 | 244 | 228 | 198 | 159 | 85 | - |

Eighty five percent of Group N and 65% of group F were alive at 15 years. Groups T and B had a similar survival at 12 years (60%) but due to small numbers there were no data for group T after 12 years. Only 20% of group B were alive at 15 years. There was a very different survival between groups K and T with group K having a better survival than group T.

Cox regression models were derived using the 4 phenotypes that we have previously defined. Firstly when the groups were defined using Kco only, groups F and B had a significant hazard ratio for death 2.6 (95% Confidence Limit (CL) 1.0 to 6.8) and 3.6 (95% CL 1.5 to 8.9) respectively. When the Cox regression models were derived using TLco a better fitting model was found (chi-squared value increased from 19.4 to 36.4) and groups T and B were the only groups to have a significant hazard for death at 9.0 (95% CL 1.5 to 54.4) and 6.4 (95% CL 2.0 to 20.3) respectively.

Table 5.3 illustrates the results of the univariate Cox regression models for predicting the survival of the subjects from the date of the first complete lung function tests.

Table 5.3

| Index | Num | χ^2 | p value | HR | (95%CL) | Harrell's C |
|-----------------------------|-----|----------|----------|------|----------------|-------------|
| VA%TLC | 523 | 48.5 | p<0.0001 | 1.73 | (1.47 to 2.04) | 67.8 |
| TLcoSR | 530 | 42.1 | p<0.0001 | 1.65 | (1.41 to 1.93) | 66.3 |
| Activity SGRQ | 508 | 48.4 | p<0.0001 | 1.75 | (1.48 to 2.07) | 66.3 |
| Total SGRQ | 503 | 43.9 | p<0.0001 | 1.70 | (1.44 to 2.00) | 65.3 |
| FVCSR | 530 | 26.7 | p<0.0001 | 1.50 | (1.28 to 1.77) | 63.3 |
| Impacts SGRQ | 504 | 35.1 | p<0.0001 | 1.61 | (1.37 to 1.90) | 63.2 |
| FEV1SR | 530 | 26.0 | p<0.0001 | 1.48 | (1.27 to 1.73) | 62.9 |
| LZ910 | 300 | 25.0 | p<0.0001 | 1.57 | (1.31 to 1.88) | 61.9 |
| FEVRSR | 530 | 21.9 | p<0.0001 | 1.43 | (1.22 to 1.66) | 61.6 |
| KcoSR | 530 | 19.4 | p<0.0001 | 1.41 | (1.20 to 1.64) | 61.3 |
| Age | 530 | 24.8 | p<0.0001 | 1.49 | (1.27 to 1.74) | 60.0 |
| UZ910 | 300 | 15.9 | p=0.0001 | 1.43 | (1.19 to 1.71) | 59.9 |
| Symptoms SGRQ | 508 | 9.0 | p=0.0027 | 1.25 | (1.08 to 1.45) | 56.6 |
| δ FEV1 mls/Yr | 351 | 2.0 | p=0.1536 | 1.15 | (0.95 to 1.40) | 55.6 |
| δ TLco mmol/min/kPa | 328 | 0.9 | p=0.3402 | 1.11 | (0.89 to 1.38) | 53.7 |
| PackYrs | 530 | 2.1 | p=0.1431 | 1.12 | (0.96 to 1.30) | 53.7 |
| δ Kco mmol/min/kPa/L | 334 | 0.8 | p=0.3721 | 1.10 | (0.89 to 1.35) | 52.7 |
| Male sex | 530 | 0.1 | p=0.7287 | 0.94 | (0.67 to 1.33) | 49.3 |

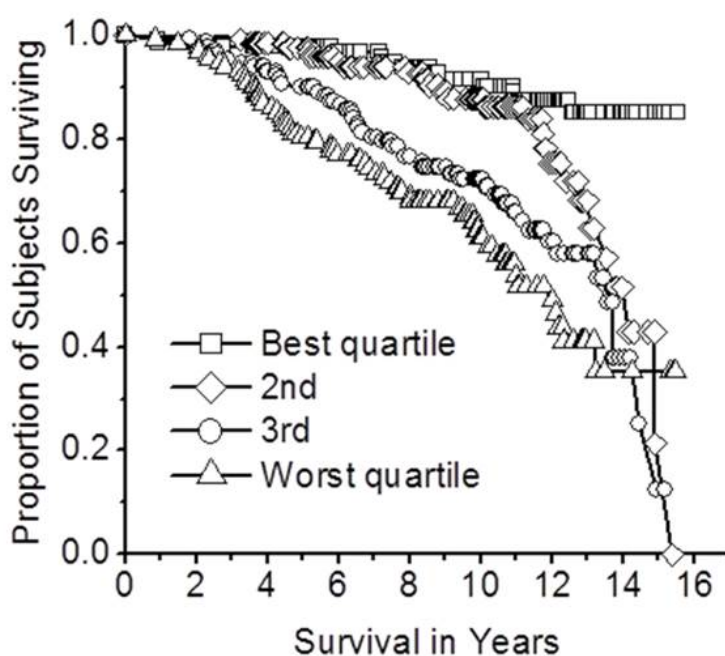
The table shows the chi-square values for the univariate Cox regression for predicting survival from the date of the first (baseline) lung function tests as well as the hazard ratio (HR) for all-cause mortality, its 95% confidence limits and is ordered using the Harrell's C statistic which is the percentage of agreement between the actual and predicted deaths. All the indices were expressed as quartiles so HR could be directly compared.

VA%TLC was the strongest predictor of subsequent survival as illustrated by the highest Harrell's C of 67.8% followed by TLcoSR, then the activity domain of the SGRQ, total SGRQ score followed by FVCSR. TLco decline, smoking pack years, Kco and FEV₁ decline and male sex were not significant contributors to the model (chi-squared values <2.2). TLcoSR was better at predicting survival compared to KcoSR (Harrell's C 66.3%

and 61.3% respectively). All the univariate predictors were divided into quartiles to explore the relationship with mortality further. Figure 5.3 shows the Kaplan Meier survival curves for quartiles of VA%TLC on entry to the study. Subjects in the best 2 quartiles of VA%TLC behave similarly over the first 12 years but subsequently deviate.

Figure 5.3

Estimated Kaplan Meier survival plots for the subjects according to quartiles of VA%TLC on entry to the study with the best quartile (\square), 2nd quartile (\diamond), 3rd quartile (\circ), worst quartile (Δ) plotted separately. The absolute patient numbers surviving at each 2 year interval are shown at the bottom of the figure.



| | | | | | | | | | |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|---|
| Best quartile | 128 | 125 | 125 | 124 | 119 | 112 | 109 | 109 | - |
| 2nd | 128 | 128 | 125 | 120 | 114 | 110 | 87 | 27 | 0 |
| 3rd | 129 | 123 | 116 | 104 | 97 | 86 | 76 | 16 | - |
| Worst quartile | 128 | 120 | 103 | 95 | 86 | 68 | 52 | 44 | - |

Table 5.4 illustrates the best two multivariate Cox regression models for predicting mortality from the date of the first complete lung function test.

Table 5.4

| | Quartile | Mean | (SD) | Model 1 | | Model 2 | |
|---------------|----------|------|--------|---------|-----------------|---------|-----------------|
| | | | | HR | 95%CL | HR | 95%CL |
| VA%TLC | Best | 93.9 | (4.0) | 1 | | | |
| | 2nd | 84.9 | (2.2) | 1.54 | (0.76 to 3.13) | | |
| | 3rd | 78.0 | (2.0) | 2.63 | (1.36 to 5.09) | | |
| | Worst | 66.4 | (6.2) | 3.42 | (1.75 to 6.66) | | |
| TLcoSR | Best | -0.1 | (0.8) | | | 1 | |
| | 2nd | -1.6 | (0.3) | | | 2.45 | (1.16 to 5.17) |
| | 3rd | -2.5 | (0.2) | | | 4.38 | (1.99 to 9.64) |
| | Worst | -3.6 | (0.6) | | | 5.44 | (2.21 to 13.42) |
| KcoSR | Worst | -3.6 | (0.6) | | | 1 | |
| | 3rd | -2.3 | (0.2) | | | 1.11 | (0.65 to 1.90) |
| | 2nd | -1.5 | (0.3) | | | 0.96 | (0.50 to 1.84) |
| | Best | 0.0 | (0.8) | | | 2.50 | (1.12 to 5.56) |
| Activity SGRQ | Best | 24.1 | (14.6) | 1 | | 1 | |
| | 2nd | 54.9 | (7.0) | 3.09 | (1.33 to 7.17) | 2.68 | (1.20 to 5.98) |
| | 3rd | 76.9 | (5.9) | 3.80 | (1.66 to 8.69) | 3.61 | (1.65 to 7.89) |
| | Worst | 93.9 | (4.4) | 4.96 | (2.16 to 11.37) | 4.78 | (2.19 to 10.43) |
| Age | Youngest | 37.1 | (4.8) | 1 | | 1 | |
| | 2nd | 47.4 | (2.1) | 1.31 | (0.73 to 2.35) | 1.36 | (0.76 to 2.43) |
| | 3rd | 54.2 | (2.1) | 1.67 | (0.95 to 2.94) | 1.65 | (0.94 to 2.90) |
| | Oldest | 64.3 | (4.8) | 2.41 | (1.38 to 4.22) | 2.37 | (1.35 to 4.15) |

Results for the best two multivariate Cox regression models for predicting mortality from date of first lung function tests using quartiles of VA%TLC, TLcoSR, KcoSR, age and activity SGRQ. For each model the hazard ratio (HR) for death and its 95% confidence limits (95%CL) are shown as well as the mean SR value (SD) in each quartile of the indices. Harrell's C statistic was 72.0% for Model 1 and 71.9% for Model 2.

The models both used the activity domain of the SGRQ and age and then either VA%TLC, TLcoSR or KcoSR. Model 1 had a similar Harrell's C statistic 72.0% compared to 71.9% in model 2. In model 2 the hazard ratio for the TLcoSR quartiles increased as the mean TLcoSR in each quartile decreased but the opposite was seen when KcoSR was used. So the best KcoSR quartile (highest mean KcoSR value) had the worst hazard ratio (2.66 (95% CL 1.15 to 6.15)). The survival was similar between the other 3 quartiles using KcoSR. The apparent paradoxical survival disadvantage of a higher (better) KcoSR in A1AD was only seen after TLcoSR was taken into account in the models for each subject.

To further explore these findings we compared the number of subjects and the number who died when grouped as TLcoSR quartiles and KcoSR quartiles (see Table 5.5). When the subjects were grouped by TLcoSR quartiles the mortality increased step-wise as the TLcoSR decreased (from 12.8% in the best group to 40.9% in the worst group).

Table 5.5

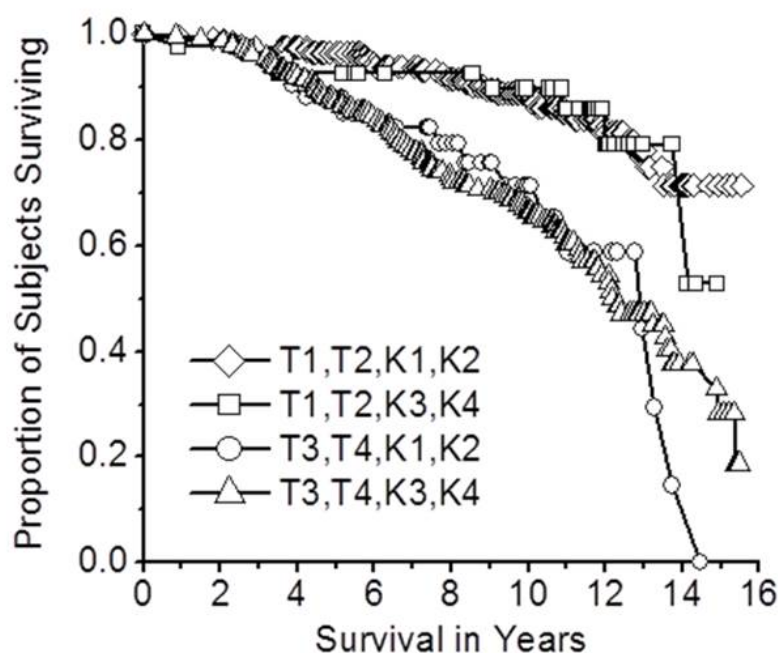
| | | KcoSR quartiles | | | | | |
|------------------|-----------|-----------------|-------|---------|---------|----------|-----------|
| TLcoSR quartiles | | Best | | 2nd | 3rd | Worst | Mortality |
| | Best | 106 (16) | 14.3% | 23 (1) | 4 (0) | 0 (0) | 12.8% |
| | 2nd | 21 (6) | | 74 (9) | 35 (7) | 2 (0) | 16.7% |
| | 3rd | 5 (3) | 39.0% | 32 (11) | 73 (25) | 23 (8) | 35.3% |
| | Worst | 1 (1) | | 3 (1) | 21 (8) | 107 (44) | 40.9% |
| | Mortality | 19.5% | | 16.7% | 30.1% | 39.4% | |

Table illustrates the number of subjects in KcoSR quartiles and TLcoSR quartiles with the number of subjects in each cell who died shown in brackets. The mortality in each row and column is also shown and in the middle of each quadrant is the mortality percentage for that quadrant.

The mortality increased for the KcoSR quartiles from best to worst but it was not as clear cut in its stepwise increase. The mortality for the worse KcoSR and TLcoSR quartiles was comparable to the worst TLcoSR quartile and best KcoSR quartile (37.9% versus 39.0% respectively). The best 2 quadrants i.e. the best TLcoSR quartiles had a similar mortality irrespective of the KcoSR and these 2 quadrants had a better overall survival than the quadrants which included the worst TLco. To further illustrate this apparent paradox Figure 5.4 shows the estimated Kaplan Meier survival plots for the subjects in the 4 quadrants that were illustrated in Table 5.5.

Figure 5.4

Estimated Kaplan Meier survival plots for the subjects in the four quadrants of Table 5.5: those in the best two quartiles of TLco and Kco (\diamond), those in the best two quartiles of TLco but worst two quartiles of Kco (\square), those in the worst two quartiles of TLco but best two quartiles of Kco (\circ) and those in the worst two quartiles of both TLco and Kco (Δ). The absolute patient numbers surviving at each 2 year interval are shown at the bottom of the figure.



| | | | | | | | | | |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|---|
| T1,T2,K1,K2 | 224 | 221 | 218 | 211 | 205 | 199 | 182 | 161 | - |
| T1,T2,K3,K4 | 41 | 40 | 38 | 38 | 38 | 37 | 32 | 32 | - |
| T3,T4,K1,K2 | 41 | 40 | 36 | 34 | 32 | 29 | 24 | 6 | - |
| T3,T4,K3,K4 | 224 | 221 | 206 | 188 | 162 | 149 | 122 | 84 | - |

The survival for those in the best 2 quartiles of TLco and Kco was 70% at 15 years which was similar to those in the best 2 quadrants of TLco and worst 2 quartiles of Kco

until 14 years of follow-up. A worse survival was seen in all the quadrants with the worst TLco irrespective of the Kco.

5.4 Discussion

This study has illustrated two novel and important findings with respect to COPD clinical phenotypes in subjects with A1AD. Firstly that $VA_{\%TLC}$, which is an index of gas mixing that is derived from the measurements of diffusing capacity, was the best predictor of survival and secondly, that TLco was the better predictor of survival compared to Kco. Previous studies have shown that survival was predicted best by TLco rather than indices of airway obstruction or lung size in the general population (Neas and Schwartz, 1998) and in a broad range of patients from a tertiary lung function dataset (Ward et al., 2012). This study showed that in patients with A1AD, after TLco was taken into account the survival was paradoxically better with lower (worse) Kco values. Monitoring Kco in lung disease can be complex as it can decrease or increase (Hughes and Pride, 2012). For example as COPD and emphysema progresses the Kco may increase if the disease is characterised predominantly by a deterioration in airflow obstruction which then results in a reduction in $VA_{\%TLC}$ as a consequence of poor gas mixing (Hughes and Pride, 2012) or the Kco may decrease if the main effect is loss of alveolar surface area.

We used $VA_{\%TLC}$ in this study instead of VA_{eff} as there are no valid prediction equations which would accommodate for age and sex. $VA_{\%TLC}$ takes in account VA_{eff} by including the subject's TLC and so taking into account size and sex effects. Lower values of

$VA\%TLC$ indicate worse gas mixing as a result of airflow obstruction. In emphysema the total lung capacity increases as a result of gas trapping and accompanied by the presence of airflow obstruction and poor gas mixing the $VA\%TLC$ will reduce and together increases the potential for respiratory failure. In this study the lung volumes were measured using the helium dilution method. In this particular group of patients the TLC may well be larger if measured by body plethysmography which potentially may lead to a greater $VA\%TLC$ signal. As a consequence, monitoring Kco may not be the best way to assess disease progression in A1AD or COPD and therefore defining physiological clinical phenotypes using Kco may be potentially misleading or problematic. A better physiological phenotype definition may therefore be achieved using $TLco$ or $VA\%TLC$.

$VA\%TLC$ takes into account TLC which is measured using the multi-breath or plethysmographic method and VA which is measured using a single breath method (VA_{eff}). In healthy subjects there is a good correlation between TLC and VA_{eff} (Pecora et al., 1968) but lower readings may be produced in patients with airflow obstruction (Rodarte et al., 1976). Roberts et al (1990) measured TLC using single and multi-breath methods along with their ratio (single breath TLC divided by multi-breath TLC) which was used as an index of incomplete gas mixing. This ratio was found to be sensitive and specific for detecting the presence of airflow obstruction and was independent of the absolute volumes. Other techniques have been developed that measure gas mixing efficiency including the lung clearance index (LCI). The LCI is derived from multiple breath washout tests and is a measure of the washout of an inert tracer gas from the lungs during tidal breathing (Horsley, 2009). The index is a measure of the number of

times the lung gas volume at the start of the test is turned over so that the tracer is washed out to below 1/40th of its starting concentration and after each breath the peak of the exhaled tracer concentration is reduced. As the lung disease severity increases the LCI increases. Advantages of LCI include that as it has a narrow range of normal values over a wide age range it is suitable for long term follow-up studies and it is useful in children as it does not involve any complex manoeuvres. It has an advantage over spirometry in that it is a sensitive measure to small airways dysfunction. But a significant disadvantage is that it takes longer to complete (Kraemer et al., 2005) the test compared to spirometry and can take up to 30 minutes in an adult. In addition a good mouth piece seal is essential for the accuracy of the measurement.

In this study TLco was found to be the best predictor of survival with the activity domain score from the SGRQ being the next best predictor which is consistent with other studies (Domingo-Salvany et al., 2002). The activity score in the SGRQ measures the impact of the lung disease on the patients' daily physical activity particularly limited by shortness of breath. The activity domain has been shown to correlate with CT evidence of airway disease in patients with usual COPD (Martinez et al., 2012) but also the CT quantification of emphysema in A1AD (Dowson et al., 2001). Makita et al (2007) studied 274 patients with COPD and characterised the subjects into three clinical phenotypes determined by the severity of emphysema on HRCT assessed visually by three independent pulmonologists. All dimensions of the SGRQ increased as emphysema became more severe, but the activity score remained significantly worse when those with FEV₁ <60% of predicted were compared. The impacts score was also significant

but was a less good predictor of survival in our study. These observations may help us when defining clinically meaningful groups for COPD phenotypes.

Disease progression as measured by decline in FEV₁, TLco and Kco did not predict survival of our population in this study. Despite a mean follow-up of 8.9 years the significant lung function decline may have occurred prior to the entry into the study. 10% of our subjects had lung function within normal range and 19% were non-index which suggests that these subjects in particular may need a longer follow-up period to observe whether lung function decline relates to survival.

There are a couple of limitations to this study. Firstly when a model is defined in a population it ideally should be replicated in a second population group to ensure the indices are valid and reliable. We are unable to do this at the current time as we have used the largest cohort of A1AD in this study and significantly more time would be needed but it could be explored again in the future.

The main limitation of our study is that due to the nature of the referral criteria being uncontrolled, subjects entered at different stages of their disease. There was no bias as a consequence so our results could be applicable to other populations and it only limited the power of the study due to the differing lengths of the follow-up periods. Some of the phenotype groups were small due to the unusual manifestation of the disease but this again did not affect the validity of the main findings of the study.

In conclusion when defining COPD phenotypes it is important that the best and most appropriate indices are used that relate closely to clinical meaningful outcomes for

example survival. In A1AD we found that TLco is better to use rather than Kco for defining the clinical phenotypes and $V_A\%TLC$ and activity SGRQ should be considered for A1AD and COPD phenotypes.

Chapter 6

GENERAL DISCUSSION

There are three main conclusions from this study which will now be discussed more broadly than within the chapters.

6.1 Choosing the best prediction equations for lung function data

When using lung function data in a clinical and research context the results need to be standardised by using reference equations that take into account the effects of sex, age and height. How to select which equations are the most appropriate for a specific population has always been problematic. In the studies reported here I have used a novel approach to confirm that the usual reference equations for our laboratory could be problematic and equations from the US fitted our data better.

Recently there has been progress towards reducing the number of different prediction equations used worldwide. The Global Lung Function Initiative (GLI) was launched in 2008 as a European Respiratory Society Task force (Quanjer et al., 2012b). Its aim was to establish one set of equations for spirometry which could be used worldwide thereby removing the issues of using many different equations such as ethnicity and dealing appropriately with the transition of lung function from childhood through teens to adulthood. These issues have not been completely solved by the GLI equations but are

work in progress. Stanojevic et al (2008) first published the early GLI equations in 2008 based on 3,598 non-Hispanic white subjects ranging between 4 and 80 years old. This Lambda-Mu-Sigma (LMS) method performed well in our study (Ward et al., 2012) but as there are currently no equations for TLco we used the Miller equations (Miller et al., 1983) to ensure we had a coherent set of equations for all lung function indices. The GLI 2012 prediction equations for FEV₁, FVC and FEV₁/FVC followed 4 years later (Quanjer et al., 2012a) and were based on a larger population of 74,187 records (77% Caucasians) across 33 countries with a wider age range (3 to 95 years old) and again used the LMS method. Quanjer et al (2013) have looked at the implications of adopting the GLI 2012 prediction equations. They compared two commonly used sets of prediction equations; National Health and Nutrition Examination Survey (NHANES) III (Hankinson et al., 1999), which is recommended by the ATS, and the European Community for Coal and Steel (ECCS) prediction equations (Quanjer et al., 1993) which are used mainly across Europe. They compared the predicted values to the GLI 2012 equations. NHANES III and GLI 2012 were the best matched for FEV₁ and FVC. Using the ECCS equations the predicted values for both the indices were consistently smaller by 220 to 470mls when compared to NHANES III and GLI 2012. The predicted FEV₁/FVC was also slightly lower for NHANES III and ECCS compared to GLI 2012, as the GLI equations take into account that the FEV₁/FVC ratio reduces with increasing standing height. So using the new equations may slightly increase the prevalence of airway obstruction depending on which prediction equations were previously used by the lung function laboratories (Quanjer et al., 2013). The Global Lung Function Initiative is

now working on prediction equations for TLco and hopefully will in due course tackle equations for static lung volumes.

Our method of using survival analysis to test which equations best fit a population has recently been used for GLI 2012 and predicting survival in the elderly (Miller et al., 2014). GLI 2012 included 'normal' subjects aged over 90 years old and this recent work found that these subjects tend to be 'supra normal' compared to the usual population of subjects in their 10th decade of life. The authors suggested future revision of the GLI equations should consider extending the application of the equations from a younger age group. Our novel methodology may therefore be of help in refining and adapting prediction equations in the future.

This study used the novel approach of survival as an endpoint for determining the most appropriate prediction equation to be used for our analysis. Choosing an endpoint is a critical part of designing a study to ensure that it is powered correctly and that the results adequately answer the hypothesis. Clinical meaningful outcomes can be subjective, objective or include health related outcomes i.e. survival and hospitalisations (de Benedictis et al., 2011). The ideal endpoint needs to be well defined, reliable, interpretable and measurable (Raghu et al., 2012) with minimal risk and expense. Survival is ideal as an endpoint as it relates to lung function and therefore allows the validation of the lung function equations. Several studies have shown that FEV₁ is related to survival in the general population (Peto et al., 1983, Beaty et al., 1982, Schunemann et al., 2000, Chinn et al., 2007) and has been linked with increased mortality in cardiovascular diseases (Lange et al., 1991). FVC has been linked to

survival in respiratory and non-respiratory diseases (Anderson et al., 1988, Olofson et al., 1987) including amyotrophic lateral sclerosis (Czapinski et al., 2006) and TLco has been identified as a predictor of all-cause mortality in a general population without symptomatic respiratory disease (Neas and Schwartz, 1998). Other endpoints that have been used in respiratory clinical trials include hospitalisations, exacerbations, functional status and mortality. Hospitalisations are a hard endpoint but can be influenced by other patient factors such as level of home support and motivation, and collecting the data can be challenging across different hospital trusts due to coding and technical issues. Exacerbations are an important feature in COPD and some studies have shown they affect lung function decline (Donaldson et al., 2002) and mortality (Soler-Cataluna et al., 2005) but others have not found a link with lung function decline (Vestbo et al., 2011). Current smokers in the Lung Health Study had an annual increased rate in FEV₁ decline of 7ml/year for each additional self-reported lower respiratory infection (Kanner et al., 2001). Donaldson et al (2002) found a similar rate of FEV₁ decline (8mls/year) in patients with COPD who had a history of more frequent exacerbations compared to those who had less frequent exacerbations. The ECLIPSE study, on the other hand, showed the baseline FEV₁ was lower in patients with more reported exacerbations in the year prior to the study and exacerbations were only weakly associated with a faster FEV₁ decline during the study (Vestbo et al., 2011). Exacerbations are used commonly as endpoints in COPD clinical trials but the definition of exacerbations is controversial as it is not standardised which means defining exacerbations in the context of a clinical trial may make it difficult to adjudicate.

All-cause mortality, as used in this study, is an accurate endpoint which is precise, reliable and easy to define (as the NHS Central database keeps all records) but if it is to be used in clinical trials the sample size needs to be adequately powered to enable the hypothesis to be answered. This means trials would need to run over a longer duration to achieve any clinically meaningful information (Raghu et al., 2012). Survival is highly correlated with age and this needs to be taken into consideration when interpreting results.

6.2 Spirometric and gas transfer discordance in A1AD: patient characteristics and progression

The lower limit of normal for Kco and FEV₁/FVC was used to define 4 distinct physiological phenotypes in a large cohort of untreated patients with A1AD. The patient's characteristics, demographics, radiology, lung function and quality of life differed between the groups and thought likely to influence disease progression. Longitudinal data was available for up to 11 years and illustrated the greatest Kco decline was in group F and the greatest movement was from group F to B.

The second main finding was that in A1AD there can be discordant lung function. In A1AD Kco was reduced uniformly whereas FEV₁/FVC reduction was bimodal suggesting FEV₁/FVC was affected differentially by other factors including smoking and male sex. Parr et al (2004) illustrated that lower zone emphysema was associated best with reduction in FEV₁ and upper lobe emphysema best with reduction in Kco. In A1AD the

usual initial distribution of emphysema is in the lower zones of the lungs but as the disease progresses and the emphysema extends to the upper zones the Kco becomes increasingly affected. Hereditary A1AD has been shown to present with symptomatic obstructive lung disease at the mean age of 32 – 41 years in smokers (ATS/ERS, 2003) and is rarely seen under the age of 25 years. There have been case presentations of teenagers with emphysema (Griese and Bruggen, 2009) but most of the observations of early childhood progression in A1AD have been based on the Swedish newborn screening programme which has monitored 127 PiZZ subjects since birth (1972-1974) with annual liver and lung function tests. In the first 2 decades the spirometry remained normal (Sveger et al., 1994). CT densitometry was performed at the age of 32 and compared to age-matched controls and no emphysema was observed (Bernspang et al., 2011). In more recent work Holme et al (2013) observed the natural history of spirometry, gas transfer, health status and CT densitometry in 591 A1AD ZZ deficient patients using data observation and logistic regression models. The aim of the study was to identify when the parameters were consistently worse than a healthy population. For non-index patients the gas transfer and health status deviated from normal at around 16 years of age, upper zone densitometry and FEV₁/FVC at the age of 29 and lower zone densitometry and FEV₁ at 37. A similar order of decline was observed in never smokers but at an older age. Further longitudinal studies of these subjects will help better understand the natural progression of lung function, in particular the development of emphysema and obstructive lung disease with associated factors.

As discussed in the Introduction (Section 1.3.6) there has been much controversy about the spirometric definition of airflow obstruction. Supporters of the fixed ratio feel that it is simple to use and pragmatic (Mannino et al., 2007) and is independent of reference equations but the fixed ratio does not take into account the age related decline in the absolute ratio or the FEV₁/FVC difference between the sexes. More recent work has looked at the group who are termed the discordant group. The discordant group are those defined as having COPD by the fixed ratio (FEV₁/FVC<0.7) but above the lower limit of normal. Mannino et al (2007) studied 5201 subjects over the age of 65 years from the Cardiovascular Health study. They observed that the adjusted risk of death in the discordant group was 1.3 and the adjusted risk of COPD hospitalisation 2.6 which were both higher than the healthy cohort with no symptoms, normal spirometry and a similar age. But both these risks were less than those whose FEV₁/FVC was below normal defined by LLN and fixed ratio <0.7. Unfortunately there were no causes of death available in this paper to help understand whether patients were dying from respiratory or non-respiratory causes in the discordant group. Mannino and Diaz-Guzman (2012) looked at this discordant group again but causes of death were now available. As before they showed the discordant group (obstructed by fixed ratio and normal by LLN) had an increased risk of death (1.46) which was lower than those with airflow obstruction by both methods. The discordant group had an increased proportion of patients dying due to cardiovascular disease but less respiratory deaths than the airflow obstruction group by LLN and fixed ratio. Lamprecht et al (2011) found the discordant subjects were older, more likely to be male and never smokers and had less severe FEV₁. The discordant group also had higher reported levels of heart disease (after adjusting for age) and less

cough and phlegm than those with normal lung function. But 81% of this discordant group were male compared to 48.7% of the concordant group and this sex difference was not taken into account which is important as it is known that males are more likely to suffer from heart disease (Miller, 2012). Regan et al (2010) looked at several different COPD phenotypes and showed the discordant group had a higher rate of reporting mild respiratory exacerbations and more emphysema on CT (4% versus 2% in smokers with normal spirometry). They concluded that LLN misses patients who have respiratory symptoms with emphysema who may benefit from early diagnosis and management. These respiratory exacerbations were not verified in the medical notes so it is unclear whether they were as a result of exacerbations of COPD, heart failure or viral infections for instance. It is important to determine the cause for these deteriorations (Enright, 2014). The concern expressed in an editorial by Enright (2014) highlighted the potential danger of treating the discordant group with inhalers with potential side effects particularly in those with heart disease (Singh et al., 2011) and the only effective intervention that can be offered early in the diagnosis of COPD is smoking cessation.

There is much uncertainty about the term 'phenotype' especially in the field of medicine. 'Phenotype' is classically defined as the observable structural and functional characteristics of an organism that are determined by the combined influence of genotype and environment (Rice et al., 2001). There is increasing interest in phenotypes particularly in COPD but it is essential that phenotypes are defined appropriately. Han et al (2010) proposed the definition of phenotypes in the context of COPD as "a single or combination of disease attributes that describe differences between individuals with

COPD as they relate to clinically meaningful outcomes.” Clinically meaningful outcomes include symptoms, exacerbations, response to treatment, prognosis and death. This definition ensures that phenotypes have unique therapeutic and prognostic characteristics that define the management of the patient in the clinical and research context. In this body of work the physiological phenotypes did have differing symptoms as well as prognosis and mortality. The exacerbation data were only available for a subgroup of the dataset described here and no differences were observed. Further work is required to explore possible differences in exacerbation rates between these 4 main phenotypes.

Unfortunately there is currently no NICE approved treatment for A1AD in terms of alpha-1-antitrypsin replacement. The main stay of management is as for usual COPD with pharmacological treatment including inhaled corticosteroids, long acting B2 agonists etc. and complemented by non-pharmacological treatment including pulmonary rehabilitation. Current pharmacological therapy for each subject on the A1AD dataset was not collated to explore treatment differences at baseline between the physiological groups.

What is the evidence that the groups are subtypes and not just differences in severity? This current study along with several previous studies has shown that CT distribution is different between the groups and relates to physiology which alone suggests different subtypes (Parr et al., 2004, Holme and Stockley, 2007). Longitudinal data has shown that there is some movement between specific groups (for instance Group F to B) but some do not seem to change so it cannot be assumed that there is merely a progression

of severity between the groups. In addition the age of individuals in F and B was similar, the average FEV₁ for groups F and B were also similar as was the FEV₁ decline in patients in group F who stayed in group F and those in group F who moved to B. Whereas the group who moved from F to B had a much more rapid decrease in Kco, not a sequential one, suggesting it is a disparate progression not just the natural progression affecting everyone.

Overall group K only contributed 1.5% (8/530) of the patient cohort. As a consequence it is difficult to determine whether these subjects are a separate group in their own right or whether it is an extension of group N. To define abnormality for the lung physiological indices the LLN was used. The LLN is an estimate of the lower 5th percentile within a population and so this approach implicitly introduces a possible 5% false positive rate when applied to a 'normal' population. In this A1AD dataset 26 patients might be misclassified wrongly as abnormal using KcoSR if they were from a 'normal' population and so this inherent false positive rate may account for some of the patients in group K. When compared to group N, group K had similar physiology (in terms of airflow obstruction (FEV₁ and its ratio) both groups were within the normal range as defined by LLN), demographic and clinical parameters but due to small numbers a Type 2 error may have contributed to the failure to reject the null hypothesis. However it is important to continue to observe these patients as they may provide unique data on the early evaluation of lung disease in A1AD.

6.3 Improving the definition of lung function COPD phenotypes in A1AD

The third main finding was that $V_A\%TLC$, a measure of gas mixing, was the most important predictor of survival in our population. Gas mixing occurs in the terminal airways and during breath holding the dead space volume reduces which reflects the gas mixing between the alveolar and conducting airways. The mechanism of gas mixing is unclear. It was initially thought to be due to molecular diffusion (Engel et al., 1973) but this is not thought to be the only explanation. Engel et al (1973) observed that the heart may contribute to gas mixing as a consequence of the cardiac impulse. The alveolar volume (V_A) measures the lung volumes that are 'accessible' to the gas exchange surface in the alveoli (Hughes and Pride, 2012). Volume measurements made using the single breath and multi-breath technique correlate very well in normal subjects (Pecora et al., 1968, Hamer, 1962) but this correlation declines when there is increased airflow obstruction as a consequence of ventilation distribution inhomogeneity. The multi-breath washout method measures nitrogen elimination during the breathing of oxygen and is performed with relaxed breathing and without the need for a forced effort. So this test is potentially feasible within all age groups but is time consuming. The single breath measurement of alveolar volume involves breathing in a mixture of a tracer gas to TLC and breath holding for 9 seconds. Then on expiration the first 500-1000ml is discarded, this is from the dead space that is not involved with gas exchange, and then gas is sampled from the alveolar plateau part of expiration when the sample is from the alveoli which are the main site of gas exchange. The differences between the single and multi-breath method for measuring TLC and RV have been defined in differing ways, which

include the ratio (TLCr and RVr) and the absolute difference of between both methods (TLCd and RVd). Roberts et al (1990) studied 179 non-smokers, 100 asthmatics and 100 COPD patients to explore the normal ranges and within subject reproducibility of these 4 variables. TLCr was overall the best variable as it was independent of the absolute volume measured, had better reproducibility and there was a single normal range for both sexes. There was also no significant dependence on the age, height or weight of the subjects. Our study has used VA%TLC, which is internally standardised and therefore does not require prediction equations, to explore gas mixing further and has shown it was the best predictor of survival.

6.4 Limitations and Generalisability

6.4.1. Population

A1AD is estimated to account for between 1 and 2% of the total number of people who are diagnosed with COPD (DeMeo and Silverman, 2004). Further work needs to determine whether the physiological phenotypes used in this study are valid in usual COPD. One advantage for using patients with usual COPD would be the potential for a much larger dataset thereby enabling more hypotheses to be proposed and potentially answered. The mean age of the population in this study was 50.4 ± 0.76 SEM in females and 51.0 ± 0.57 SEM in males. In usual COPD the subjects are more likely to be older (Tidy, 2014) therefore it would be essential to use the lower limit of normal for establishing the phenotypes as it takes into account the subject's age. But also the

timespan for monitoring of lung function progression in usual COPD would be shorter than for A1AD as they are older on diagnosis which may impact the observation of movement between the phenotypes.

The distribution of emphysema in A1AD is typically seen in the lower zones of the lungs (Eriksson, 1964) but in usual COPD it is mainly in the upper zones. Parr et al (2004) showed that A1AD subjects with emphysema predominantly in the lower zones of the lungs had a greater amount of airflow obstruction and if the emphysema was in the upper zones a trend towards a worse Kco. As a consequence of the differing emphysema distribution between usual COPD and A1AD there may also be a different subject distribution across the physiological phenotypes in usual COPD.

When considering the 4 physiological phenotypes in usual COPD no patients would be grouped in Group N or K as these groups have a normal FEV₁/FVC by either definition (FEV₁/FVC ≥ 0.7 or $\geq \text{LLN}$) so therefore technically do not have COPD. So it would be more appropriate to repeat this phenotype study in patients with confirmed emphysema on CT who do not have A1AD. This would enable a large dataset to be collated and all the phenotypes are likely to be appropriate.

6.4.2 Methods

One limitation of the studies within this body of work that included patients with A1AD is the possibility of selection bias. In our dataset 81% of subjects were identified as a consequence of reporting respiratory symptoms or evidence of disease and 19% were

identified from family screening. There were only a few patients identified by presentation with liver disease and due to the low numbers they were excluded from the dataset. To be recruited to the ADAPT programme a referral is required from a chest physician or general practitioner who is aware of the programme. So the dataset is unlikely to be a true representation of the whole A1AD population. Despite the World Health Organisation (1997a) recommending that all patients with COPD and adult onset asthma should be assessed for the presence of A1AD and that the early detection increases awareness of the damages of smoking and environmental pollution (Sveger et al., 1997) there is currently no population screening for A1AD. The ATS/ERS taskforce (2003) have published evidence based recommendations for screening but unfortunately financial factors and other controversies have obstructed the launching of large scale screening programmes in the United States and UK. If screening is initiated in the future this would lead to the identification of increasing numbers of group N patients (normal FEV₁/FVC and Kco) and would enable the observation of this group over a longer period with respect to physiology and movement between the phenotypes.

A further limitation in this study is that the subjects have been entered into the study at different stages in their disease. Therefore we do not know the exact 'disease journey' the subject's had undergone prior to entering the study and being allocated to one of the physiological groups. For example someone with supra-normal lung function on completing their lung development around the age of 25 may have deteriorated a lot but still be above the LLN whereas someone with less good lung function development may have deteriorated much less but now be below the LLN and so allocated to a different

phenotype. Twelve years of longitudinal data were available which has enabled the observation of some movement between the groups. It would be essential to observe these groups over a longer period to explore further how these groups move over time.

6.4.3 Analysis

The physiological phenotypes used in this thesis were determined by previous studies detecting differences between lung function parameters and distribution of emphysema on CT (Parr et al., 2004, Holme and Stockley, 2007). An alternative approach would be to apply cluster analysis to find the discrete groupings using the physiological indices. Cluster analysis is a set of statistical methods used to define groups within a population based on measured characteristics. The groups are defined by the differences or similarities within the groups or clusters (Weatherall et al., 2010). Cluster analysis is used mainly for generating rather than testing hypothesis (Everitt, 1993). There are several different considerations that need to be taken into account before cluster analysis is performed; firstly the selection of the individuals or population needs to be carefully considered. If the population has too many differences then the number of clusters may be small with many outlying subjects which is unlikely to indicate a clinically meaningful disease and if the population is too similar the results may be misleading (Weatherall et al., 2010). The ideal scenario would be to have a randomly selected population. Secondly, when membership of the test population is pre-determined then the variables that will be used to define the clusters need to be considered carefully.

This is crucial to ensure appropriate and clinically relevant variables are included and to avoid collinearity. It has been suggested that around 10 variables are an appropriate number to be included in the analysis (Weatherall et al., 2010). As cluster analysis is determined using statistics it has less bias susceptibility apart from the initial decision of the inclusion variables.

Cluster analysis is fraught with mainly different methods available at each stage of the analysis; for instance there are an infinite numbers of measures for detecting similarity or differences between the subjects thereby the meaning of the clusters may be different depending on the method used (Everitt, 1993). The inclusion of different indices with different scales would also make comparing data more difficult.

There has been much interest in cluster analysis in COPD as it is thought to be a good tool for studying heterogeneous disease (Weatherall et al., 2010). There have been several cluster analyses published in COPD (Wardlaw et al., 2005, Weatherall et al., 2009, Burgel et al., 2010). These studies used different population sources and different cluster analyses methods thereby resulting in cluster groups which were not comparable. This makes validating and reproducing the findings in a clinical setting more challenging as all the groups will be very diverse.

6.5 Future Experiments

With the increased interest in identifying biomarkers in COPD further work is required to determine whether there are other biomarkers (apart from lung function indices) that

relate specifically to these different phenotypes. In A1AD Carter et al (2011) showed that A- α -Val360, a neutrophil elastase specific biomarker, related to COPD disease activity and treatment in A1AD patients. Further work is required to explore whether there are differences between the physiological phenotypes in terms of this biomarker and whether these differences would identify those who may benefit from A1AD replacement therapy.

Further work is needed to explore whether there are differences between the physiological groups in terms of underlying biological mechanisms. There seems to be a difference in how FEV₁ and Kco progress over time in A1AD with a bimodal distribution in FEV₁ compared to a normal distribution in Kco. The imbalance in anti-protease/protease caused by A1AD is thought to contribute to the general parenchymal damage and male sex and smoking to decline in airflow obstruction. Understanding this better may help target specific therapies to each of the physiological phenotypes.

Phenotypes should have a 'real predictive value' (Han et al) and whilst this work has shown differences in the rate of disease progression and symptoms between the phenotypes there were no statistical differences in the percentage of patients who died between the phenotypes. The predictions of prognosis or mortality are an important element of a clinically applicable phenotype. Our work has shown that TLco is a better predictive of survival than Kco and so further work is required to look at the physiological phenotypes defined using FEV₁/FVC and TLco instead of Kco. It would be important to explore the differences in the demographics, clinical characteristics and radiology but also mortality rates as well. The final results chapter showed that VA%TLC, a measure of

gas mixing, was the overall best predictor of survival so future work on phenotype definition should consider using FEV_1/FVC and $VA\%TLC$ thereby studying subjects with airflow obstruction but also evidence of poor gas mixing. Defining the physiological phenotypes in these 2 different ways may give added predictive value concerning mortality that is expected of a phenotype.

Several studies have shown that spirometric measurements are associated with all-cause mortality independent of smoking (Hole et al., 1996, Bang et al., 1993, Lange et al., 1990). $TLco$ has also been shown to be a predictor of all-cause mortality independent to spirometry and respiratory symptoms (Neas and Schwartz, 1998). Further work is needed to explore whether $VA\%TLC$ predicts all-cause mortality independent to gas transfer or spirometry and whether it is potentially an early marker of lung disease prior to observed changes in spirometry and gas transfer. In those who have confirmed lung disease could $VA\%TLC$ predict a sub-group who are more likely to go on to develop respiratory failure at an earlier stage in their disease thereby indicating earlier or more aggressive intervention? Specifically more work is needed to explore whether $VA\%TLC$ is as good a predictor of survival in usual COPD as was found in my study. In addition whether it correlates with symptoms and quality of life in A1AD and usual COPD and whether pharmacological and non-pharmacological treatment can be tailored for the patient depending on the $VA\%TLC$. Finally figure 5.3 suggests that at a certain time point there is a sudden worsening in survival for those in the 2nd best quartile of $VA\%TLC$ which needs further exploration to understand the underlying mechanisms and whether timely therapeutic intervention could influence the outcome.

6.6 Conclusion

In conclusion discordant lung function enabled the observation of 4 distinct physiological phenotypes in A1AD. This included identifying a subgroup that had a faster Kco decline which may indicate an appropriate future target for A1AD replacement therapy. Our study has illustrated that it is essential to use the most appropriate reference equations for the study population otherwise subjects may be wrongly interpreted as having worse lung function which has an impact on the determination of prognosis. Finally it is essential to include lung function indices that best reflect the most appropriate endpoint. This ensures the phenotypes are as clinically robust and meaningful as possible.

APPENDIX 1

Papers and Abstracts published related to this thesis

Validation of lung function prediction equations from patient survival data

Ward H, Cooper B and Miller MR ERJ 2012; 39:1181-7.

Research article

**Spirometric and gas transfer discordance in alpha-1-antitrypsin deficiency;
patient characteristics and progression**

Ward H, Turner AM and Stockley RA Chest February 2014; Epub ahead of print.

Research article

**Improving the definition of lung function COPD phenotypes in alpha-1 antitrypsin
deficiency**

Ward H, Miller MR and Stockley RA – being prepared for submission

Research article

How survival can help determine which TLco prediction equations to use

Ward H, Miller MR and Stockley RA

Oral presentation ARTP conference 2012

Abstract

**Survival analysis can help determine which TLco prediction equations to use for
patient data**

Ward H, Miller MR and Stockley RA

Oral presentation ERS conference 2011

Abstract

Change in physiological phenotypes in alpha-1-antitrypsin deficiency over time

Ward H, Miller MR and Stockley RA

Oral presentation BTS conference 2010

Abstract

**Alpha-1-antitrypsin deficiency COPD phenotypes are clinically more valid when
defined as lower limit of normal rather than % of predicted**

Ward H, Miller MR and Stockley RA

Poster presentation ERS conference 2010

Abstract

Discordant lung function groups in A1AD

Poster presentation ATS 2010 and COPD 7 conference 2010

Oral presentation ARTP conference 2011

Abstract

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