CLINICAL STUDIES IN ALPHA-1 ANTITRYPSIN DEFICIENCY

by

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ABSTRACT

Four clinical studies in subjects with alpha-1 antitrypsin deficiency were undertaken.

The first examined CT densitometry and health status in 4 groups with discordant pulmonary physiology. It demonstrated that subjects with an isolated gas transfer abnormality had more emphysema, the least basal predominant emphysema and a worse health status than those with normal physiology. Subjects with an isolated spirometry abnormality had the most basal emphysema.

The second study examined the age at which radiological, physiological and health status measures started to deviate from values expected for a normal population, and concluded that CT densitometry and gas transfers measures were the first to deviate from normal, while spirometry was the last.

The third study examined the relationship of γ-glutamyl transferase to physiology, symptoms, mortality and liver disease in alpha-1 antitrypsin deficiency, and demonstrated associations between γ-glutamyl transferase and spirometry, mortality and cirrhosis after correction for associated factors.

The final study described subjects with the PiSZ phenotype compared to matched PiZ subjects. CT revealed emphysema (mainly panacinar) in 46% of PiSZ index and 15% of non-index subjects. Health status was impaired in PiSZ subjects, who had less lower zone emphysema, better health status, pulmonary physiology and symptom profile compared with PiZ subjects.

This thesis is dedicated to my daughter Isabelle.

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LIST OF DEFINITIONS AND ABREVIATIONS

AAT	Alpha-1 antitrypsin
AATD	Alpha-1 antitrypsin deficiency
ADAPT	Antitrypsin Deficiency Assessment and Programme for Treatment
ALP	Alkaline Phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of variance
ARTP	Association for Respiratory Technology and Physiology (UK)
AST	Aspartate transaminase
ATS	American Thoracic Society
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BODE	Body mass index, airflow obstruction, dyspnoea and exercise capacity index
BTS	British Thoracic Society
CI	Confidence interval
CO	Carbon monoxide
CO2	Carbon dioxide
COPD	Chronic obstructive airways disease
CRP	C-Reactive protein
CT	Computed Tomography
CXR	Chest X-Ray

DLCO	Diffusing capacity of the lung for carbon monoxide
DNA	Deoxyribonucleic acid
ERS	European Respiratory Society
FEV1	Forced expiratory volume in one second
FEV1:FVC	Ratio of forced expiratory volume in one second to forced vital capacity
FRC	Functional residual capacity
FVC	Forced vital capacity
GGT	Gamma-glutamyl transferase
GP	General practitioner
GSH	Gamma-glutamyl-cysteinylglycine (also known as glutathione)
GSSG	Oxidised glutathione
HRCT	High resolution computed tomography scan
HU	Hounsfield Units
IC	Inspiratory capacity
IL6	Interleukin 6
IL8	Interleukin 8
IQR	Interquartile range
KCO	Diffusing capacity of the lung for carbon monoxide corrected for alveolar volume
KPa	Kilopascals
LTB4	Leukotriene B4
LZVI	Lower zone voxel index
µM	Micro Molar

MMP	Matrix metalloproteinase
MPO	Myeloperoxidase
MRC	Medical Research Council
mRNA	Messenger ribonucleic acid
NE	Neutrophil elastase
NICE	National Institute for Clinical Excellence
NO	Nitric oxide
PaCO2	Partial pressure of carbon dioxide
Pa02	Partial pressure of oxygen
PAS-D	Periodic acid-Schiff diastase
Perc 15	Fifteenth percentile point
PiM	Protease inhibitor M
PiMM	Homozygous for protease inhibitor M
PiMS	Heterozygous for protease inhibitors M and S
PiMZ	Heterozygous for protease inhibitors M and Z
PiS	Protease inhibitor S
PiSS	Homozygous for protease inhibitor S
PiSZ	Heterozygous for protease inhibitors S and Z
PiZ	Protease inhibitor Z
ROS	Reactive oxygen species
RV	Residual volume
RV:TLC	Ratio of residual volume to total lung capacity
S.D.	Standard deviation
S.E.	Standard error of the mean

SF-36	Short Form 36 Questionnaire
SGRQ	St Georges Respiratory Questionnaire
SMR	Standardised mortality ratio
TLC	Total lung capacity
TLCO	Transfer factor of the lung for carbon monoxide
TNFα	Tumour necrosis factor alpha
U.K.	United Kingdom of Great Britain and Northern Ireland
USA	United States of America
UZVI	Upper zone voxel index
VI	Voxel Index
WHO	World Health Organisation

1. INTRODUCTION

This thesis explores four separate clinical studies based on subjects with the genetic condition alpha-1 antitrypsin deficiency which predisposes to chronic obstructive airways disease[1] and liver disease[2-4].

1.1 Alpha-1 Antitrypsin

Alpha-1 antitrypsin (AAT) is a 394 amino acid glycoprotein coded for on chromosome 14q3-32.1[5]. It is the principal inhibitor of serine proteases, such as neutrophil elastase[6]. Alpha-1 antitrypsin consists of 3 β-sheets and an exposed mobile reactive loop, onto which the target protease docks. After docking, the protease cleaves a peptide bond on the loop, releasing it to move from the upper to the lower pole of the protein, inserting into β-sheet A, thereby 'trapping' and inactivating the protease[7]. This process is depicted in figure 1.1 below.

Proteases are an important host defence mechanism in the lung. The protease, neutrophil elastase, plays a particularly important role by catalysing the breakdown of micro-organisms that can lead to infection in the lung, and in the clearance of necrotic lung tissue[8]. However, if left unopposed, studies have shown that neutrophil elastase can also cause damage to the host lung similar to that seen in chronic obstructive pulmonary disease (COPD) [see section 1.2.2.2.2.3.]. These changes include alveolar destruction leading to emphysema[9], loss of ciliated epithelium[10], squamous cell metaplasia[11], reduced ciliary beat frequency[12] and mucous gland hyperplasia[13]. In health, alpha-1 antitrypsin blocks these harmful effects of proteases.

FIGURE 1.1: The mechanism of protease deactivation by alpha-1 antitrypsin.

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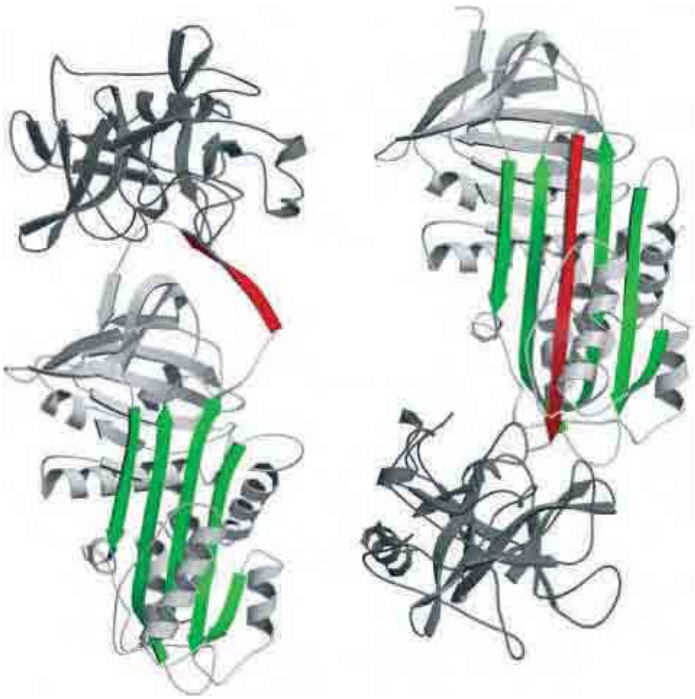
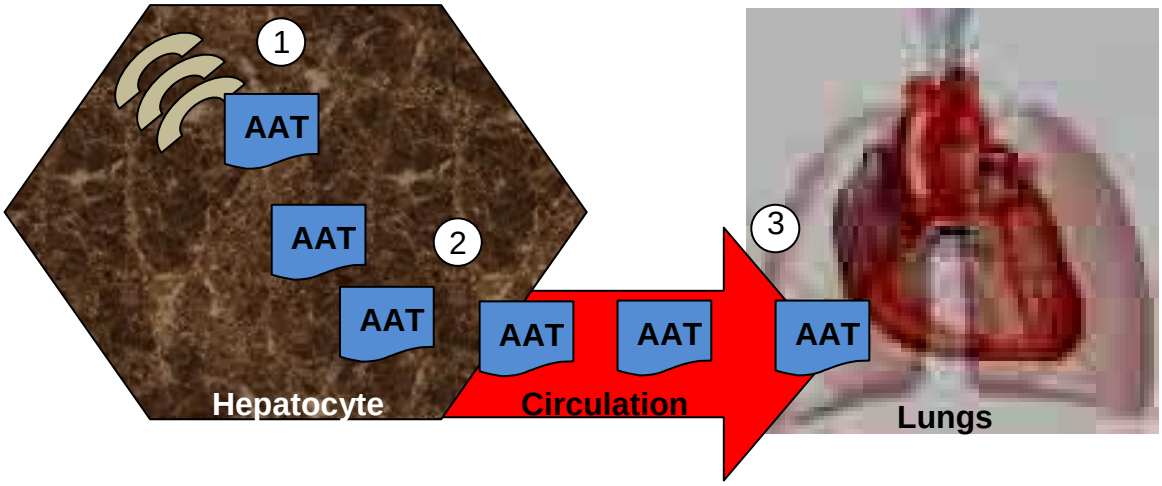


Neutrophil elastase (dark grey) docks onto the mobile reactive loop of alpha-1 antitrypsin (red) and cleaves a peptide bond on the loop (picture on left). The loop moves and inserts into β-sheet A (green), thereby 'trapping' and inactivating the protease (picture on right).

AAT is produced mainly by hepatocytes[14], but small amounts can also be synthesised by macrophages[15], intestinal[16] and respiratory epithelial cells[17]. In subjects who have the usual protease inhibitor M (PiM) type of AAT, newly

synthesised monomeric AAT enters the circulation and is transported to the lung where it diffuses into the tissues and inhibits neutrophil elastase released by migratory neutrophils, thereby minimising the associated tissue damage and hence subsequent development of features of COPD (Figure 1.2).

FIGURE 1.2: The production and dissemination of alpha-1 antitrypsin in a healthy subject with the normal 'M' type of alpha-1 antitrypsin protein.



The normal 'PiM' type of alpha-1 antitrypsin protein is synthesised mainly by hepatocytes (1). The 'PiM' type of alpha-1 antitrypsin protein exists mainly as a monomer and is able to pass out of the hepatocyte and into the circulation through the normal secretory pathway (2). The 'PiM' type of alpha- antitrypsin monomers enter the lung tissue from the circulation by simple diffusion (3) where they inhibit serine proteases.

1.2 Alpha-1 Antitrypsin Deficiency

Alpha-1 antitrypsin deficiency (AATD) was first described in 1963 by Laurell and Eriksson[18]. It is a genetic condition resulting in a major reduction of serum and hence lung concentrations of the protein alpha-1 antitrypsin. AATD is associated with a markedly increased risk of developing chronic obstructive pulmonary disease[1] and liver disease, including neonatal jaundice[2], cirrhosis [3] and primary hepatocellular carcinoma[4].

1.2.1 Genetics of alpha-1 antitrypsin deficiency

More than 100 genetic mutations of AAT have been identified and around 30 of these lead to AATD[19]. Each of these 30 mutations code for an AAT protein that is either abnormal in structure or function, or genes that are not transcribed or translated.

Subjects inherit one AAT allele from each of their parents in a co-dominant manner. The most common allele is M (95% of alleles), and this produces normal levels of circulating AAT[19]. The most common AAT alleles associated with a circulating deficiency of AAT in Caucasians are Z (1-3% of alleles), S (2-3% of alleles) and null (<0.1% of alleles)[20]. Table 1.1 summarises the circulating levels of AAT associated with these genotypes[19].

TABLE 1.1: Typical plasma concentrations of AAT (μM) associated with various genotypes[19].

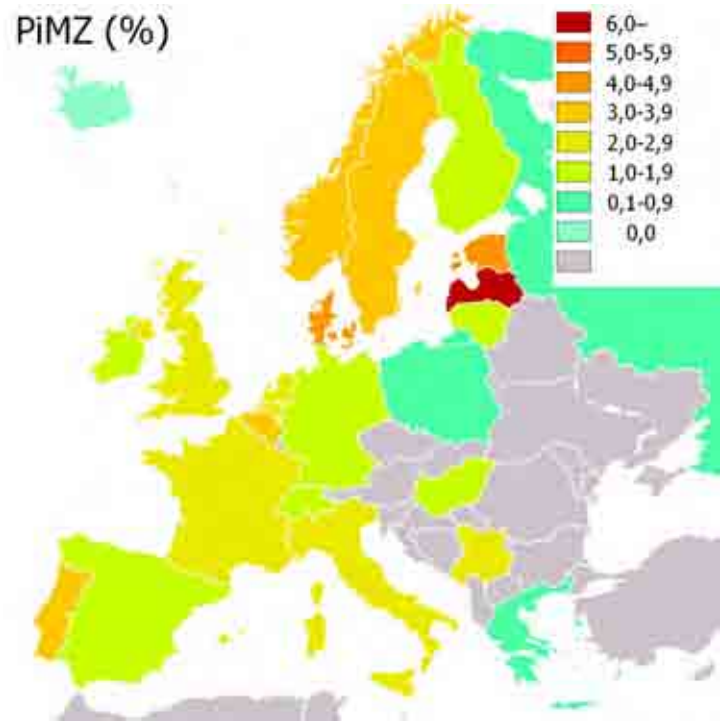
AAT Genotype	AAT level (μM)
MM	20-48
MZ	17-33
SS	15-33
SZ	8-16
ZZ	2.5-7
Null	1.3-3.5
NullNull	0

1.2.2 PiZ Alpha-1 Antitrypsin Deficiency

1.2.2.1 PiZ Alpha-1 Antitrypsin Protein

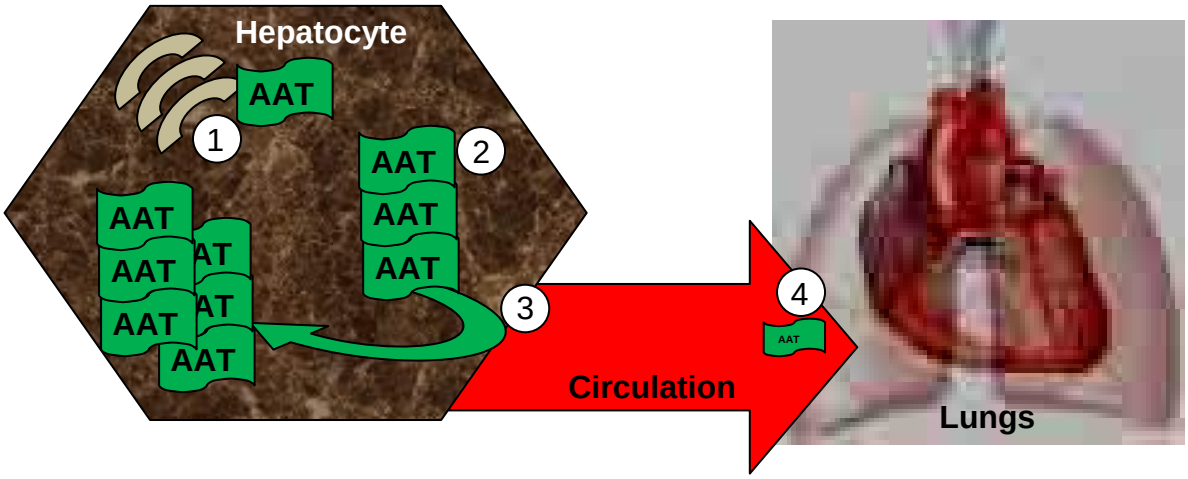
The most common significant genetic variant of AAT in the Caucasian population is the PiZ (protease inhibitor Z) variant. The Z allele is more prevalent in northern Europe, and it becomes less common towards the south west of Europe, as demonstrated in figure 1.3, in relationship to the heterozygous PiMZ phenotype.

FIGURE 1.3: European distribution by country of the PiMZ phenotype of alpha-1 antitrypsin deficiency. The figure illustrates the percentage of the population estimated to have the PiMZ phenotype of alpha-1 antitrypsin in each European country. *(Figure by Olve Utne and reproduced from http://upload.wikimedia.org/wikipedia/commons/0/0d/PiMZ_Europe.png. Creative Commons Attribution ShareAlike 2.5 Licence).*



The PiZ allele occurs as the result of a point mutation causing the substitution of glutamine for lysine in position 342 of the protein[7]. The abnormal PiZ AAT protein is prone to polymerisation after being produced in the liver[21], and as a result it becomes blocked in the terminal secretory pathway of the hepatocytes[22], with only a small percentage reaching the circulation (Figure 1.4).

FIGURE 1.4: Mechanisms by which the PiZ type of alpha-1 antitrypsin causes a deficiency state in the circulation and the lungs.



The abnormal PiZ type of alpha-1 antitrypsin protein is produced in hepatocytes (1). The PiZ alpha-1 antitrypsin protein is prone to polymerisation within the hepatocytes (2). The PiZ alpha- antitrypsin polymers are unable to enter the circulation, and are retained within the endoplasmic reticulum of the hepatocytes (3). This significantly reduces the plasma level and hence the delivery of alpha-1 antitrypsin to the lung (4).

This leads to a subsequent deficiency in the lungs, and consequently neutrophil elastase released by migratory neutrophils becomes relatively unopposed at this site, leading to the pathological changes seen in COPD. Furthermore, the small amount of PiZ AAT that does reach the circulation and subsequently the lung is approximately five times less potent as an inhibitor of neutrophil elastase compared with the normal PiM type of AAT protein[23-26] decreasing the protective role even further.

1.2.2.2. The Respiratory System in PiZ Alpha-1 Antitrypsin Deficiency

1.2.2.2.1. Respiratory System Anatomy

The respiratory system is the site of gas exchange between the circulation and the air. The delivery of oxygen from the circulation to respiring tissues and the removal of carbon dioxide (CO2) from tissues back to the circulation, and subsequently the lung, is critical to support the metabolic needs of cells.

The respiratory system includes airways through which air is transported to and from the lung parenchyma. The airways start at the nasal or oral cavities and include the pharynx, larynx and trachea which splits into the left and right main bronchi. The main bronchi give rise to the segmental and sub-segmental bronchi, and, after some 15 to 25 generations, become bronchioles. Each bronchiole branches into several alveolar ducts, which each lead to an alveolar sac and such a unit is termed the primary pulmonary lobule. Alveolar sacs, alveolar ducts and respiratory bronchioles are surrounded by capillaries which provide the interface through which gas exchange occurs. These capillaries are supplied with deoxygenated blood from the right side of the heart via the pulmonary arteries[27]. The ratio of ventilation of the alveoli to perfusion of capillaries tends to be greater at the apices of the lungs compared with the bases due to a higher basal pulmonary artery pressure as a result of the interaction of gravity with the physiological effects of the cardio-respiratory system[28].

1.2.2.2.2. COPD

AATD is known to be a risk factor for the development of COPD[18], and approximately 1- 4% of subjects with COPD have severe AATD[29-31], whereas 5-10% have at least moderate AATD[29-31]. The definition of COPD differs slightly depending on which national or international guidelines are used. However, all state that COPD is a chronic inflammatory condition characterised by airflow obstruction that is not fully reversible and usually progressive[32-34].

1.2.2.2.2.1. Protease / anti-protease balance in COPD

The protease / anti-protease hypothesis of COPD pathogenesis arose from the observation by Laurell and Eriksson in 1963 that deficiency of alpha-1 antitrypsin was associated with a susceptibility to the development of early onset emphysema[18]. Since this time, many studies in animal models have shown that serine proteases such as neutrophil elastase, proteinase 3 and the cysteine protease, cathepsin B, can reproduce the pathological features of emphysema[13,35-37]. The serine proteases are contained in the azurophilic granules within the neutrophil and are made early in differentiation. Neutrophils are recruited from the vascular compartment into lung tissues in response to chemoattractants such as leukotriene B4 (LTB4) and interleukin-8 (IL8), which are released by alveolar macrophages or respiratory epithelial cells in response to stimuli including cigarette smoke, pollutants or microorganisms[38,39] (Figure 1.5).

FIGURE 1.5: The pathogenesis of COPD – Protease / Antiprotease balance.

(See next page for legend).

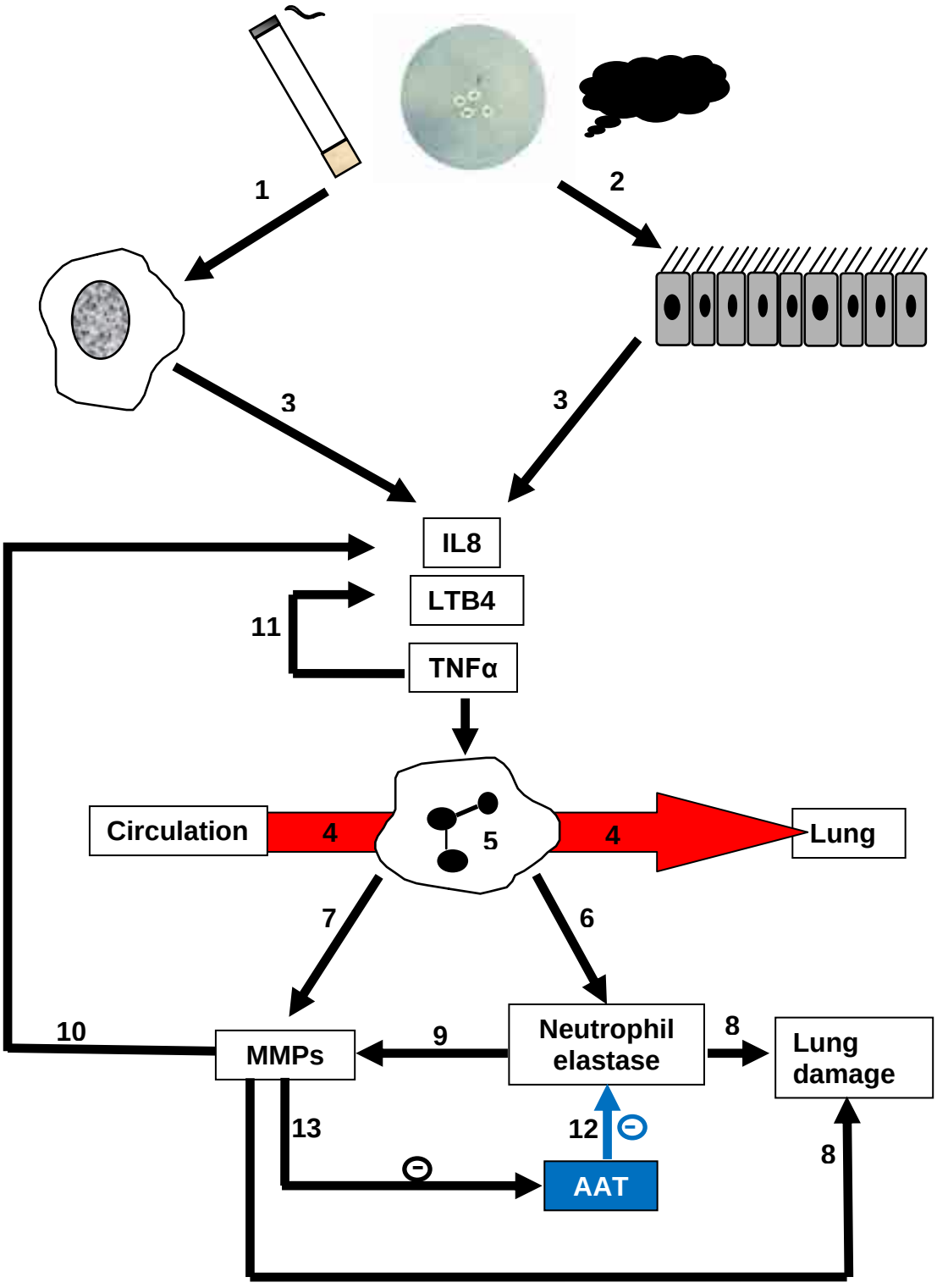


FIGURE 1.5 (Legend): The pathogenesis of COPD – Protease / Antiprotease balance.

Stimuli such as cigarette smoke, microorganisms and air pollution in the airways activate alveolar macrophages (1) and respiratory epithelial cells (2). These cells produce chemoattractants and cytokines such as IL8, LTB4 and tumour necrosis factor alpha [TNFα] (3), which are instrumental in the movement of neutrophils from the pulmonary circulation into the lung parenchyma and airways(4). IL8 and LTB4 activate the neutrophil (5) and lead to the release of proteases such as neutrophil elastase (6) and matrix metalloproteinases [also released by macrophages](7), which cause lung damage such as that seen in COPD (8). Serine proteases further activate MMPs (9), and in turn, MMP (10) and TNF (11) increase and activate proinflammatory cytokines like IL6 and IL8, amplifying the inflammatory process. The effects of serine proteases are blocked by antiproteases such as AAT (12). MMP is also capable of inactivating these antiproteases causing further amplification of the inflammatory process by an alternative pathway(13). The net effect is excessive protease activity leading to the pathological changes of COPD.

TNFα is released from leucocytes, airway epithelial cells and endothelial cells, and is capable of increasing the expression of adhesion molecules on leukocytes and the pulmonary endothelium, thereby aiding migration of the leucocytes into the lung[40]. TNFα also increases levels of many other inflammatory cytokines, thereby potentiating the inflammatory process further[40].

Activated neutrophils release proteases such as neutrophil elastase and matrix metalloproteinase (MMP) from the relevant granules, and these proteases are thought to break down connective tissue (including elastin) in the lung to create a passage through which they can pass until they reach their destination within the lung tissue or airways[41,42]. As proteases dissipate, their concentration decreases as the distance from the neutrophil increases. Protease inhibitors in the tissues form complexes with proteases with a 1:1 ratio, and inhibit their activity. As the distance of the protease from the activated neutrophil increases, the concentration of the protease is reduced to a level that equals that of protease inhibitors such as alpha-1 antitrypsin, and at this point, protease activity is stopped[43]. However, in alpha-1 antitrypsin deficiency, the concentration of the protease inhibitor is much reduced, and therefore the distance over which the concentration of protease exceeds that of its protease inhibitor is greatly increased, leading to a marked increase in the area of tissue destruction which may result in the accelerated development of emphysema[43].

Serine proteases also activate MMPs, which in turn activate proinflammatory cytokines and inactivate protease inhibitors like AAT, further potentiating the inflammatory and hence destructive process[41].

Hence, the balance between protease and anti-protease plays a central role in determining which subjects will develop emphysema[42].

Alpha-1 antitrypsin deficiency is an example of a specific protease anti-protease imbalance. Usual COPD is caused mainly by smoking and it is known that lung tissue in smokers contains a high number of neutrophils which in turn release proteases[44]. In addition the neutrophils of smokers who develop COPD have a greater response to chemoattractants, and a greater ability to destroy lung tissue

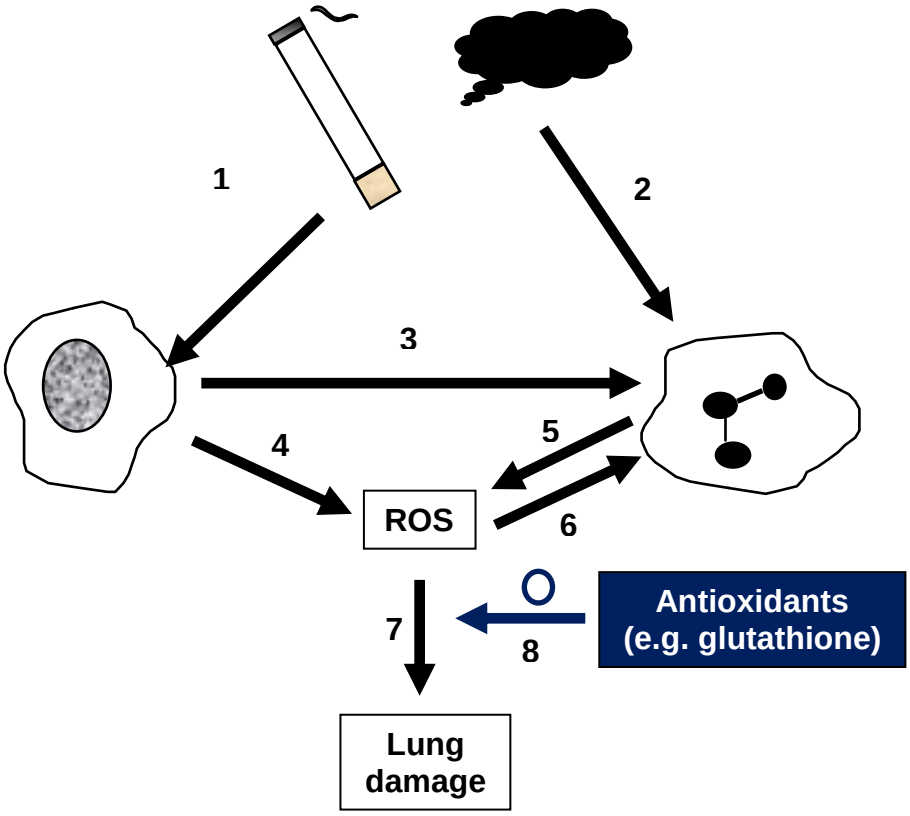
than those without COPD. Therefore, in usual COPD there is also an imbalance between proteases and anti-proteases caused largely by the excessive protease load rather than inhibitor deficiency, but the net effect is similar leading to the development of destructive lung disease[45].

1.2.2.2.2.2. Oxidant /antioxidant balance in COPD

The protease / anti-protease hypothesis described in section 1.2.2.2.2.1. is thought to be a key pathogenic process in COPD. In addition, there is increasing evidence that the balance of oxidants and antioxidants is also important and interrelated, involving leucocytes such as neutrophils and alveolar macrophages (Figure 1.6).

Reactive oxygen species are released from these cells when activated[46], and are directly inhaled in cigarette smoke[47] and as air pollutants[48]. These oxygen species have several effects that can result in tissue damage similar to that seen in COPD. They have been associated with direct tissue damage involving elastin and collagen breakdown[49], cell membrane damage via lipid peroxidation, epithelial cell detachment, and lysis[50]. In addition, they play a role in potentiating inflammation as described below.

FIGURE 1.6: The pathogenesis of COPD – Oxidant / Antioxidant balance.



Cigarette smoke and air pollution deliver oxidants to the lung which in turn stimulate alveolar macrophages (1) and neutrophils in the lung (2), both directly and indirectly via LTB4 and IL8 released by alveolar macrophages (3). Reactive oxygen species are released by activated alveolar macrophages (4) and neutrophils (5). Reactive oxygen species also facilitate further neutrophil migration from the vascular compartment into the lung, which perpetuates the process (6). Reactive oxygen species in the lung cause lung damage directly (7) which, in health, is blocked by antioxidants such as glutathione (8). Again, an imbalance of the oxidants and antioxidants can result in an excess of oxidant activity and hence influence the pathogenesis of COPD.

Neutrophils are relatively large cells, and must be able to change shape in order to pass through the pulmonary microcirculation. Reactive oxygen species are thought to affect the cell exoskeleton and make it more rigid[51]. The neutrophil is not able to 'deform' adequately and therefore passes more slowly through the pulmonary microcirculation[52]. This increases the likelihood that the neutrophil will adhere to the vessel wall and migrate into the lung tissue where it may cause damage as described in section 1.2.2.2.2.1.

Reactive oxygen species in cigarette smoke are also thought to upregulate adhesion molecules and perpetuate this effect further[52-54]. Furthermore, cigarette smoke reduces elastin synthesis and repair, thereby compounding tissue damage[55]. It is also possible that reactive oxygen species are responsible for the reduced activity of antiproteases such as AAT[56,57], although this concept remains controversial.

Various antioxidants are present in the lung, which counteract the effects of the oxidants described above. The most prevalent antioxidants are reduced glutathione (gamma-glutamyl-cysteinylglycine or GSH), mucin, uric acid, albumin and ascorbic acid[58]. GSH is known to be elevated in the bronchoalveolar lining fluid (BALF) of smokers, but this protective response is not thought to be sufficient to counteract the quantity of oxidants present in cigarette smoke[58]. Therefore, in smokers, an oxidant/antioxidant imbalance is thought to exist which will increase the inflammatory processes central to the development of COPD. Levels of the other anti-oxidants are either greater or less than normal in cigarette smokers compared with non-smokers, although the nature of this has not been precisely determined. However, it is thought that some antioxidants may be consumed due to the increased

oxidant load, and others upregulated as a protective response. The balance between oxidants and antioxidants is therefore believed to be important in the pathogenesis of COPD[58], although what influences the 'susceptibility' in this pathway remains unknown.

1.2.2.2.2.3. Pathological changes in COPD

COPD is associated with many pathological changes affecting the airways, parenchyma and vasculature.

Changes in the airways include loss of ciliated epithelial cells[59], reduced ciliary beat frequency, mucous gland hyperplasia[13], increased intraluminal mucus[60], squamous cell and goblet cell metaplasia[11], airway wall thickening resulting from a combination of subepithelial fibrous tissue, airway smooth muscle hyperplasia & thickened fibrotic adventitia[61], and loss of peribronchial alveolar attachments[62].

Emphysema is defined as an 'abnormal permanent enlargement of the airspaces distal to the terminal bronchiole accompanied by destruction of their walls without obvious fibrosis'[63]. Several pathological types of emphysema are recognised including centrilobular emphysema (figure 1.7) which is located in the central part of the lobule of Miller, panlobular emphysema (figure 1.8) which is located throughout the lobule and paraseptal emphysema which is located at the periphery of the lobule[62].

FIGURE 1.7: Histology slide showing centrilobular emphysema. *(Reproduced with permission from J. L. Wright & A. Churg. Histopathology 2006;49:1-9[62])*

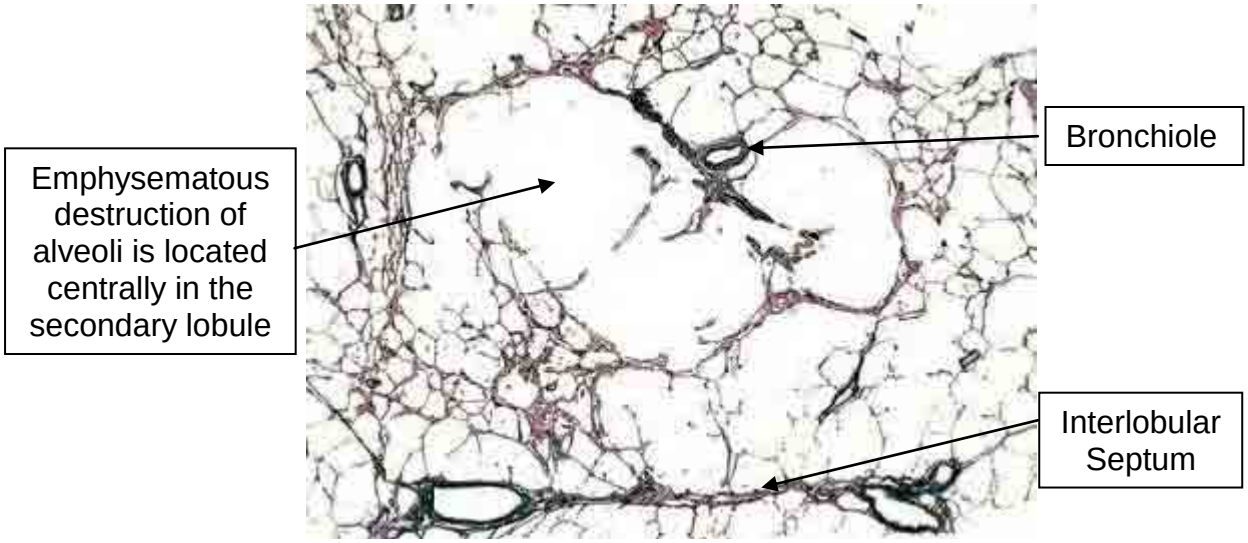
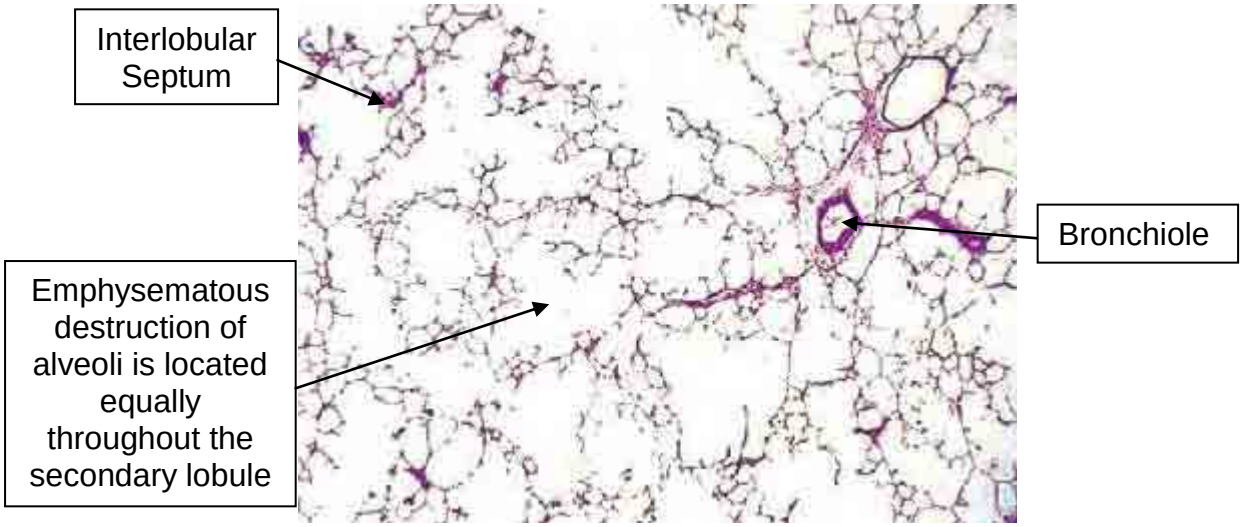


FIGURE 1.8: Histology slide showing panlobular emphysema. *(Reproduced with permission from J. L. Wright & A. Churg. Histopathology 2006;49:1-9[62])*



Panlobular emphysema is classically associated with alpha-1 antitrypsin deficiency[64] and the centrilobular pattern with usual smoking-related emphysema[65].

It is often possible to differentiate centrilobular and panlobular (also called panacinar) emphysema in vivo, by the appearance of the CT scan[66]. Emphysema is characterised by areas of abnormally reduced attenuation which lack a distinct wall on an HRCT scan. Centrilobular emphysema is characterised by small focal, approximately circular areas of lung destruction, seen centrally in a secondary pulmonary lobule. Panlobular emphysema is characterised by areas of uniform hypoattenuated lung involving the whole of the secondary pulmonary lobule[67]. Figures 1.9 and 1.10 show CT scan slices from subjects with typical appearances of centrilobular and panacinar emphysema respectively.

FIGURE 1.9: CT scan slice showing centrilobular emphysema.

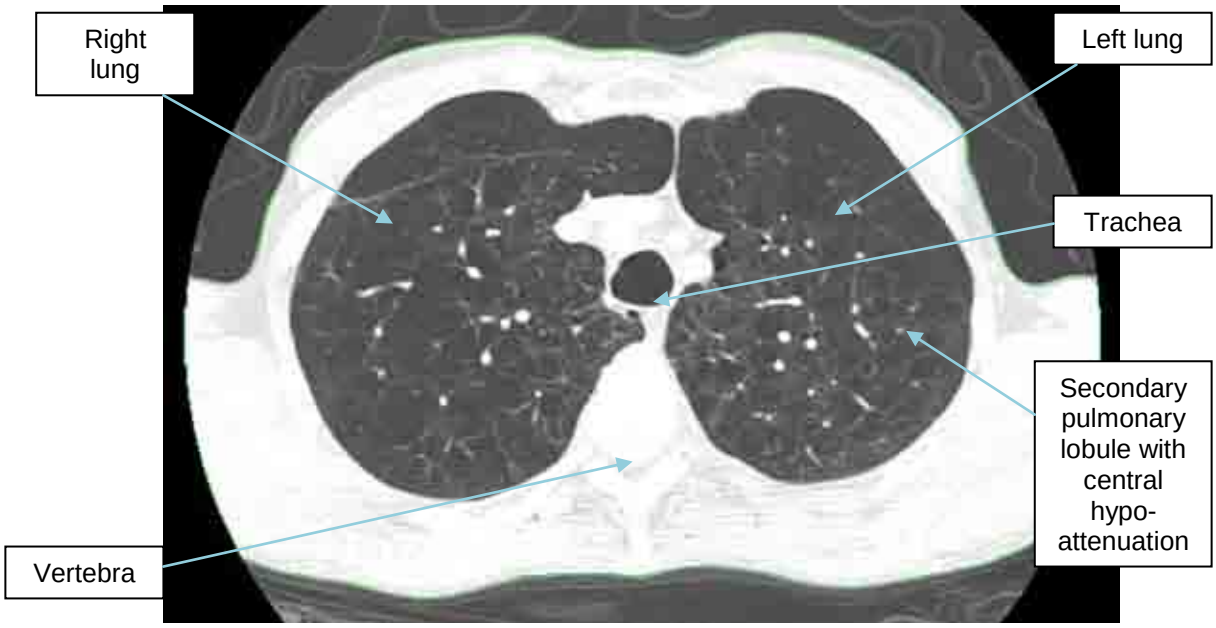
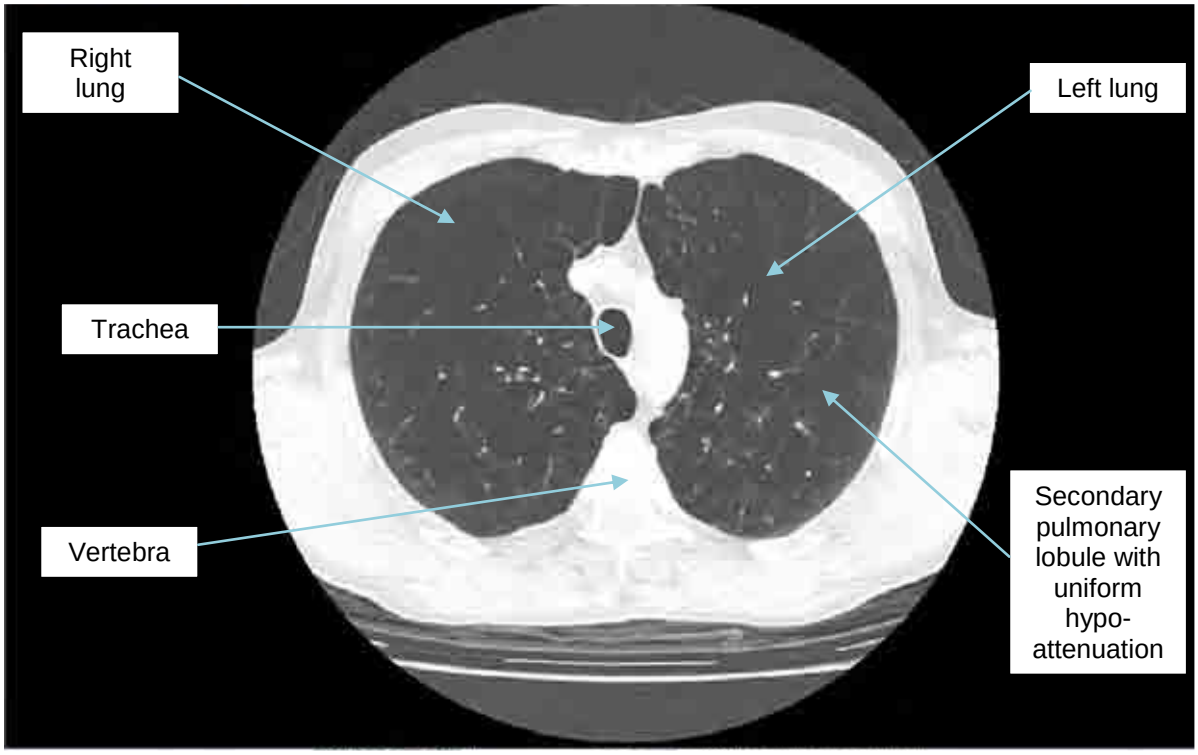


FIGURE 1.10: CT scan slice showing panlobular emphysema.



In addition to these pathological changes, the pulmonary vasculature exhibits intimal and muscular thickening[62] in COPD.

1.2.2.2.2.4. Clinical features of COPD

Subjects with COPD may complain of exertional dyspnoea, exercise limitation, cough and chronic sputum production (which fulfils the criteria for 'chronic bronchitis' if it occurs on most days for 3 months in 2 consecutive years, as defined by the Medical Research Council [MRC][68]).

COPD is associated with periodic exacerbations, which are defined as, "a sustained worsening of the subject's condition, from the stable state and beyond

normal day-to-day variations, necessitating a change in regular medication"[69]. The classical features of these episodes were described by Anthonisen and colleagues[70]. In general the three major features are an increase in dyspnoea, sputum volume and sputum purulence supported by a series of minor symptoms such as fever, cough and upper respiratory symptoms. These episodes are classed as type I (all three major criteria present), type II (two major criteria present) and type III (one major criterion present together with at least one minor criterion).

On clinical examination, subjects with COPD may be centrally cyanosed, exhibit physical signs of hyperinflated lungs, have an expiratory wheeze and reduced air entry. Early in the disease there may be none or few clinical signs, but as COPD becomes more advanced, it may be associated with respiratory failure, evidence of cor pulmonale and episodes of right heart failure.

Anxiety and depression are often associated with COPD and there is an increased incidence of co-morbidities including cardiovascular disease, osteoporosis, peptic ulcer disease, diabetes, cachexia and muscle wasting which may be associated with systemic inflammation[71].

The most informative data concerning the natural history of lung disease in AATD is a Swedish cohort study[72], in which 200, 000 infants born over a two year period from 1972 to 1974 in Malmo, Sweden were phenotyped. 127 PiZ and 48 protease inhibitor SZ (PiSZ) subjects were identified, and have been followed up periodically. The PiZ subjects were 30 years of age at the last full assessment[73], at which time 42% reported dyspnoea, 31% wheeze and 17% sputum production. These proportions were greater in those who had smoked (65%, 48% and 30%

respectively). Despite this, subjects generally had spirometry in the normal range. However, gas transfer was lower in PiZ smokers compared with PiM smokers[74].

1.2.2.2.2.5. Pulmonary physiology in COPD

Spirometry is the basis of all assessment of COPD as, by definition, the ratio of forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC) defines airflow obstruction. These measurements change with age, gender, size and ethnic group and hence are usually expressed in both absolute terms and corrected for these factors as a percentage of the predicted normal value (% predicted)[75].

Airflow obstruction that is not fully reversible is currently a defining feature of COPD, and is characterised as a post-bronchodilator FEV1:FVC ratio of less than 70%. Spirometry is also used to characterise COPD severity[32-34]. A lower FEV1 has been associated with worse symptoms[76,77], worse quality of life[78,79], a greater frequency of exacerbations[80], a more rapid decline in exercise capacity[81] and increased mortality[82] in subjects with usual COPD. Similarly, in subjects with AATD, a lower FEV1 has been associated with more severe symptoms[83], more frequent acute exacerbations[84], and greater mortality[85,86]. However, the relationship of FEV1 to health status in AATD remains controversial[87].

Previous observational cross-sectional studies suggest that spirometry in AATD becomes abnormal from age 50-65 in never-smokers[88,89] and from 25 years in smokers[90], although definitive data from the prospective Swedish cohort study described in section 1.2.2.2.4.[72] are unlikely to be available for several decades. The most recent complete report from this cohort suggest that spirometry is generally normal in PiZ subjects at 30 years of age[74].

The diffusing capacity (DLCO) or transfer factor (TLCO) for carbon monoxide (CO) measures the gas exchange in the lung and are often used as interchangeable terms. These measures reflect both the distribution of gas to the alveoli as well as the efficiency of uptake in the alveoli. KCO is a measure of DLCO corrected for alveolar volume and as such is more informative of the alveolar efficiency and hence more specific to the emphysema component of COPD[91].

In general there is a broad relationship between FEV1 and gas transfer for COPD subjects. However many subjects do not have emphysema and hence disparity may occur between the results of spirometry and gas transfer.

KCO has been less well characterised compared with FEV1 in COPD. Nevertheless, gas transfer is related to symptoms[92]and exercise capacity[93]in usual COPD. A small study[94] has also shown that subjects with a KCO of less than 75% of predicted have a more rapid decline in FEV1 over time than those with a KCO of greater than 75% of predicted. Few studies have examined the implications and associations of reduced gas transfer in subjects with AATD. However, impairment in gas transfer is associated with an increase in mortality in AATD[85], and is generally lower in AATD subjects who have more frequent acute exacerbations although this relationship is lost when gas transfer is corrected for alveolar volume[84].

There are little previous data available regarding the age at which gas transfer starts to deviate from normal in AATD. However, it was measured in a small cohort of PiZ subjects (4 current smokers and 52 subjects who had never smoked) from the Swedish cohort at the age of 30[74]. The mean KCO was 81% of predicted in smokers and 99% in those who had never smoked, suggesting that it had already started to

deviate from expected normal values in the smoking subset by the age of 30, when it was first measured in this group.

The measurement of lung volumes such as total lung capacity (TLC), functional residual capacity (FRC) and residual volume (RV) can be measured by either inert gas dilution or body plethysmography. The former method compares a known concentration of inert gas present in a closed system prior to the test, to the concentration present after a steady state has been achieved after several minutes of tidal breathing to allow concentration equilibrium. However, due to inefficient gas distribution in COPD the achievement of steady state may take a long time, and measurements are often made *near* rather than *at* plateau. Plethysmography compares the pressure in a closed box when the subject is at FRC with an open airway and when the subject is making respiratory effort with a closed airway[91]. As such it is more rapid and more likely to reflect the true volume of gas in the lung.

In COPD, these investigations often reveal evidence of gas trapping, with increased RV:TLC ratio, and reduced inspiratory capacity (IC). This can be associated with marked breathlessness[95], reduced exercise capacity[96] and mortality[97]. In AATD, gas trapping has been associated with more frequent exacerbations[84], although it has yet to be assessed against the parameters above.

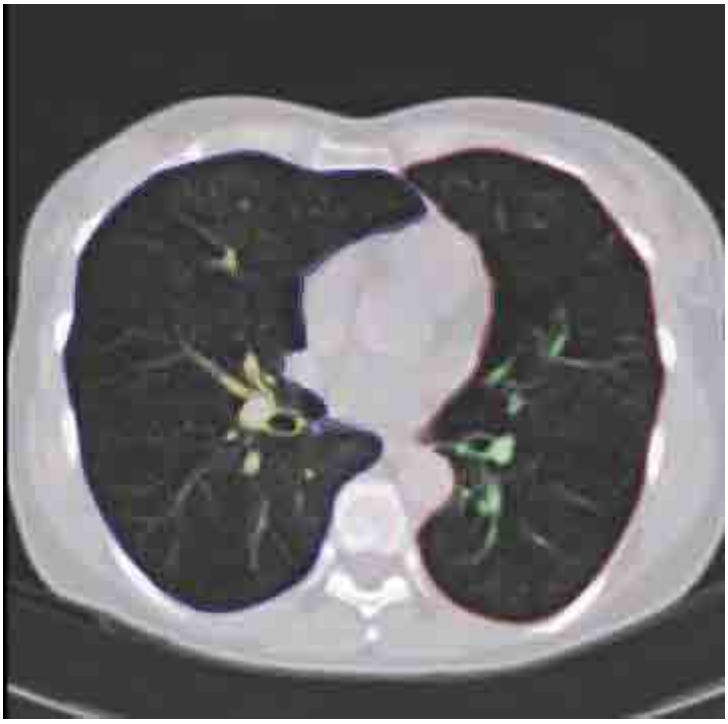
Arterial blood gas measurements give the partial pressures of oxygen (PaO2) and carbon dioxide (PaCO2), pH, and bicarbonate level in oxygenated blood. Subjects with usual COPD are often hypoxic and eventually develop respiratory failure. A reduction in PaO2 is associated with dyspnoea[92], reduced exercise capacity[98] and mortality[99] in subjects with usual COPD. Indeed, survival can be

increased by administering long-term oxygen therapy to subjects with severe chronic hypoxia who meet specified criteria[100]. Similar data have not been reported in AATD.

1.2.2.2.2.6. CT scans in COPD

Computed tomography (CT) scans were shown to detect emphysema in 1978 by Rosenblum et al[101]. The extent of emphysema can be characterised using CT scans subjectively with visual scoring systems[102] or objectively by assessing the density of the scanned images[103]. In 1988, Muller et al.[103] described the method of densitometric analysis using CT scans. The technique uses information derived from the cumulative frequency distribution histogram of lung voxel densities measured using specialised software either integrated into the CT scanner, or available externally. For example, figure 1.11 shows a CT scan slice through the lungs. Specialised software (in this case Pulmo-CMS software (MEDIS Medical Imaging Systems BV, Leiden, The Netherlands)) has been used to identify and outline the margins of the lungs.

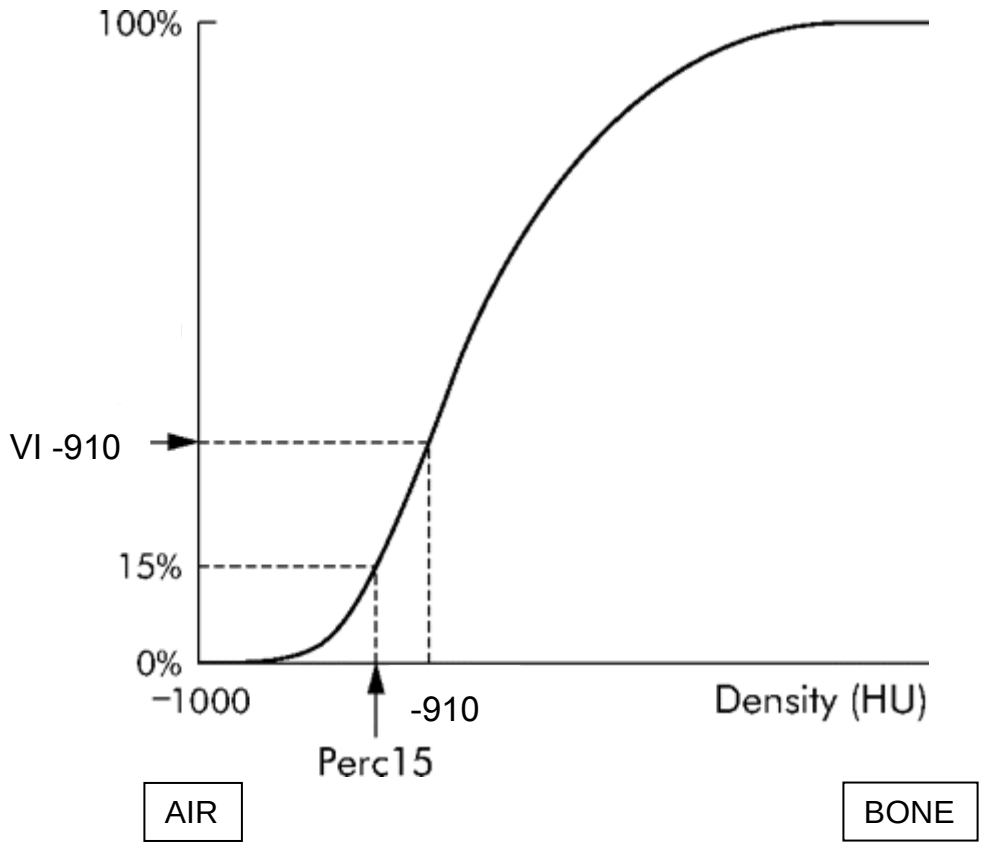
FIGURE 1.11: CT scan slice through the lungs.



Pulmo-CMS software has been used to identify the right (blue surround) and left (red surround) lung fields.

The software can then be used to assess the density of each CT voxel in Hounsfield Units (HU), and a cumulative frequency distribution histogram of the densities is constructed similar to that shown in figure 1.12. Density mask analysis describes the proportion of voxels that are less dense than a given threshold of Hounsfield Units (-910 in the example shown in figures 1.12 and 1.13 below), while the percentile point method describes the density in HU below which a defined percentage of voxels are distributed (in this case 15% or Perc 15). These points are labelled on the cumulative frequency distribution histogram in figure 1.12.

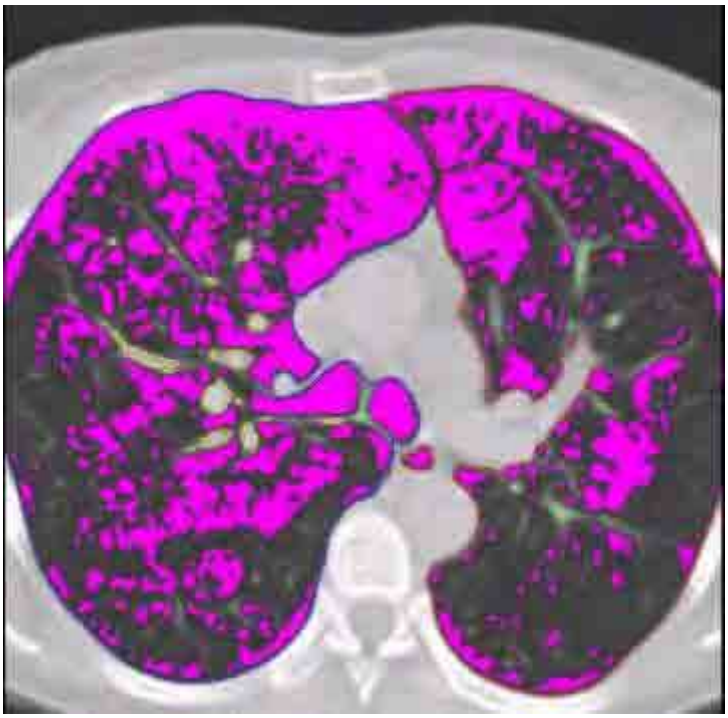
FIGURE 1.12: Example of a cumulative frequency distribution histogram of CT scan slice voxel densities.



The cumulative proportion of voxels (y-axis) of increasing densities (x-axis) is plotted for a CT scan slice. The 15th percentile point (Perc15) and the voxel index (VI) using the -910HU threshold (VI -910) are shown.

Figure 1.13 highlights the process of density mask analysis where specialised software (in this case Pulmo-CMS) has highlighted any voxel that is less dense than -910 HU. In this case, the Voxel Index (VI) describes the percentage of voxels that are less dense than -910 Hounsfield Units (VI-910).

FIGURE 1.13: CT scan slice showing density mask analysis of the lung using a threshold of -910HU.



Each CT voxel with a density of less than -910 HU has been highlighted in purple using Pulmo-CMS software.

Muller[103] demonstrated that densitometric measurements correlated well with the pathological grade of emphysema in resected lungs, and Gevenois et al.[104] subsequently proved this relationship existed when both macroscopic and microscopic pathological features of emphysema were examined using computer assisted techniques.

Kinsella et al.[105] also demonstrated that CT densitometry correlated well with spirometry, lung volumes and gas transfer measurements derived from physiological

testing[105]. Soejima et al.[106] added to this work by reporting that KCO related better to upper zone than lower zone densitometry, and conversely, FEV1 related better to lower zone compared with upper zone densitometry, at least in normal subjects. A similar relationship was also seen by Dowson et al.[107] in subjects with AATD, and persisted even after correction for mean lung density, gender, method of ascertainment, smoking and the presence of chronic bronchitis[108]. However, most of the subjects in the latter studies had *both* airflow obstruction and reduced gas transfer factor.

Very recently, Bernspang et al.[109] described CT densitometry findings in a small group of non-index (identified to have AATD as a result of screening tests, rather than diagnostic tests) PiZZ AATD subjects from the Swedish cohort described in section 1.2.2.2.2.4.[72] compared with PiM subjects (mean age 32), and concluded there was no difference between the two groups. However, less than one fifth of the study population consented to the examination, introducing a large selection bias. Furthermore, only a small proportion of these subjects had ever smoked, making the results difficult to interpret and apply to the general AATD population (albeit index [identified to have AATD as a result of diagnostic tests following the development of symptoms] or non-index). Furthermore, the PiZ smoking subjects with reduced gas transfer who had been previously described by the Swedish investigators[74] were not included in this study. Nevertheless, the subject with the most abnormal CT densitometry results was a PiZ smoker.

1.2.2.2.2.7. Heterogeneity of COPD

COPD is a heterogenous condition in terms of clinical, pathological, radiological and physiological features, and it is possible that different inflammatory mediators and processes are involved in the pathophysiology of the different phenotypes, as in animal models with COPD[110]. If so, it is increasingly important to identify these different COPD phenotypes reliably, in order to understand the pathophysiology and design clinical trials of treatments targeting the relevant inflammatory process and site[111].

Clinically, only 17 to 40% of subjects with COPD have chronic bronchitis[112], and approximately 40 to 60% do not develop regular acute exacerbations[113]. In addition, pathologically, different morphological types of emphysema exist. Centrilobular emphysema affects the central parts of the primary pulmonary lobule and is common in usual COPD[65], whereas panlobular emphysema affects the whole lobule and is the most common type of emphysema seen in AATD[64]. After examining pathological specimens, Thurlbeck et al.[65] described that centrilobular emphysema occurred more frequently in the upper lobes of the lungs, and that panlobular emphysema was more common in the lower lobes, consistent with this observation, emphysema in usual COPD, is classically described as apical, whereas AATD related panlobular emphysema is more frequently basal. However, despite these classical descriptions, subjects with usual COPD can develop emphysema in the lower lobes[65] and those with AATD can develop upper lobe emphysema in up to one third of cases[114].

Although part of the definition of COPD includes airflow obstruction (postbronchodilator FEV1:FVC ratio of less than 70%[32-34]), subjects are recognised

who have no evidence of airflow obstruction but have visible emphysema on CT scanning, with or without an isolated abnormality of gas transfer[115]. Recognising this phenomenon is becoming more common as CT scanners become more widely used. In addition it is becoming appreciated that significant bronchiectasis can be identified in 29% of scans from subjects with usual COPD[116] and up to 40% from subjects with AATD[117]. A further defining characteristic of COPD is that the airflow obstruction is largely irreversible. However, around one third of subjects with usual COPD[116] and up to half of those with AATD[118] have evidence of significant bronchodilator reversibility suggesting a further COPD phenotype.

Some relationships between these clinical, pathological, radiological and physiological phenotypes have already been established, in order to define phenotypes of COPD more specifically. One example, described above, is the association of centrilobular emphysema with the upper lobes of the lungs and with smoking, and conversely the association of panlobular emphysema with the lower zones of the lungs and with AATD.

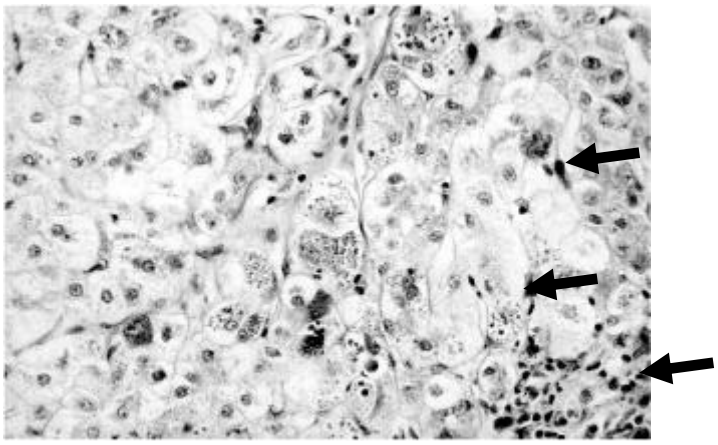
In order to understand the mechanisms behind these various phenotypes and develop effective therapy that is specifically targeted, it is becoming increasingly important to define the different aspects of COPD effectively.

1.2.2.3 The Liver in PiZ Alpha-1 Antitrypsin Deficiency

1.2.2.3.1. Pathological changes of Liver Disease in alpha-1 antitrypsin deficiency

Liver disease in AATD is thought to be related to the presence of AAT polymers in the hepatocytes, although the exact mechanism remains unclear[119]. Pathologically, the retained AAT polymers appear as periodic acid-Schiff diastase (PAS-D) resistant globules (figure 1.14)[120], which are present in all subjects with PiZ AATD, irrespective of whether they have clinically significant liver disease or abnormal liver function tests.

FIGURE 1.14: Liver biopsy specimen (x400) showing periodic acid-Schiff diastase (PAS-D) resistant globules (arrows) in peri-portal hepatocytes from a subject with PiZZ AATD. *(Reproduced with permission[121]).*



The factors that influence the development of clinically relevant liver disease are largely unknown. There may be genetic factors[122], and environmental factors such as co-infection with hepatitis B or C viruses[123]. Furthermore, it has been

hypothesised that breast feeding a child with AATD protects against the development of liver disease[124]. However, no clear consensus regarding the influence of these environmental factors has yet been reached.

1.2.2.3.2. Clinical features Of Liver Disease In PiZ Alpha-1 Antitrypsin Deficiency

The most informative study of the natural history of liver disease in AATD is the Swedish cohort described in section 1.2.2.2.2.4.[72]. Of the 127 PiZ infants identified by population screening, 14 (11%) developed neonatal cholestasis (most often caused by extrahepatic biliary atresia), and a further 8 (6%) had other signs of liver disease. Of the 14 who developed cholestasis, 2 subsequently developed liver cirrhosis between the ages of 2 & 4 and eventually died. A further child developed aplastic anaemia and died, but was found to have cirrhosis at post mortem, and a fourth child with liver disease in infancy died in an accident. The other infants with neonatal cholestasis or hepatomegally improved and by the age of 26, none had clinical evidence of liver disease[2,72,125-128].

In young adults, cohort studies have suggested that the risk of developing chronic liver disease is around 2%[129], but in older adults this figure increases to 15-20%[129-131]. However, due to the nature of these cohort studies it is likely that selection bias exists which may lead to overestimation of the presence of liver disease. Despite this, in post-mortem studies, the pathological incidence of liver cirrhosis is often even greater, and largely asymptomatic hepatomas have been found in around 16% of PiZ subjects[132]. Selection bias is likely to also lead to an overestimation of liver disease in these post-mortem studies, as they study only

subjects who have died (possibly in relation to their liver disease). Subjects who have never smoked are at increased risk of developing liver cirrhosis as they generally survive long enough for it to become clinically overt, as opposed to smokers who are more likely to die at a younger age because of lung disease[133].

Despite the relatively low levels of clinical liver disease in the Swedish cohort described above[72], abnormal liver function tests (either gamma-glutamyl transferase [GGT], aspartate transaminase [AST] or alanine transaminase [ALT]) were observed in all of the PiZ infants[72], reducing to 40%in 4-year olds[127], 3-15% in 12 - 18 year olds[134,135] and 5-8% in 26 year old subjects[126].

1.2.2.3.3. Gamma-glutamyl transferase in PiZ alpha-1 antitrypsin deficiency

Most current knowledge about GGT in subjects with AATD comes from the Swedish cohort identified via population screening[72]. These subjects have been followed up periodically from birth and are currently in their thirties. At 2–4 months of age, all infants had an abnormally high serum GGT activity, but only 17% had clinical evidence of liver disease[72]. By the age of 4, serum GGT activity was only abnormal in 4%, and by the age of 26, the proportion with an abnormal GGT remained between 1 and 8%, and abnormalities were usually transient in any one individual. From the ages of 8 to 26 years, none of the subjects had clinical evidence of liver disease[2,125,126]. Serum GGT activity has not been described in older adult subjects with AATD.

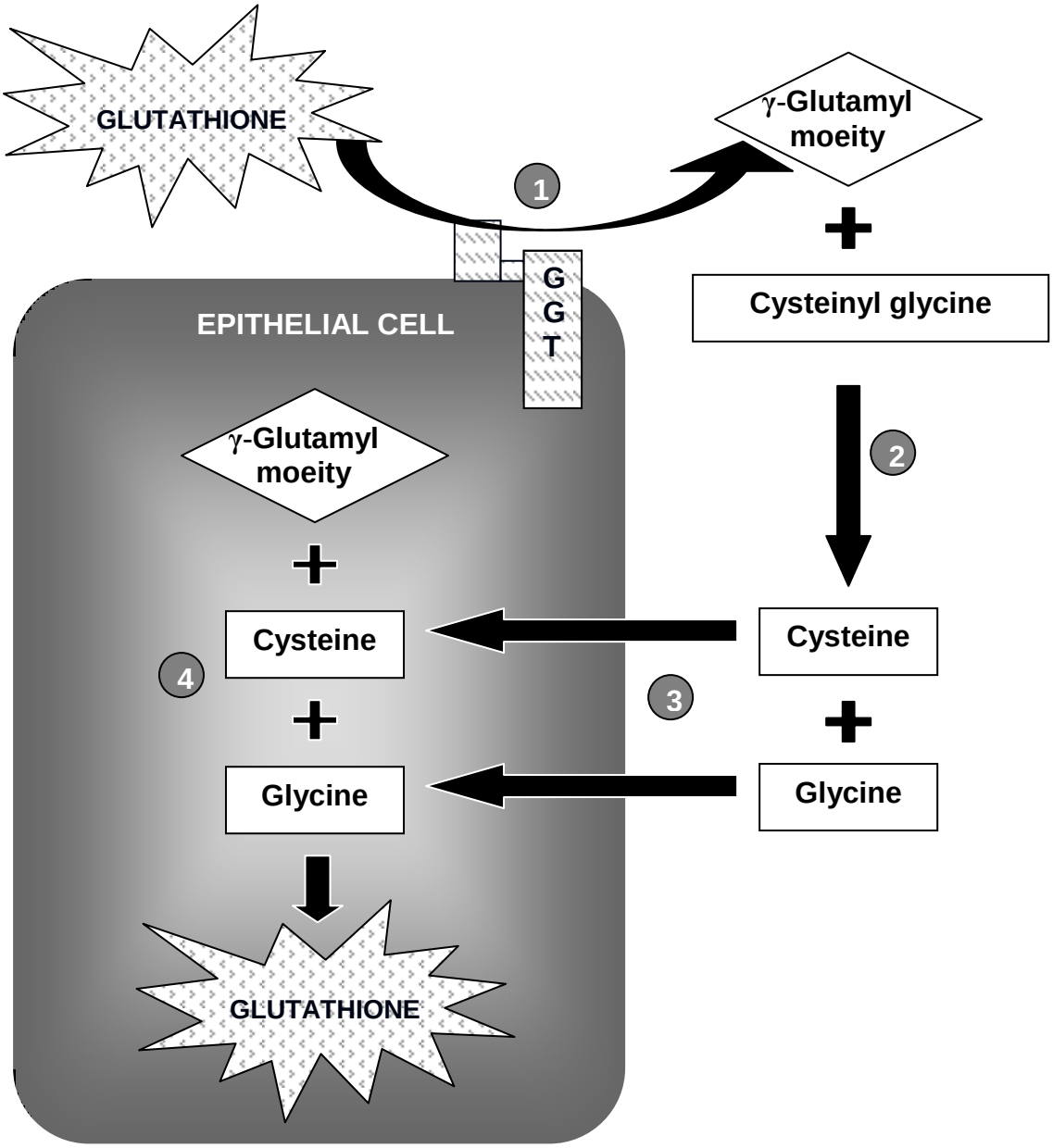
1.2.2.3.3.1. The structure of gamma-glutamyl transferase

Gamma-glutamyl transferase is a glycoprotein consisting of a heavy chain comprising of 351 amino acids, which is covalently bound to a light chain comprising 189 amino acids. The two chains are derived from the cleavage of a single precursor peptide[136]. The active site of GGT is situated on the hydrophilic light chain, while the heavy chain is hydrophobic and anchors GGT to the cell membrane. The deoxyribonucleic acid (DNA) that codes for GGT is located on chromosome 22q11 in humans, and several transcript variants are seen in different tissues, with variation usually occurring in untranslated sequences in the 5' region. Two mRNA populations exist in human lung. The first is similar to the transcripts seen in the liver, but the second appears unique to the lung, and is half the size of the first transcript[137]. Although, these transcripts both encode for the same protein[138,139], GGT is known to undergo various post-translational changes, including glycosylation, and sialylation and it may exist complexed to various substances including lipoproteins and immunoglobulins. Such post-translational changes are likely to result in a hydrophilic form of GGT as found in the circulation and various bodily secretions[140-143]. GGT activity is present in the kidney, pancreas, intestine, liver and lung[144].

1.2.2.3.3.2. The function of gamma-glutamyl transferase

GGT plays an important role in the synthesis of intracellular glutathione, which is the main antioxidant in the lung (see section 1.2.2.2.2.2.)[145]. This process is shown in figure 1.15.

FIGURE 1.15: The role of GGT in the metabolism of gluthathione.



1: Membrane bound GGT catalyses the breakdown of glutathione to cysteinylglycine plus a γ–glutamyl moiety.

2: Cysteinylglycine dissociates into its constituents cysteine and glycine.

3: Cysteine and glycine can pass easily into the cell, whereas glutathione cannot.

4. Cysteine, glycine and a γ–glutamyl moiety combine to form the antioxidant, intracellular glutathione.

Intracellular glutathione (GSH) is utilised rapidly after exposure to oxidative stress, and GGT subsequently catalyses the breakdown of extracellular GSH, thereby releasing the amino acids cysteine and glycine. Unlike extracellular GSH, these amino acids can then pass easily into the cell, where they are reconstituted with a gamma glutamyl moiety to reform intracellular GSH, and hence provide antioxidant activity[146].

1.2.2.3.3.3. Known associations of gamma-glutamyl transferase

GGT, measured as an activity assay, has been recognised as a sensitive but non-specific biomarker of all types of liver disease since the 1960s[147], and has become a 'routine' serum liver function test since the 1970s[148]. The mechanism behind this relationship is poorly understood, although it is known that serum GGT activity increases disproportionately compared to hepatic GGT activity in subjects with liver disease[149,150], which suggests it is unlikely that GGT is simply released from damaged liver cells. Hypotheses promoted to explain this disparity include the increased release of the enzyme from surface cell membranes, the induction of GGT transcription, and the reduced clearance of GGT from the circulation, but none of these have incontrovertible supporting data.

During the many studies exploring the relationship between GGT and liver disease, it was noted that serum GGT activity was particularly elevated in subjects with alcoholic liver disease compared with other liver pathologies[150], and an independent relationship between alcohol consumption and serum GGT activity was also reported in both alcohol abusers[151] and healthy subjects[152,153].

However, serum GGT activity has subsequently been related to many other factors, including age[154] (it increases with age), race[155] (it is greater in Afro-caribbean subjects compared with white subjects), gender[155] (greater in males) and smoking[155] (greater in current smokers than in ex-smokers and those who have never smoked). GGT in the circulation may have several other tissue sources (kidney, pancreas, brain, spinal cord, reproductive system[156]) and may be a marker of systemic inflammation with increased oxidative stress. Inflammation is being increasingly implicated in the pathophysiology of many systemic diseases[71], and this is supported by the relationship of these diseases to circulating GGT. In 1988, Perry et al.[157] reported the results of a prospective study of 7458 British men (age 40-59), and demonstrated that higher GGT activity levels at baseline were associated with an increased future risk of developing diabetes over the mean follow-up period of 12.8 years. Lee et al.[158-160] performed 3 further studies using large cohorts of healthy subjects, and demonstrated a similar relationship between GGT (with values that were mainly within the normal reference range), and diabetes together with impaired glucose tolerance, even after correction for confounding factors such as age, gender, race, alcohol consumption and smoking.

Serum GGT activity has also been shown to be associated with myocardial infarction since as early as the 1970s[161,162]. However, confounding factors were often present causing difficulties in interpreting the results of studies. In 2001, Emdin et al.[163] prospectively studied the relationship between serum GGT activity and myocardial disease over 6 years, and found that subjects with an elevated GGT activity at baseline had a greater risk of future myocardial infarction or cardiac death even after correcting for confounders. The relationships were particularly strong for

subjects who had a history of previous myocardial infarction and those who had multi-vessel disease. Subsequently, GGT was implicated in the pathological processes of atherosclerotic plaque formation[164], and has been shown to be associated with hypertension, independently of other confounding factors[159].

Other studies have also established relationships between serum GGT activity and the metabolic syndrome along with its individual components (insulin resistance, hyperinsulinaemia, dyslipideamia, central obesity and hypertension)[165-167]. Furthermore, serum GGT activity has been shown to relate to all-cause mortality, even after correction for confounding factors[167-169].

COPD is becoming increasingly recognised to be related to several co-morbid conditions, including ischaemic heart disease[170], osteoporosis[171] and diabetes[172]. The development of these co-morbidities is thought to be mediated by a common systemic inflammatory process involving the cytokine tumour necrosis factor alpha (TNFα), oxidative stress, and associated with high serum measurements of high sensitivity C-reactive protein (CRP)[71]. Serum GGT activity has been shown to relate to serum CRP in healthy subjects[173], as well as the co-morbidities described above.

1.2.2.3.3.4. Gamma-glutamyl transferase and the lung

Previous studies have been performed using rat alveolar epithelial cells to examine the characteristics of GGT and its relationship to the antioxidant, glutathione, in the lung. In 1994, Kugelman et al.[174] exposed such cells to oxidative stress using menadione. Intracellular glutathione levels initially decreased, but subsequently increased to a greater level than at baseline, in conjunction with an increase in GGT mRNA and GGT activity. These changes were prevented by the specific GGT inhibitor acivicin. This study suggested that GSH was initially consumed in the pro-oxidant conditions, but the expression of GGT was subsequently increased and the enzyme was involved in the breakdown of extracellular GGT and re-synthesis of intracellular GGT, as a protective response to oxidative stress. Other similar studies have since been performed using different substances to induce oxidative stress, and yielded similar results[175,176].

Moreover, a GGT deficient mouse model was studied by Jean et al. in 2002[177], and demonstrated a six-fold reduction in glutathione levels within alveolar macrophages in conjunction with an intense signal for 3-nitrotyrosine (a marker of oxidative stress). When mice were exposed to pro-oxidant conditions, their survival was reduced by 25%, due to severe bronchiolar cellular injury and pulmonary oedema, associated with glutathione depletion and diffuse oxidant stress measured by nitrotyrosine staining.

In 2004, Payne et al.[178] exposed alveolar macrophages and type II epithelial cells from human lung to airborne particulate matter collected in the aftermath of the World Trade Centre collapse, (which contained pro-inflammatory, pro-oxidant inducing substances), and examined the release of GGT, IL-8 and interleukin 6 (IL6).

The release of GGT was related to the concentration of particulate matter in a dose dependant manner.

In 1974, Barton et al.[179] showed that GGT activity was greater in human subjects with chronic bronchitis compared to healthy subjects, and that sputum GGT activity correlated with the DNA content of the sputum, which was used as a marker of inflammation. Furthermore, children with an inflammatory phenotype of cystic fibrosis had greater GGT activity in bronchoalveolar lavage fluid compared with those with a non-inflammatory phenotype of cystic fibrosis[180].

Despite these associations between lung disease and GGT in lung secretions, and the relationships of serum GGT activity to co-morbidities associated with COPD, which are associated with oxidative stress, no studies have explored serum GGT activity in human subjects with COPD, and no attempt has been made to examine the relationship between lung secretions and serum GGT levels.

1.2.2.3.3.5. Biomarkers

A biomarker is a molecule or substance that can be measured and reflects disease process[181]. Biomarkers should be involved in the pathophysiological process of the disease, they should be stable, (only vary with events known to relate to the disease), predict disease progression and be sensitive to effective therapeutic interventions[182]. Biomarkers should not only be sensitive, specific, but ideally, non-invasive, acceptable to the subject, inexpensive and easy to obtain and analyse. There is currently much research into biomarkers in COPD, including markers in the serum, sputum, exhaled breath condensate, bronchoalveolar lavage fluid (BAL) and

tissue. A recent meta-analysis revealed increasing trends with disease severity for sputum neutrophils, macrophages and IL8, serum CRP and TNFα and BAL IL8. Serum CRP also relates to the MRC dyspnoea score, exercise tolerance[183], acute exacerbations[184], hospitalisation[185], disease progression and all cause mortality[186] in subjects with COPD.

AATD is generally associated with evidence of increased airway inflammation. Elastin breakdown (as part of the inflammation) is a by-process thought to be central to the development of emphysema. However, inflammation is also detectable in the airways in subjects with AATD. It is present to a greater extent in subjects with AATD compared with those with usual COPD[187], (especially in the presence of chronic bronchitis[188]), and is reduced in subjects receiving augmentation therapy[189].

Biomarkers of inflammation may be important in order to identify subjects at risk of developing COPD, or at risk of clinical deterioration such as progression of disease severity or to predict or characterise exacerbations. GGT is an oxidative stress component of inflammation and hence has the potential to be an important biomarker in lung disease.

1.2.3. The PiSZ Phenotype of Alpha-1 Antitrypsin Deficiency

1.2.3.1 Epidemiology of the PiSZ Phenotype of AATD

The S allele of AAT exists throughout the world but is most common in Europe, where the prevalence increases towards the most South-western parts, especially around the Iberian Peninsula[190]. This is thought to result from population movements eastwards from the presumed origin of the mutation in the North of the Iberian peninsula[191]. The geographical distribution of the S allele as part of the PiMS phenotype in European countries is demonstrated in figure 1.16. This figure can be contrasted to the distribution of the Z allele represented by the PiMZ phenotype in figure 1.3, which tends to be more common in northern Europe and less so towards the south west.

It is estimated that over 70,000 people in the U.K. have the PiSZ phenotype of AATD and around 130,000 have the PiSS phenotype, and these phenotypes are more common than the PiZ phenotype, which is estimated to be present in over 10,000 subjects[190].

The PiS type of AAT results from a substitution of glutamate for valine at position 264[192] which leads to increased intracellular degradation of the protein within hepatocytes and subsequently a reduction in secretion into the circulation[193] (Figure 1.17). The PiS AAT also has the capability of forming polymers in a similar way to PiZ AAT albeit less readily[194]. And has also been shown to form heteropolymers with PiZ AAT[195].

FIGURE 1.16: European distribution by country of the PiMS phenotype of alpha-1 antitrypsin deficiency. The figure illustrates the percentage of the population estimated to have the PiMS phenotype of alpha-1 antitrypsin in each European country. *(Figure by Olve Utne and reproduced from http://commons.wikimedia.org/wiki/File:PiMS_Europe.png. Creative Commons Attribution ShareAlike 2.5 Lisence).*

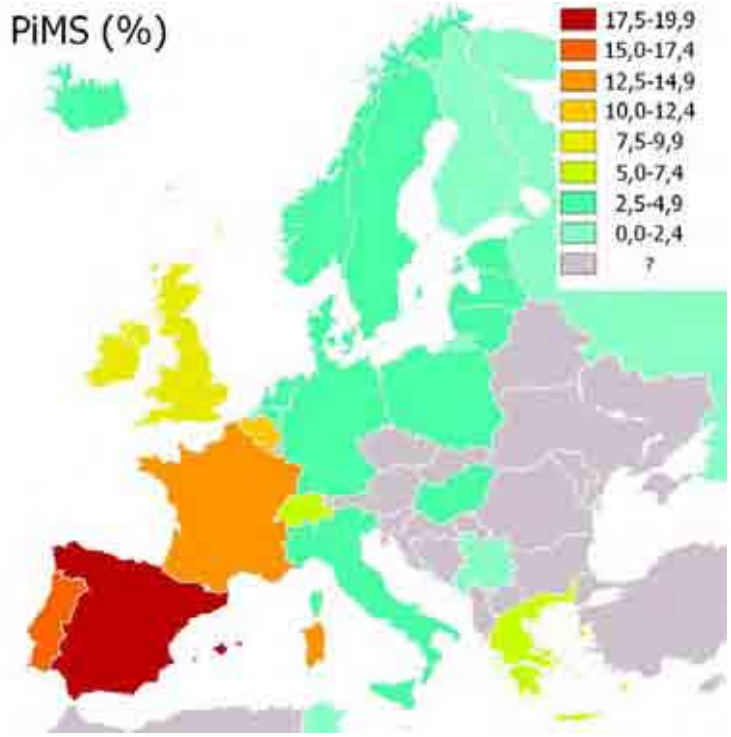
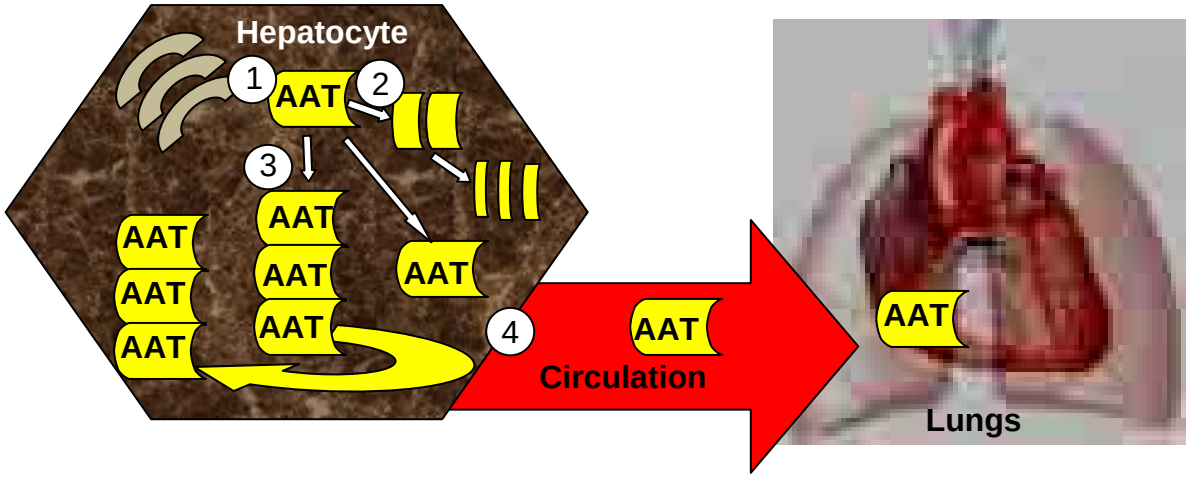


FIGURE 1.17: Mechanisms by which the PiS type of alpha-1 antitrypsin causes a deficiency state in the circulation and the lungs.



The abnormal PiS type of alpha-1 antitrypsin protein is produced in hepatocytes (1). The PiS alpha-1 antitrypsin is more likely to undergo intracellular degradation compared with the normal PiM protein (2). The PiS alpha-1 antitrypsin protein also has a mild tendency to polymerise within the hepatocytes (3). Despite points 2 and 3, which inevitably cause a reduction in the levels of circulating protein, some active PiS alpha-1 antitrypsin is able to enter the circulation and subsequently the lung to provide a slightly reduced level of antiprotease activity (4).

1.2.3.2. Risk of developing lung disease with PiSZ phenotype of AATD

The circulating level of AAT is thought to be an important determinant for the risk of developing lung disease. Previous epidemiological studies suggested that serum AAT levels above a threshold of 11microMolar (μM) were not associated with a significant risk of developing emphysema[196,197]. In 1987, Wewers et al. demonstrated that AAT could be infused at a dose of 60mg/kg weekly, and trough serum levels were consistently maintained above this 11μM threshold in recipients[198]. If subjects with severe AATD received AAT replacement at this dose, the decline in FEV1 over time tended to be lower[199,200] and sputum inflammatory mediators fell in conjunction with an increase in sputum AAT[189], suggesting that serum AAT at intermediate levels reduces inflammation and hence protects against the development of lung disease.

In addition, in 1999, Campbell et al.[43] demonstrated a non-linear relationship in vitro between serum AAT level and the extent of proteolytic damage produced in vitro, suggesting this may mimic events critical in the development of emphysema. It was shown that at AAT levels of less than 10μM, there was a dramatic increase in the area of proteolytic damage produced by polymorphonuclear degranulation both theoretically and in vitro compared to levels of greater than 10μM, where relatively large increases in AAT concentration result in only a small decrease in the area of damage.

Subjects with the PiSZ phenotype of AATD have circulating AAT levels of 8 to 16μM[19], and therefore some fall above the 'protective threshold' of 10-11μM,

whereas others fall below. Hence, it is possible that some PiSZ subjects may be at increased risk of developing COPD as a result of low levels alone.

However, some AAT phenotypes also have impaired inhibitory function irrespective of their serum level[20]. This is possibly relevant for the PiSZ phenotype, as the Z variant has a reduced association rate constant for neutrophil elastase. Whether this is critical for the borderline SZ level remains unknown. As such the AAT level per se cannot be related directly to the risk of lung disease in AATD, although an association with the AATD level is clearly important.

The neutrophil elastase inhibitory capacity is also slightly lower in both plasma and lung epithelial lining fluid with PiS AAT compared with PiM AAT[201], and this may in turn influence the susceptibility to develop emphysema in subjects with S alleles. The interstitial concentration of AAT are predicted to be about 80% of the plasma level[202] so the critical concentration of 10-11μM would be expected in subjects with a plasma level of 13μM, which would include at least the majority of the PiSZ subjects.

A further factor that should be considered when relating AAT level to the risk of lung disease is the fact that alpha-1 antitrypsin is an acute phase protein[203]. Therefore, its' level may be elevated in the presence of inflammation such as that seen in COPD[42], especially during acute exacerbations[204].

Previous case control and cohort studies have tried to assess the risk of developing COPD for subjects with the PiSZ phenotype. In 1969, Fagerhol et al. conducted the first case control study and reported a greater frequency of the PiSS, PiSZ and PiZZ phenotypes of AATD in Norwegian subjects with lung disease in general, when compared with Norwegian blood donors and pregnant women[29]. Two subjects with the PiSZ phenotype were identified from 503 with pulmonary disease.

One had chronic bronchitis and the other had a primary lung cancer. Three subjects with the PiSS phenotype were also identified - one had asthma, the second had sarcoidosis and the third a primary malignant tumour. However this study examined subjects with lung diseases of different types, and not exclusively COPD. Furthermore, no objective measures, such as pulmonary function were obtained. However, some ten years later, the same group attempted to identify the risk of developing obstructive lung disease for various phenotypes[205]. Three subjects with the PiSZ phenotype and 2 with the PiS phenotype were identified from 1258 people from the population of Oslo. Of these, 1 PiSZ subject had COPD, and the numbers were too small to draw firm conclusions regarding any increase in risk of COPD in subjects with these phenotypes.

In 1985, Bartmann et al.[206] provided the first case control study to show that the proportion of PiSZ individuals was greater in German subjects with COPD (18/526) compared with blood donors and a group of healthy employees (1/642). In addition, vital capacity was lower and airways resistance higher in PiSZ subjects compared with PiMM controls matched for age, gender, disease duration, smoking, environmental exposure and serum immunoglobulin E. However, the S allele frequency was not significantly different between the 2 groups. Nevertheless, this was the first study that provided reasonable support that the PiSZ phenotype was associated with an increased risk of COPD. The following year, Lieberman et al.[30] in a larger study, reported no difference in the proportion of American subjects with severe COPD and the PiSZ (2/965) or the PiSS (3/965) phenotypes compared with controls (5/1380 and 1/1380 respectively). Alvarez-Grandez et al.[207] produced similar results to Lieberman when studying a Spanish population in 1997.

Subsequently, Dahl et al. performed a cohort study in a general Danish population using data from the Copenhagen City Heart Study. From 9187 subjects, 10 were found to have the PiSZ phenotype. When compared to subjects with the normal PiMM phenotype, the odds ratio for airflow obstruction was 5.4 (CI: 1.6-40), even when corrected for age, gender, smoking, occupational exposure and childhood infections at 5.3 (CI:1.0-26). However, the confidence interval for the corrected data included 1.0, thus failing to achieve statistical significance. Nevertheless, a greater proportion of PiSZ cases had airflow obstruction (40%) compared with PiM controls (15%)[208], and PiSZ subjects had a lower mean FEV1 and a more rapid decline in FEV1 (56ml per year) if they had previously smoked, compared with PiMM subjects (21 ml/year)[209]. Twelve PiSS subjects were identified from this study, but no differences were observed between these subjects and those with the PiMM phenotype. These data suggested that the PiSZ but not the PiSS phenotype conferred an increased risk of developing lung disease although the risk is low.

As these studies had produced conflicting results, Dahl et al. performed a meta-analysis in 2005 to attempt to answer the question, "Is there an increased risk of COPD in individuals with the PiSZ genotype?"[210]. Six studies were included, yielding 42 subjects with the PiSZ phenotype, of whom, 27 had COPD. They reported an odds ratio of 3.26 (95% CI: 1.24-8.57) of PiSZ subjects having COPD compared with PiMM subjects. However, sensitivity analysis revealed that the removal of one unusually positive study[206] from the analysis meant that this result was no longer significant. In addition, not all studies adjusted for confounding factors such as smoking, patient acquisition or ethnicity. Therefore, despite this further

analysis, uncertainty still remains regarding the risk of developing COPD for the PiSZ phenotype.

Although several studies have reported a greater than expected frequency of asthma with the PiMS phenotype compared with healthy subjects in Hispanic [211,212] and white American[213] populations, little data are available regarding the risk of asthma in the PiSZ, PiSS and PiSVar phenotypes of AATD. In 1990, Lindmark et al.[214] described that the frequency of the PiSZ phenotype in 172 Swedish children with asthma was similar to that expected in the general population, inferring that the PiSZ phenotype was not associated with an increased risk of asthma in minors. Similar conclusions were reached by Miravittles et al.[215] while studying Spanish adult asthmatics. Contrary to this, Prados et al.[216] showed that the PiSZ phenotype was more prevalent ($p<0.001$) in 31 Spanish subjects with intrinsic asthma compared with 200 subjects representative of the general Spanish population. In 2000, Sigsgaard et al.[217] grouped farming students according to their phenotype - PiMM, PiMZ, PiMS, or rare (PiSS, PiSZ, PiZZ). Male farming students with rare phenotypes had a higher prevalence of doctor diagnosed asthma than subjects with the PiMM phenotype, and the odds ratio of having bronchial hyperresponsiveness was increased with the rare phenotypes (4.34; 95%CI:1.19-15.8) in farming students but not in non-farming rural based control subjects. The authors concluded that the S and Z alleles were associated with asthma but only in a specific group of subjects, suggesting that the relationship between phenotype and asthma was not simple, but a more complex gene/environment interaction.

The incidence of bronchiectasis in subjects with the PiSZ phenotype is unknown, as CT scan appearance has only been reported in 4 such subjects in total, as described in more detail in the next section.

1.2.3.3. Characterisation of lung disease in PiSZ AATD

Few PiSZ subjects have been characterised in detail involving more complete physiology, symptoms and quality of life. Radiological features and specifically CT scans have never been described in a cohort of such subjects.

The first study to provide a more detailed description of pulmonary physiology in subjects with the PiSZ phenotype was by Larsson et al.[218] in1976. Seven PiSZ and 6 PiZ subjects who were diagnosed with AATD due to abnormal serum electrophoresis after investigation for conditions other than lung disease were described, along with 4 symptomatic PiZ subjects. These asymptomatic PiSZ subjects all had a normal FEV1:FVC ratio, but 2 showed evidence of gas trapping with an elevated RV/TLC ratio expressed as a % predicted, and 1 had an abnormal gas transfer measurement. Tests of elastic recoil were abnormal in most PiSZ subjects, but pulmonary artery pressures were normal in all subjects.

In 1982[114] and 1983[219], Hutchinson et al. described details of 25 PiSZ subjects from the UK (14 index and 11 non-index cases). Of the 8 non-index cases who had smoked, one (age 67) had an abnormal FEV1(% predicted) and 2 (age 45 and 63) had abnormal gas transfer values, the oldest of whom also had radiological evidence of emphysema. Spirometry or gas transfer was impaired in all 12 index subjects who had smoked, and in one of the 2 subjects who had never smoked. There were signs of emphysema on the chest X-ray (CXR) in 9/25 (36%) PiSZ subjects of whom 5 had generalised changes, 3 lower zone changes and 1 upper zone changes, compared with 85% of PiZ subjects from the same registry. No radiological signs of emphysema were visible in PiSZ subjects who had never smoked.

In 1996, Turino et al.[220] described the clinical characteristics of 50 PiSZ subjects compared with 1090 PiZ subjects from the National Institute for Health combined registries. 10 PiSZ subjects had a serum AAT level less than the protective11μM threshold, and 36% were non-index cases. A smaller proportion of the total 50 PiSZ subjects reported respiratory symptoms (productive cough - 44%; wheeze – 42%; dyspnoea – 64%) compared with PiZ subjects (50%, 66%, 85% respectively). However, PiSZ *index* subjects had a similar incidence of the two latter symptoms compared with the PiZ group. Only 4/14 (29%) PiSZ subjects who had never smoked had abnormal spirometry, compared with 51% of PiZ subjects who had never smoked. A similar proportion of PiSZ and PiZ ex or current smokers had evidence of airflow obstruction (approximately 70% & 80% respectively). Pack years of cigarettes smoked correlated with FEV1% predicted (r = -0.68) and FEV1:FVC % predicted (r = -0.7) more closely in PiSZ subjects compared with PiZ subjects (r = -0.42 & r = -0.41 respectively). Evidence of hyperinflation (42%) or bullae (12%) were seen less frequently on chest X-rays (CXR) in PiSZ subjects compared with PiZ subjects (73% & 29% respectively), and a greater proportion of PiSZ ex or current smokers (76%) had an abnormal CXR compared with PiSZ subjects who had never smoked (17%). No differences were found between PiSZ subjects with an AAT level <11μM or >11μM when analysed categorically, although multivariate logistical analysis revealed an increased prevalence of cough and wheeze with *increasing* serum AAT levels when modelled as a continuous predictor. The authors suggested that cigarette smoking may be a more important risk factor than the AAT level or phenotype per se for the development of airflow obstruction in PiSZ subjects compared with PiZ subjects.

However, there were difficulties regarding the interpretation of this work, as fewer PiSZ subjects had smoked compared with PiZ subjects, and an obvious selection bias existed, as most data were derived from index subjects who were initially diagnosed with AATD due to the presence of lung disease.

This latter issue was addressed (at least partly) by Seersholm et al. in 1998[221], who compared 66 index PiSZ cases to 28 non-index PiSZ cases from the Danish registry. It was shown that index subjects had an elevated standardised mortality ratio (SMR) of 18 (95% CI: 1.9-171), but non-index subjects did not. Mean FEV1 was also higher in non-index subjects (94 %predicted) compared with index subjects (59 %predicted) and in never-smokers (94 %predicted) compared with ex or current smokers (73 %predicted). However, spirometry data were only available for 62% of non-index and 86% of index cases, again introducing a potential selection bias. It is also unclear how subjects diagnosed with AATD due to liver disease or after investigation of other diseases were categorised (index / non-index).

The most informative study regarding the natural history of PiSZ subjects with AATD is likely to be the Swedish cohort study described in section 1.2.2.2.2.4.[72], where 200,000 neonates underwent population screening for AATD in 1972 to 1974, resulting in the identification of 127 PiZ and 53 PiSZ subjects. These subjects have been followed up since, and data published at the age of 30 showed that PiSZ subjects who had never smoked were more likely to produce sputum (29%) than PiM controls (11%), but spirometry values remained normal, even in subjects who had smoked[73]. Data from a very small subset (n=11) of these PiSZ subjects has very recently been made available[109] in regards to gas transfer and CT densitometry, which was generally comparable with PiM subjects of the same age (mean 32 years).

However, significant selection bias exists in this very small subset, making generalisation to the PiSZ population as a whole difficult. Complete spirometric and symptomatology follow-up data throughout life will not be available for some decades.

The gross appearance of the thoracic CT scan has only been described in 4 subjects with the PiSZ phenotype. The first as part of a case control study by Cuvelier et al.[222] to determine whether the incidence of AAT phenotypes was different in 204 subjects with bronchiectasis compared with healthy controls. One of the 204 subjects was identified as having the PiSZ phenotype and 3 had the SS phenotype. These proportions were no different to control subjects, suggesting these phenotypes did not confer an increased risk of bronchiectasis. The second PiSZ subject was presented as a case report and had bronchiectasis in the absence of emphysema[223]. Finally, McMahon et al. described CT scan findings in 21 subjects with the PiZ, 3 with the PiMZ and 2 with the PiSZ phenotypes. One of the PiSZ subjects had widespread emphysema, but neither had bronchiectasis[224].

Overall the studies do not support an increased risk of PiSZ subjects developing emphysema or asthma.

1.2.3.4. Risk of developing liver disease in PiSZ AATD

The risk of developing neonatal, childhood and adolescent liver disease with the PiSZ phenotype has been addressed by Sveger et al. and Pittscheiler et al. using 2 separate cohorts identified by neonatal screening[72,225]. Neither have reported cases of PiSZ infants developing clinical evidence of liver disease. However, serum biochemistry tests such as alanine transaminase (ALT) and GGT were abnormal in

approximately one quarter of PiSZ infants at the age of 3 months The proportion with such abnormal tests decreased to 5% at the age of 2[2] and 2% at the age of 4[127], and remained at this level until the age of 16[2,128,226]. However, from 18 years of age[226], around 10% of PiSZ adolescents had abnormalities of either ALT or GGT, although these abnormalities appeared transient, as rarely had the same subjects had abnormal results at the ages of 18, 22 and 26[126].

The risk of developing liver disease in older adult subjects with the PiSZ phenotype of AATD has not been studied. However, Rakela et al.[227] reviewed 19 subjects with AATD and liver disease, 3 of whom had the PiSZ deficiency, from a clinic in the United States of America (USA). It was noted that PiSZ subjects were older than PiZ subjects (mean age 66 in PiSZ compared with 58 in PiZ), and that 2 PiSZ subjects had liver cirrhosis and 1 had steatohepatitis. As there are likely to be a greater number of PiSZ than PiZ subjects in the USA[228], yet fewer subjects with liver cirrhosis and AATD have the PiSZ compared with the PiZ phenotype, it seems likely that although PiSZ subjects can develop liver disease, it is a far less frequent occurrence than in PiZ subjects. However, as the numbers are small comparisons with the incidence of the phenotype in the general population is unlikely to be informative.

1.2.4. Testing For Alpha-1 Antitrypsin Deficiency

The World Health Organisation (WHO) recommended that all subjects with adult onset asthma or COPD should be tested for AATD[64]. However, this approach would delay the diagnosis until disease and symptomatology are established. The alternative is neonatal screening although this raises some ethical issues.

Nevertheless when such an approach was initiated in the Swedish cohort described previously, subjects responded positively to advice about the hazards of smoking[229], with little psychological impact. In practice, it is unlikely that the WHO advice is adhered to, possibly due to cost implications and general unawareness of its existence.

1.3 Background to the current research projects

ADAPT (Antitrypsin Deficiency Assessment and Programme for Treatment) is the U.K. registry for alpha-1-antitrypsin deficient subjects, and was established in 1996. Subjects with alpha-1-antitrypsin deficiency are referred to ADAPT by their general practitioner or hospital consultant (predominantly because of the presence of lung disease), or are identified by family screening of an index subject, organised through the ADAPT programme.

Subjects attend annually and clinical, social, physiological, radiological, biochemical and microbiological data are collected as detailed in section 2. Subjects undergo a full medical review and examination, following which, advice is given to the subject and their general practitioner (GP) regarding current and future management of their condition.

Data are stored in medical records and protected electronic databases, and analysed as required. The ADAPT programme is approved by the South Birmingham Research and Ethics Committee (LREC Number 3359), and all subjects provide written informed consent.

Four separate studies have been performed using the data acquired from ADAPT as summarised below. Some of the data included in this thesis has previously been published[230-232].

1.4 Purpose of the Research

It is clear that various clinical, radiological and physiological phenotypes of COPD exist, in both the smoking population and the alpha-1 antitrypsin deficient population. In order to understand the mechanisms behind these various phenotypes and develop effective therapy that is specifically targeted, it is becoming increasingly important to define the different aspects of COPD effectively. The current thesis contains projects to further our understanding of the condition.

1. Of the subjects who attend ADAPT, approximately 27% have a normal FEV1 and a normal KCO (greater than or equal to 80% of predicted), 14% have an abnormal FEV1, but a normal KCO, 7% have a normal FEV1 and an abnormal KCO and 52% have abnormalities of both FEV1 and KCO. The aim of the first study presented here was to assess the influence of different physiological impairments on radiological and clinical factors in subjects with each of these physiological patterns, in order to understand the implications of different physiological phenotypes. It was hypothesised that subjects with an abnormality of KCO would have more upper zone emphysema and those with an abnormality of FEV1 would have more lower zone emphysema.

2. The clinical data from the Swedish cohort of AATD subjects described in section 1.2.2.2.2.4.[72] suggests that subjects become symptomatic prior to spirometry becoming abnormal[73], suggesting that spirometry may be

insensitive to early change. Furthermore, we have identified subjects with established emphysema and reduced gas transfer but normal spirometry[230], suggesting that other tests may be more informative.

The UK database of AATD subjects (ADAPT) includes smokers, non-smokers, index and non-index subjects who have undergone extensive assessment including spirometry, gas transfer, HRCT scanning and health status. In the second study, these parameters were related to age to determine, by retrograde analysis, when they deviated from normal values in order to provide a more informed approach to monitoring for early lung disease. It was hypothesised that measures of gas transfer and CT densitometry would start to deviate from normal prior to spirometry.

3. Biomarkers of inflammation may be important in order to identify subjects at risk of developing COPD, or at risk of clinical deterioration such as progression of disease severity or before / during exacerbations. GGT as a member of the oxidative stress component of inflammation has the potential to be an important biomarker in lung disease. The third study investigated the association of GGT with the inflammation of lung disease in order to establish if it had the potential to be a biomarker of lung disease as well as liver disease in AATD. It was hypothesised that serum GGT would be related to the severity of lung disease in subjects with AATD, and

that this would be independent of an anticipated, primary association with liver disease.

4. Neither CT scan appearance nor health status have been characterised in subjects with the PiSZ phenotype of COPD. Although physiology and clinical features have been described in a small number of subjects and compared to subjects with the PiZ phenotypes, confounding factors such as differences in smoking history have been present. Hence the final study describes CT scan appearance, CT densitometric analysis and health status in PiSZ subjects for the first time. Physiology and clinical features are also described, and those are compared to subjects with the PiZ phenotype matched for age, gender, smoking status, pack years and finally ascertainment method to determine any differences in disease phenotype and its impact.

2. GENERAL METHODS

This chapter describes the general methodologies used in ADAPT to perform the biochemical, social, physiological and radiological assessments on the subjects studied. The manner in which the data were collated and analysed to perform each individual study is described in sections 3, 4, 5 and 6, which detail the individual research projects.

2.1 AAT Level and phenotype

The AAT level (μM) is measured at the baseline visit, and the phenotype is determined by isoelectric focusing at a central USA laboratory (Salt Lake City, Utah).

2.2 Questionnaires

Each subject completes an annual questionnaire including demographics and current symptoms such as the Medical Research Council (MRC) dyspnoea score[233], the presence of chronic bronchitis as defined by the MRC criteria [68], sputum colour in the stable state using the Bronko Test chart (BronkoTest, Middlesex, U.K.)[234], and the number and features of exacerbations of COPD using the Anthonisen criteria[70]. Subjects are asked to recall the reason they were initially tested for AATD. Those who were tested because of the presence of respiratory or liver disease are termed 'index' subjects, and those who underwent testing primarily due to family screening or population screening are termed 'non-index' subjects. All 'index' subjects described in the current studies refer to those index subjects who were initially tested for alpha-1 antitrypsin deficiency due to lung disease unless stated otherwise in the

relevant section. The database also captures information about medication use, past medical history, smoking, alcohol consumption, occupational history and family history.

At the baseline visit, subjects complete a St Georges Respiratory Questionnaire (SGRQ)[235] and the Short Form-36 (SF-36)[236]. The SGRQ is a reproducible 76 item questionnaire to assess quality of life, and is validated for use in COPD subjects[235]. The questionnaire provides 3 separate domain scores (symptoms, activity and impacts) along with a total score. The greater the score, the worse the health status, and an increase of 4 points in the total score is suggestive of a clinically significant difference[235].

The SF-36 is a 36 item questionnaire used to assess general health. It produces 8 health profile scales, which can be used to calculate a physical health summary score and a mental health summary score. The lower the score, the worse the health status, and normal values are available for the general population[237].

2.3 Pulmonary physiology

All pulmonary physiology tests are performed in accordance with the ARTP/BTS Guidelines[238]. Spirometry is measured at the initial visit, using Vitalograph Wedge Bellows (Vitalograph, Bucks, U.K.), before and after the administration of 5mg of nebulised salbutamol and again following the addition of 500 µg of nebulised ipratropium bromide 30 minutes later. For subsequent visits, spirometry is only undertaken after these medications have been administered. A significant bronchodilator response is defined as an increase in FEV1 of greater than

200ml from baseline and more than 12 % of the predicted value as per the American Thoracic Society (ATS) guidelines[239]. Carbon monoxide transfer is determined using the single breath method described by Ogilvie[240] using Benchmark TT501 (Morgan Medical, Kent, U.K.), and corrected for alveolar volume (KCO). Lung volumes are measured by the helium dilution technique described by Meneely[241] using Benchmark TT501 (Morgan Medical, Kent, U.K.).

Values are expressed as a percentage of the average value for normal subjects (% predicted) as defined by the European Community for Steel and Coal[75]. The partial pressure of oxygen (PaO2) and carbon dioxide (PaCO2) are determined in arterialised capillary blood from the earlobe, using a Radiometer ABL5 (Radiometer Ltd, Copenhagen, Denmark).

2.4 Computed tomography scans

High resolution computed tomography (HRCT) scans of the thorax are performed at the initial visit in most subjects unless a recent scan had been performed at the referring centre. A GE Prospeed Scanner (General Electrical Medical Systems, Milwaukee, USA) was utilised until March 2002, and a GE Lightspeed Scanner (General Electrical Medical Systems, Milwaukee, USA) was used subsequently. Slices of 1mm are taken at 10mm intervals through the thorax at full inspiration. A thoracic radiologist provides a radiological report for each CT scan, commenting on the presence of emphysema, bronchiectasis and any other abnormality visualised. All CT scans performed from 1996 to 2001, and CT scans for selected subjects relevant to a particular study hypothesis in subsequent years, were analysed using density mask analysis. This technique assesses the percentage of

voxels with a density of less than a fixed level, which for these studies was -910 Hounsfield units. This percentage is known as the voxel index (VI) and was calculated for the upper zone (using one slice at the level of the aortic arch) and the lower zone (using one slice at the level of the inferior pulmonary veins). These calculations were performed using a GE proprietary density mask programme integral to the CT scanner, or Pulmo-CMS software (MEDIS Medical Imaging Systems BV, Leiden, The Netherlands). These objective measures were unavailable for any scan performed at the original patient referring centres.

Emphysema distribution was assessed, with upper zone predominance defined as the upper zone voxel index (VI) being greater than the lower zone voxel index, and lower zone predominance defined as the lower zone VI being greater than the upper zone VI.

2.5 Biochemical and haematological investigations

Venous blood is taken at each visit to determine the full blood count and liver function tests, including gamma-glutamyl transferase (GGT) (Instrumentation Laboratory Ltd, Warrington, U.K.), aspartate transaminase (AST), alkaline phosphatase (ALP) and bilirubin. Serum and plasma are also stored for use in any future analyses of relevant biomarkers.

3. RADIOLOGICAL AND CLINICAL FEATURES OF SUBJECTS WITH DISCORDANT LUNG FUNCTION

3.1 Aims of the study

Of the subjects who attend ADAPT, approximately 27% have a normal FEV1 and a normal KCO (greater than or equal to 80% of predicted), 14% have an abnormal FEV1, but a normal KCO, 7% have a normal FEV1 and an abnormal KCO and 52% have abnormalities of both FEV1 and KCO.

We aimed to assess the influence of these different patterns of physiological impairment on radiological and clinical factors, in order to define further the characteristics of these phenotypes of COPD associated with AATD. It was hypothesised that subjects with an abnormality of KCO would have more upper zone emphysema and those with an abnormality of FEV1 would have more lower zone emphysema.

3.2 Method

Patients are listed on the ADAPT database in chronological order depending on the date of their baseline visit. The database was reviewed to identify the first 15 PiZ subjects to undergo a baseline visit, who had complete CT densitometry data and a normal FEV1 (> 80% of predicted), a normal FEV1:FVC ratio (>70%) and a normal KCO (>80% of predicted) at baseline (group 1); In a similar fashion, the first 10 PiZ subjects with an abnormal FEV1 and a normal KCO (group 2); the first 15 PiZ subjects with a normal FEV1, FEV1:FVC ratio and an abnormal KCO (group 3) and the first 10 PiZ subjects with an abnormal FEV1 and an abnormal KCO (group 4) were identified.

A thoracic radiologist reported the presence or absence of emphysema and bronchiectasis without previous knowledge of the subject's physiological measurements. The upper zone voxel index (UZVI) and lower zone voxel index (LZVI) were noted, and the relative distribution of emphysema was calculated as described in section 2.4. These radiological data along with the presence of chronic bronchitis defined by the MRC criteria[68], the number of exacerbations per year, the MRC dyspnoea score[233], the PaO2 while breathing room air, and the SGRQ total scores[235] were compared between the 4 physiological groups.

Statistical analyses were performed using SPSS 12.0.1 for Windows. For parametric data, the t – test was used to compare 2 categories and one way ANOVA was used to compare more than 2 categories. For non-parametric data, the Mann-Whitney test was used to compare 2 categories and the Kruskall-Wallis test was

used to compare more than 2. A p value of < 0.05 was taken as statistically significant.

3.3 Results

3.3.1 Demographic data

Data describing the demographic details and pulmonary function parameters are shown in table 3.1. 80% of subjects in groups 2 & 4 were female, but in view of the small numbers, this was not different ($p = 0.1$ and 0.1 respectively) to the proportion of females in group 1 (40%) or group 3 (53%; $p = 0.18$ and 0.23 respectively). Subjects in group 4 were older than those in group 1 ($p = 0.043$), but otherwise the groups were of a similar age. The majority of subjects from groups 1 (73%) and 3 (73%) had never smoked, whereas the majority of subjects from groups 2 (90%) and 4 (100%) were current or ex-smokers ($p<0.001$).

TABLE 3.1: Demographic data for four groups of subjects with differing patterns of lung function.

		GROUP 1 Normal FEV1 & KCO	GROUP 2 Abnormal FEV1	GROUP 3 Abnormal KCO	GROUP 4 Abnormal FEV1 & KCO
n =		15	10	15	10
Male gender	n =	9 (60%)	2 (20%)	7 (47%)	2 (20%)
Age	Mean (S.D.)	44.4 (12.6)	48.4 (4.2)	48.0 (15.6)	53.7 (6.7) **p=0.043***
Smoking status	Never n =	11 (73%)	1 (10%)	11 (73%)	0 (0%)
	Ex n =	3 (20%)	9 (90%)	3 (20%)	9 (90%)
	Current n =	1 (7%)	0 *** p=0.004**	1 (7%) **●p=0.004**	1 (10%) *** p<0.001 ¶ p=0.001**
Pack years	mean (S.D.)	2.3 (5.2)	20.7 (11.7) ***p<0.001**	4.3 (7.7) **●p=0.001**	34.1 (17.0) ***p<0.001 ¶p<0.001**
FEV1 % Predicted	mean (S.D.)	105.7 (13.2)	31.3 (5.6) ***p<0.001**	111.7 (19.6) **● p=0.001**	30.3 (8.6) ***p<0.001 ¶p<0.001**
KCO % Predicted	mean (S.D.)	97.3 (16.7)	89.0 (7.4)	69.5 (10.2) ***p<0.001 ●p<0.001**	56.8 (5.1) ***p<0.001 ●p<0.001 ¶p=0.001**
FEV1:FVC	mean (S.D.)	87.6 (10.2)	34.4 (7.4) ***p<0.001**	75.4 (11.1) **●p<0.001**	27.7 (9.3) ***p<0.001 ¶p<0.001**

The table shows demographic data for the 4 groups of PiZ subjects with differing patterns of lung function. Categorical variables are given as n= (% of group); continuous data are stated as mean (+/- S.D.). The data for pack years is derived from smokers alone.

** = significantly different from group 1.* *● = significantly different from group 2.*

¶ = significantly different from group 3.

3.3.2 CT Appearance & Densitometry

Emphysema was visible on the CT scan in 2/15 with 'normal' physiology. This proportion was greater in all groups with abnormal lung function (all of group 2, $p < 0.001$; 12/15 from group 3, $p = 0.05$; and all of group 4, $p < 0.001$).

Densitometry data are shown in table 3.2 for all groups and summarised in figures 3.1 & 3.2 for group 2 with an isolated abnormal FEV1 and group 3 with an isolated abnormal KCO.

UZ VI was greater (indicating more severe upper zone emphysema) in all groups with abnormal lung function (2, 3 & 4) than in those with normal lung function ($p = 0.003$, 0.044 & < 0.001 respectively). Subjects with an abnormality of both FEV1 and KCO (group 4) had a greater UZ VI than subjects with either an isolated abnormality of FEV1 ($p = 0.017$) or those with an isolated abnormality of KCO ($p = 0.006$).

LZ VI was also greater (indicating greater lower zone emphysema) in groups 2 and 4, compared to those with normal lung function ($p < 0.001$ & $p < 0.001$ respectively) or subjects with an isolated abnormality of KCO ($p < 0.001$ & $p < 0.001$ respectively). Those with an isolated abnormality of KCO (group 3) had a similar LZ VI to those with normal lung function ($p = 0.073$). The LZ VI in subjects with an abnormal KCO and an abnormal FEV1 was no different to those with an isolated abnormality in FEV1 ($p = 0.147$).

Groups 2 and 4, with an abnormal FEV1, had relatively more basal emphysema than group 1 with normal lung function ($p < 0.001$ and $p < 0.001$ respectively) or group 3 with an isolated abnormality of KCO ($p < 0.001$ and $p < 0.001$ respectively). Group 2 (isolated FEV1 abnormality) had the greatest relative

basal emphysema score, indicating basal dominant emphysema and group 3 had the lowest score indicating relatively more upper zone emphysema.

TABLE 3.2: CT scan densitometry data for four groups of subjects with differing patterns of lung function.

	GROUP 1 Normal	GROUP 2 Abnormal FEV1	GROUP 3 Abnormal KCO	GROUP 4 Abnormal FEV1 & KCO
Upper zone voxel index (UZVI) mean (S.D.)	13.2 (5.9)	28.1 (11.8) *p=0.003	23.0 (16.6) *p=0.044	40.4 (8.8) *p<0.001 ●p=0.017 ¶p=0.006
Lower zone voxel index (LZVI) mean (S.D.)	14.8 (9.6)	54.9 (13.3) *p<0.001	23.8 (16.1) ●p<0.001	62.8 (9.4) *p<0.001 ¶p<0.001
Relative emphysema distribution (LZVI-UZVI) mean (S.D.)	+1.6 (9.1)	+26.8 (11.2) *p<0.001	+0.8 (8.4) ●p<0.001	+22.4 (10.8) *p<0.001 ¶p<0.001

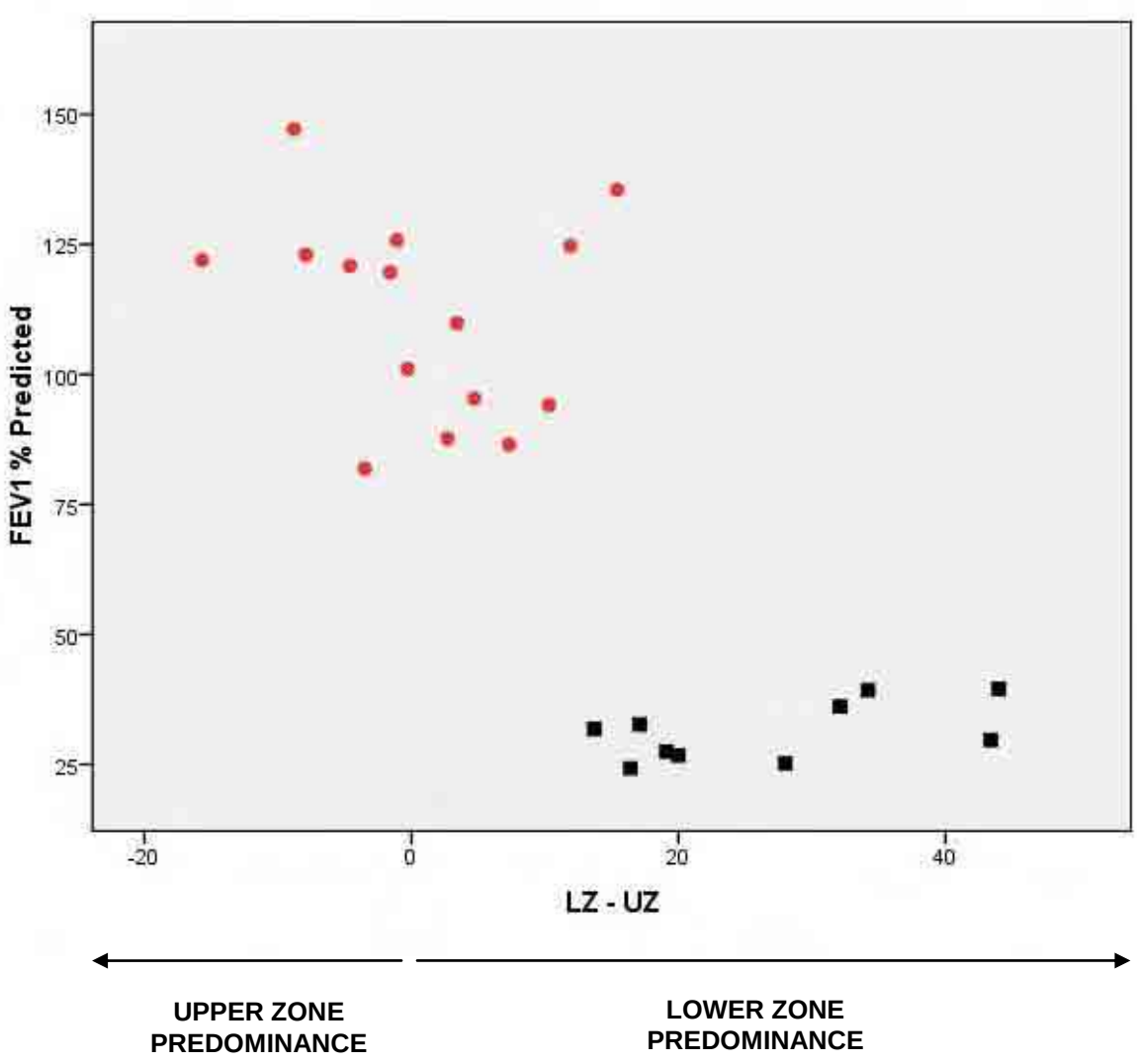
The table shows the mean (+/- S.D.) voxel index for the upper and lower zones and relative distribution of emphysema (LZ VI – UZ VI) for each group with differing patterns of lung function.

** = significantly different from group 1.*

● = significantly different from group 2.

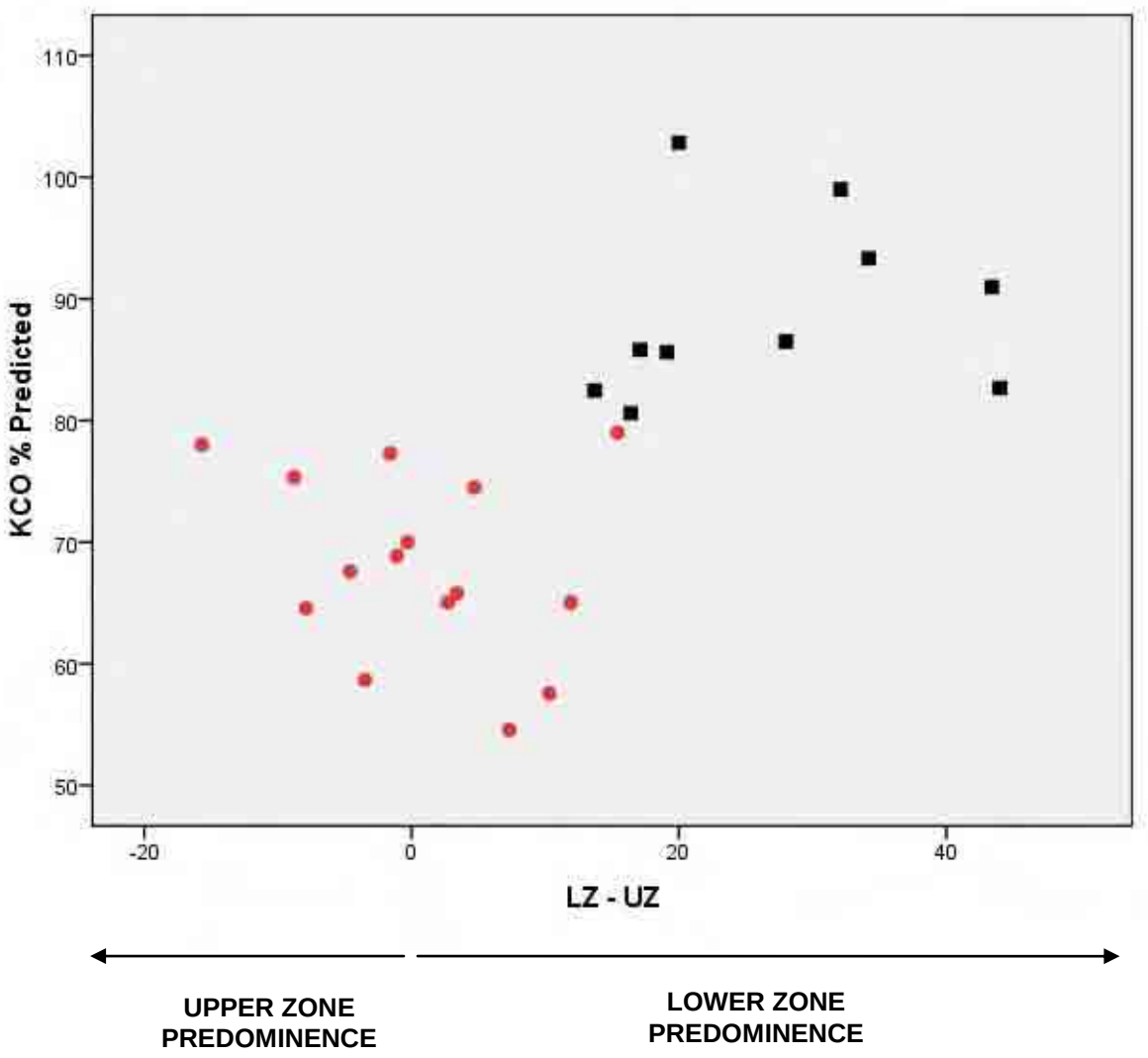
¶ = significantly different from group 3.

FIGURE 3.1: Relative emphysema distribution related to FEV1in subjects with an abnormal FEV1 & normal KCO and those with a normal FEV1 & abnormal KCO.



The figure shows the relative distribution of emphysema (LZ VI -UZ VI), and the relationship to FEV1 % predicted for each case in group 2 (■) and group 3 (●).

FIGURE 3.2: Relative emphysema distribution related to KCO in subjects with an abnormal FEV1 & a normal KCO and those with a normal FEV1 and an abnormal KCO.



The diagram shows the relative distribution of emphysema (LZ VI -UZ VI), and the relationship to KCO % predicted for each case in group 2 (■) and group 3 (●).

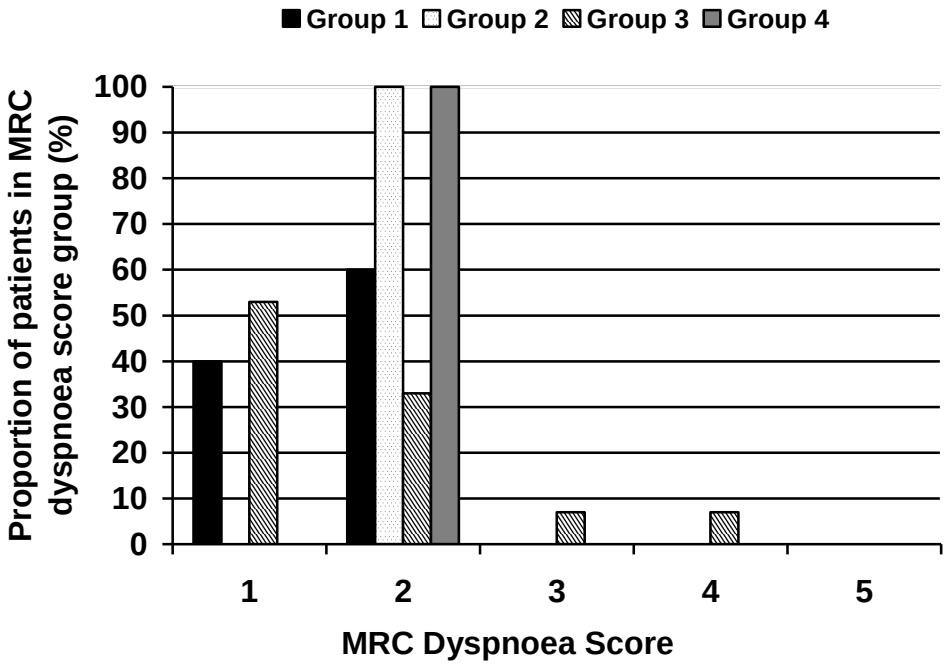
3.3.3 Symptoms

Chronic bronchitis was present in 4/15 (27%) subjects from groups 1 and 3 and in 4/10 (40%) of subjects from groups 2 and 4, but these proportions were not different between the groups (p=0.815).

The median number of annual exacerbations was 0.6 (I.Q.R. = 0 – 1.5) in group 2, compared with 0 (0 – 0) in group 1, 0 (0-1.0) in group 3 and 0 (0 – 1.1) in group 4, and again these differences between groups did not achieve statistical significance (p=0.345).

All subjects in groups 2 and 4 had mild symptomatic impairment as determined by the MRC dyspnoea score as summarised in figure 3.3. Interestingly only 40% of subjects in group 1 had a normal MRC dyspnoea score despite having normal lung function. Two subjects in group 3 had moderate to severe breathlessness as assessed by the MRC score, whereas no subjects from group 2 or 4 had this degree of dyspnoea. However, there were no statistically significant differences in MRC dyspnoea score between the four groups (p=0.248).

FIGURE 3.3: MRC dyspnoea scores in subjects with differing patterns of lung function.

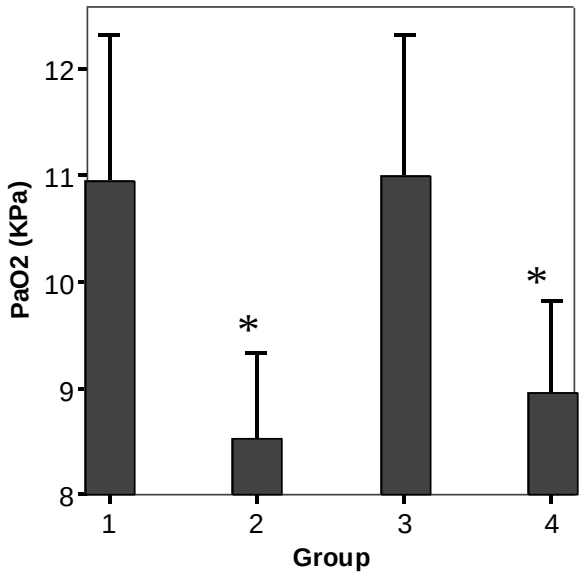


The graph shows the proportion of subjects with different MRC dyspnoea scores in each of the groups with differing patterns of lung function. A higher MRC dyspnoea score indicates more severe breathlessness.

3.3.4 PaO2

Figure 3.4 summarises the mean PaO2 for each of the groups. Groups 2 and 4 had lower PaO2 measurements than group 1 ($p < 0.001$ and $p < 0.001$ respectively) and group 3 ($p < 0.001$ and $p < 0.001$ respectively).

FIGURE 3.4: PaO2 in KPa for each group with differing patterns of lung function.



The columns represent the mean PaO2 and the bars represent the standard deviation (S.D.). The asterix marks a significant difference compared with groups 1 and 3 (see text).

3.3.5 Health status

Total SGRQ scores were greater, (indicating a worse quality of life) in group 2 (mean =56.8, S.D. = +/- 15.3; $p < 0.001$), group 3 (29.7 +/- 24.1; $p = 0.048$) and group 4 (59.2 +/- 16.7; $p < 0.001$) compared to those with normal lung function (17.7 +/- 11.5). There was no difference in total SGRQ scores between group 2 and group 4 ($p = 0.894$), although both these groups had worse scores than those in group 3 ($p = 0.01$ and $p = 0.01$ respectively).

3.3.6 Summary of the results

Differences existed between the different physiological groups with respect to age, smoking history, emphysema (prevalence, distribution and extent), PaO2 and health status, demonstrating that different physiological phenotypes are associated with different radiological and clinical features.

Generally the 2 groups with abnormalities of FEV1 consisted of more smokers, subjects with greater severity of emphysema, lower PaO2 and worse health status than groups with normal lung function or those with an isolated abnormality of KCO. However, subjects with an abnormal KCO in addition to an abnormal FEV1 had more extensive upper zone emphysema, even though it was not associated with a worse health status or PaO2 compared with subjects who had an isolated abnormality of FEV1.

Subjects with an isolated abnormality of KCO had more emphysema and a worse health status than those with normal lung function. They also had a more apical distribution of emphysema compared with subjects with an isolated abnormality of FEV1 who had the most basal distribution.

3.4 Discussion

The current findings exploring the relationship between lung function and emphysema demonstrate that FEV1 is associated with the most lower zone emphysema predominance whereas KCO is associated with the most upper zone emphysema predominance. These data support the results obtained by Dowson et al.[107] and Parr et al.[108].

Gurney et al.[242] studied 59 non-deficient subjects who had been heavy smokers, 42% of whom had a normal FEV1 and DLCO, 25% of whom had abnormalities of FEV1 and DLCO and the remainder who had either an isolated abnormality of FEV1 or an isolated abnormality of DLCO. Two thirds of the subjects had evidence of emphysema on CT scanning, presumed to be usual smoking-related emphysema, with the upper zones being affected in 59% of the subjects, and the lower zones in 39%. Gurney compared pulmonary function tests to CT density mask analysis using the -910HU threshold. Contrary to the current study results, Gurney suggested that DLCO correlated with lower zone densitometry to a greater extent than FEV1, and that lower zone densitometry generally correlated with lung function better than upper zone densitometry. However it should be noted that DLCO is not corrected for alveolar volume. It represents overall gas uptake and is influenced by both distribution (i.e. not getting to the gas exchanging areas) as well as reduced alveolar surface area. Obstruction of small airways will thus also influence this measure and may explain why lower zone changes correlate best where airflow obstruction may have a more significant effect.

Gurney's explanation for this phenomenon was that the lower zone of the lung made a much greater contribution to total lung volume than the upper zone, and

therefore, a relatively large degree of emphysema in the upper zone would have relatively little impact on lung function parameters, as the greater intact bulk of tissue in the lower zone would act as good reserve, thereby relatively sparing pulmonary function abnormality and it's decline. This would certainly be consistent with the observation that FEV1 is strongly associated with lower zone emphysema in the current study.

However, the relationship between abnormalities in KCO and upper zone emphysema demonstrated in the current study cohort are consistent with this area being important in overall gas exchange. This could be explained by changes in the ventilation perfusion ratio in the presence of basal emphysema. Normally, the ventilation perfusion ratio in the upper zones is greater than in the lower regions [243]. Therefore, deficiencies in gas transfer caused by reduced ventilation and perfusion in the presence of basal emphysema can be compensated for by the upper zones, by increasing perfusion of this region as a result of the increase in pulmonary perfusion pressure caused by basal lung disease. The result would enable KCO to be relatively preserved in predominantly basal disease, which is far more prevalent in this AATD cohort, but not if the emphysema is predominantly apical, as in the Gurney cohort.

A further explanation for the association between FEV1 and lower zone predominant emphysema demonstrated in the current study is that the lower lobe airways start to close earlier in expiration [244]. The presence of emphysema in the lower zones would exaggerate this due to dynamic collapse of the airways because of paucity of supporting tissue, and thereby also contribute to a reduction in FEV1which acts as a surrogate for emphysema in this region.

In the current cohort, subjects with an abnormal FEV1 had greater relative distribution of emphysema at the bases, which is consistent with these concepts. Also, subjects with an isolated abnormality of KCO had the least basal emphysema relative to the apical region and the addition of an abnormal KCO to an abnormal FEV1 was associated with a further increase in upper zone but not lower zone emphysema. These observations add further credence to the concept that the upper zones are more likely to reflect gas transfer abnormalities than the lower zones, at least in AATD.

The optimal density threshold for performing density mask analysis is still a matter of controversy. Although studies in usual COPD have shown that density mask analysis using a threshold of -950HU correlates best with macroscopic and microscopic emphysema measured from pathological specimens, no such pathological studies have been done in AATD. However densitometry using the threshold of -910HU has been shown to relate to physiology, health status[107], decline in lung function[245] and mortality[85] in subjects with AATD. Furthermore, in a study of 33 subjects with AATD, the correlations of voxel indices to FEV1, KCO and TLCO were better using a threshold of -910HU than -950HU [246]. Finally, the -950HU threshold sensitivity is influenced by disease severity[247]. In early disease, -950HU is relatively insensitive to small changes in lung density, whereas unpublished data from our group suggest that using a threshold of -910HU is more sensitive. For these reasons, density mask analysis was performed using a threshold of -910HU.

The current study also raises issues concerning the accepted definitions of COPD, its severity and treatment, all of which are based on the presence of airflow obstruction[33,248,249]. Subjects have been identified who have no airflow obstruction yet

have evidence of emphysema on a CT scan, with or without an isolated abnormality of gas transfer and reduced health status.

Two of the fifteen subjects in the current study with no evidence of airflow obstruction and a normal KCO had evidence of emphysema. Gurney et al.[242] also described, however, that in non-deficient heavy smokers, 40% of subjects with normal lung function had CT evidence of emphysema.

On the other hand, 80% of subjects with an isolated abnormality of KCO in the current study cohort had radiological evidence of emphysema, associated with a reduced health status. Emphysema in these subjects was generally mild in severity and evenly distributed throughout the lung. Despite the poor health status in this group compared with subjects with normal pulmonary physiology, there was no difference in the mean MRC dyspnoea score, annual number of exacerbations or the proportion with chronic bronchitis. However, there was a greater range of dyspnoea reported in this group compared to the other groups, with some subjects reporting no dyspnoea, and others reporting severe dyspnoea.

The fact that subjects can be identified who have evidence of emphysema and / or reduced gas transfer but no airflow obstruction suggests that gas transfer and CT scan should be used to assess all subjects suspected of having COPD, and investigations should not be confined to spirometry alone, particularly in the presence of symptoms.

It remains to be seen if subjects from the 4 different physiological groups progress from one group to another (for example, do subjects in group 1, with or without CT scan evidence of emphysema subsequently develop either airflow obstruction alone, or an impaired gas transfer alone, and eventually progress so that

both FEV1 and KCO become abnormal), or do they represent a completely separate phenotype. Long term prospective studies such as the Swedish study described earlier[72] are required to address this issue in AATD. However, unfortunately gas transfer and CT scans have not routinely been performed in the Swedish cohort to date.

In usual COPD, evidence that an isolated abnormality of KCO is related to early emphysema was demonstrated previously by Klein et al.[115], who found that 62.5% of smokers who had recently complained of dyspnoea, had a normal chest X-ray but had visible emphysema on the CT scan, and were shown to have an isolated abnormality of KCO. In addition, Tylen et al.[250] showed that 44% of asymptomatic smokers had evidence of emphysema on CT scanning compared to 3% of subjects who had never smoked, and those with emphysema had a significantly lower KCO but a similar FEV1 compared to the subjects without emphysema. Both of these studies in usual COPD support the current findings.

However, these studies also relate to subjects who were smokers, and the majority of subjects in the present study from groups 1 and 3 had never smoked. It is possible that AATD influences the way different physiological phenotypes develop. For example, non-smoking subjects may develop upper zone emphysema first, with an associated reduction in the KCO, but the addition of smoking leads to more extensive basal emphysema in which airflow obstruction predominates. This is contrary to the process in usual COPD, where smoking is the major risk factor, and the emphysema predominantly affects the upper zone. It is possible that other genetic modifiers exist in AATD, in a similar way to the matrix metalloproteinase (MMP) 9 (C-1562T) polymorphism in usual COPD [251]. This polymorphism may

influence the distribution of non-smoking related emphysema in AATD but this concept clearly requires further study.

Whether the different physiological groups represent subjects at different stages of lung disease or whether each physiological phenotype is a separate entity, influenced by environmental or other genetic factors requires further investigation. This may be crucial to the development of future treatment strategies for AATD in order to reduce decline in lung function at an earlier stage and / or to minimise the deterioration in health status observed in these individuals depending on their physiological phenotype.

An interesting observation from the current study is that even AATD subjects with normal lung function have impaired health status (mean SGRQ score of 17.7 +/- 11.5) compared with a general population of a similar age (mean SGRQ score 5.8). This may be related to symptoms of breathlessness, (60% described being breathless while walking on the level or up a slight hill), or chronic bronchitis, which was reported in 27%. In addition, 2 subjects with normal lung function reported having exacerbations and a further 2 had emphysema visible on the CT scan. These symptoms have previously been associated with a worse SGRQ score in subjects with COPD[252]. Furthermore, in subjects with AATD, the presence of chronic bronchitis[84], and CT measures of emphysema severity[83] have also been associated independently with the SGRQ, and these factors may also explain (at least in part) impaired health status in subjects with AATD and normal lung function.

Subjects with an isolated abnormality of KCO had a worse health status than those with normal lung function. By definition, this group had a worse mean KCO than group 1, and KCO does reflect a worse SGRQ score in subjects with AATD[107].

A much greater proportion of subjects from group 3 had emphysema on the CT scan (80%) compared to group 1 (13%), and this was reflected as a difference in the UZVI but not the LZVI between the groups. Worse UZVI scores have also been shown to be associated with worse SGRQ scores in AATD [83], but no data are available regarding the influence the subjective description of visible emphysema on the CT scan on the SGRQ. In addition, some subjects from group 3 described having moderate to severe breathlessness (MRC grade 3-4), whereas no subjects from group 1 were dyspnoeic to this level. As SOB is associated with a worse health status in COPD[252], it is possible that this factor also contributed to the difference in health status between groups 1 and 3.

Groups 2 and 4 had worse mean SGRQ scores than groups 1 or group 3. Groups 2 and 4 had worse FEV1 and LZVI measurements than groups 1 and 3, and subjects from groups 2 and 4 had smoked more. As all these factors have been independently associated with SGRQ scores in AATD, even after correction for other associated factors, the observed differences in health status remain predictable.

No difference in health status was observed between groups 2 and 4 despite differences in KCO, UZVI and age, which have previously been shown to influence the SGRQ scores in AATD[84,107]. However, compared to FEV1, LZVI and pack years smoked, the relationships of these variables to health status is relatively weak, and may not be adequate to result in a significant difference in health status.

In summary different patterns of physiological abnormalities reflect differences in the radiological distribution of emphysema in AATD. Subjects with an isolated abnormality of KCO clearly have pathological and clinical abnormalities. Furthermore

even subjects with lung function in the normal range have some evidence of emphysema and symptoms consistent with impaired health status.

4. AGE AT WHICH RADIOLOGICAL, PHYSIOLOGICAL AND HEALTH STATUS MEASURES DEVIATE FROM NORMAL IN NON-INDEX ALPHA-1 ANTITRYPSIN DEFICIENCY

4.1 Aims of the study

The aim of the current study was to determine the age at which spirometric, gas transfer, HRCT densitometric and health status measures in subjects with alpha-1 antitrypsin deficiency start to deviate from values expected in the normal healthy population, in order to provide a more informed approach to monitoring for the early identification of lung disease. It was hypothesised that measures of gas transfer and CT densitometry would start to deviate from normal prior to spirometry.

4.2 Methods

All 591 PiZ subjects who were included on the ADAPT database at the time this study was performed were included and data from their baseline visit were analysed. FEV1, the ratio of FEV1 to forced vital capacity (FEV1:FVC) and carbon monoxide transfer factor corrected for alveolar volume (KCO) were measured and expressed as a percentage of predicted[75].

A high resolution CT scan (HRCT) had been performed for 563 subjects, and density mask analysis was available for 368, to determine the proportion of CT voxels with a density of less than -910 HU (Hounsfield Units) in the upper zone (UZVI) and lower zone (LZVI) as described earlier. The presence or absence of visible emphysema on CT was determined independently by a thoracic radiologist. Subjects' St Georges Respiratory Questionnaire (SGRQ) and demographic data, including smoking history and the reason for initial diagnosis of AATD were obtained from the ADAPT database.

The proportions of subjects with abnormal results was taken as those with an FEV1, FEV1:FVC and KCO of less than 80% of the predicted values for age, gender and height[75]. There are no defined reference ranges for SGRQ scores, UZVI or LZVI, so we calculated the proportion of subjects with a score greater than 1.96 standard deviations above the mean obtained from a healthy population[106,253], based on a theoretical reference range encompassing values obtained in 95% of healthy subjects. These calculated normal values are shown in table 4.1 below.

TABLE 4.1: Mean and reference ranges calculated for St Georges Respiratory Questionnaire Total Scores, Upper Zone Voxel Index and Lower Zone Voxel Index.

	CALCULATED MEAN VALUE	CALCULATED REFERENCE RANGE
SGRQ Total score	8.41	0 – 30.61
Upper zone voxel index	5.0	0 – 16.76
Lower zone voxel index	13.0	0 - 44.36

The reference ranges are calculated as being between 1.96 standard deviations above and below the mean, based on a theoretical reference range encompassing values obtained in 95% of subjects. Data are derived from previously published values in subjects from a general population in whom lung disease had been excluded[106,253] .

The age at which test parameters start to decline was determined by 2 methods.

Firstly, the cohort was split into 5-year age strata, and the proportion of subjects in each strata who had values 'worse' than the healthy population mean was determined for each measure. The earliest age group in which this proportion was consistently greater than 50% was determined.

Secondly, a mathematical model was constructed including all subjects, using forward logistic regression (SPSS 12.0.1 for Windows, SPSS, Chicago, IL), with FEV1 <100% predicted as the dichotomous dependant variable, and age, gender, smoking status and method of ascertainment as co-variates. Using the model, an

equation was produced to determine the probability of the FEV1 being <100% predicted, expressed in terms of the covariates stated above. Coefficients for these covariates were inserted into the equation to represent the patient ascertainment, smoking status and gender. By definition, in a healthy population, it is expected that half of subjects will have an FEV1 <100% predicted. We therefore inserted different ages into the equation to determine the earliest age at which the probability of the FEV1 being <100 % predicted was consistently greater than 0.5 (i.e. started to deviate from normal).

Similar models were constructed for FEV1:FVC ratio, KCO, SGRQ total score, upper zone voxel index (UZVI) and lower zone voxel index (LZVI) being less than or greater than the respective mean for a healthy population as the dichotomous dependant variables. The mean values used for the healthy population were 100% predicted for FEV1:FVC and KCO, and previously published normal values related to age for the SGRQ[253]. No normal values for density mask analysis at a threshold of -910HU have been published. Therefore, previously published values determined using a GE Prospeed CT scanner with a density mask analysis threshold of -912HU, in subjects who had never smoked and had no evidence of lung disease on pulmonary physiology and HRCT were used[106] .

Using SPSS 12.0.1 for Windows (SPSS, Chicago, IL), data were compared between index & non-index subjects (identified by family screening) and subjects who had smoked or not. The Chi Squared and Fishers exact tests were used to compare categorical data, the one-way ANOVA test was used if continuous data were parametric and the Kruskal-Wallis test was used for non-parametric data.

Subjects gave written informed consent, and the study was approved by South Birmingham Research and Ethics Committee.

4.3 Results

4.3.1. Subject demographics

Demographic data are shown below in table 4.2 for the 591 subjects divided into index and non-index cases and smoking status groups.

TABLE 4.2: Demographic, physiological, radiological and health status data.

Data are presented as mean (± SD) if parametric and median (IQR) if non-parametric unless otherwise stated.

	NON-INDEX NEVER SMOKED (n = 54)	**NON-INDEX EX/CURRENT SMOKERS** (n = 95)	**INDEX NEVER SMOKED** (n = 90)	**INDEX EX/CURRENT SMOKERS** (n = 352)
Age	44.8 (13.8)	46.9 (11.4)	60.5 (11.4)*†	49.7 (9.1)*‡
Male gender n (%)	18 (33.3%)	49 (51.6%)*	53 (58.9%)*	227 (64.5%)*†
Pack years smoked	N/A	12.0 (5.0-21.5)	N/A	21.0 (12.8-28.0)
FEV1 %Predicted	102.9 (26.7)	76.3 (29.3)*	65.2 (28.5)*†	43.2 (19.6)*†‡
FEV1:FVC %Predicted	95.0 (20.9)	71.9 (23.2)*	63.3 (23.7)*†	45.4 (16.9)*†‡
KCO %Predicted	91.0 (20.9)	77.4 (21.5)*	73.6 (24.2)*	62.3 (19.8)*†‡
SGRQ Total score	22.4 (20.2)	37.5 (24.3)*	42.2 (17.7)*	54.2 (18.0)*†‡
Upper zone voxel index (%)	16.1 (13.9) (n = 35)	20.7 (15.4) (n=58)	28.9 (17.1)*† (n=47)	35.9 (16.4)*†‡ (n=227)
Lower zone voxel index (%)	*19.5 (16.6) (n = 35)*	*31.7 (19.8)* (n=58)*	*40.0 (22.1)* (n=47)*	*51.9 (16.2)*†‡ (n=227)*

**= p<0.05 compared with non-index never smoked.*

† = p<0.05 compared with non-index ex/current smokers.

‡ = p<0.05 compared with index never smoked.

4.3.2. Proportion of subjects with abnormal test parameters

The proportion of subjects with abnormal physiological, radiological and health status parameters (see definitions in section 4.2) are shown below in table 4.3 for index and non-index subjects depending on the smoking status.

TABLE 4.3: Percentage of subjects with abnormal physiological, radiological and health status parameters at baseline.

	NON-INDEX NEVER SMOKED (n = 54)	**NON-INDEX EX/CURRENT SMOKERS** (n = 95)	**INDEX NEVER SMOKED** (n = 90)	**INDEX EX/CURRENT SMOKERS** (n = 352)
FEV1 < 80 % predicted	16	52*	72*†	95*†‡
FEV1:FVC < 80 % predicted	16	61*	73*	96*†‡
KCO < 80 % predicted	19	54*	60*	84*†‡
SGRQ Total score >1.96 S.D. above mean	29	59*	73*†	89*†‡
UZVI >1.96 S.D. above mean	40	48	67*	86*†‡
LZVI >1.96 S.D. above mean	9	36*	52*	73*†‡
Emphysema on CT scan	20 (n=49)	54* (n=86)	64* (n=88)	69*† (n=340)

**= p<0.05 compared with non-index never smoked.*

† = p<0.05 compared with non-index ex/current smokers.

‡ = p<0.05 compared with index never smoked.

4.3.3. Age at which test parameters start to deviate from a healthy population

4.3.3.1. 5-year age strata method

The numbers of subjects in each 5 year age strata are shown below in table 4.4.

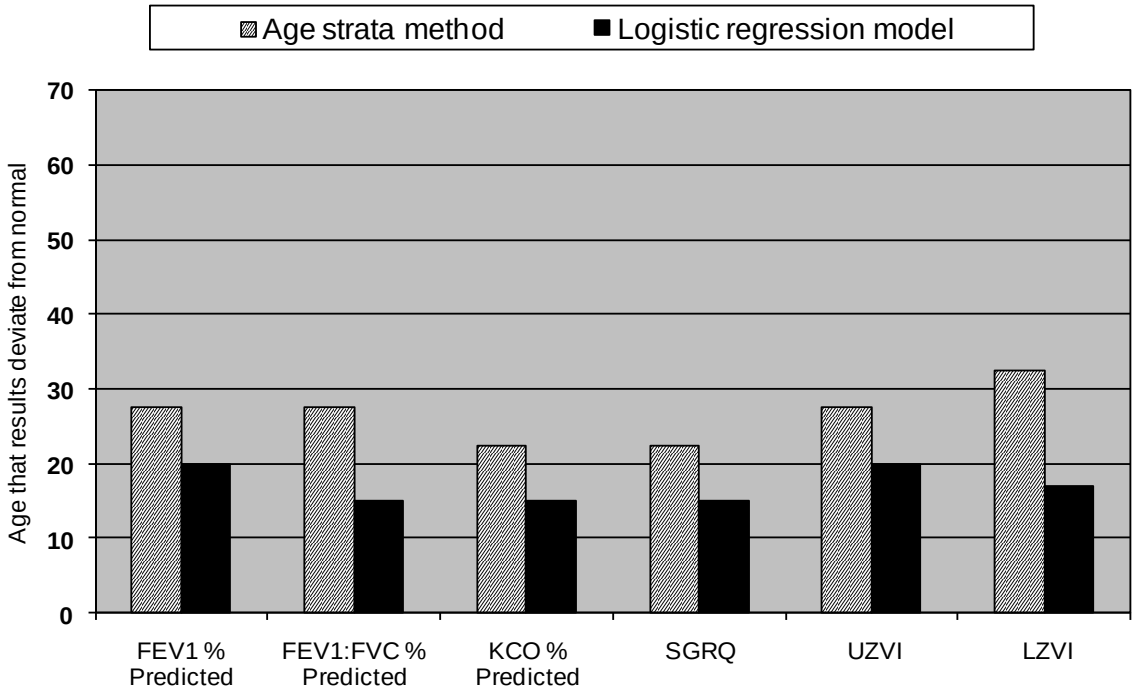
TABLE 4.4: Number of subjects in each age strata

AGE STRATA	NUMBER OF SUBJECTS IN AGE STRATA
15-20	2
20-25	7
25-30	11
30-35	27
35-40	60
40-45	75
45-50	101
50-55	107
55-60	87
60-65	56
65-70	32
70-75	18
75-80	4
80-85	2
85-90	1

Using the 5-year age strata method and forward regression analysis (see later), all parameters had deviated from the mean normal value between the ages of 20 and 32 (figure 4.1).

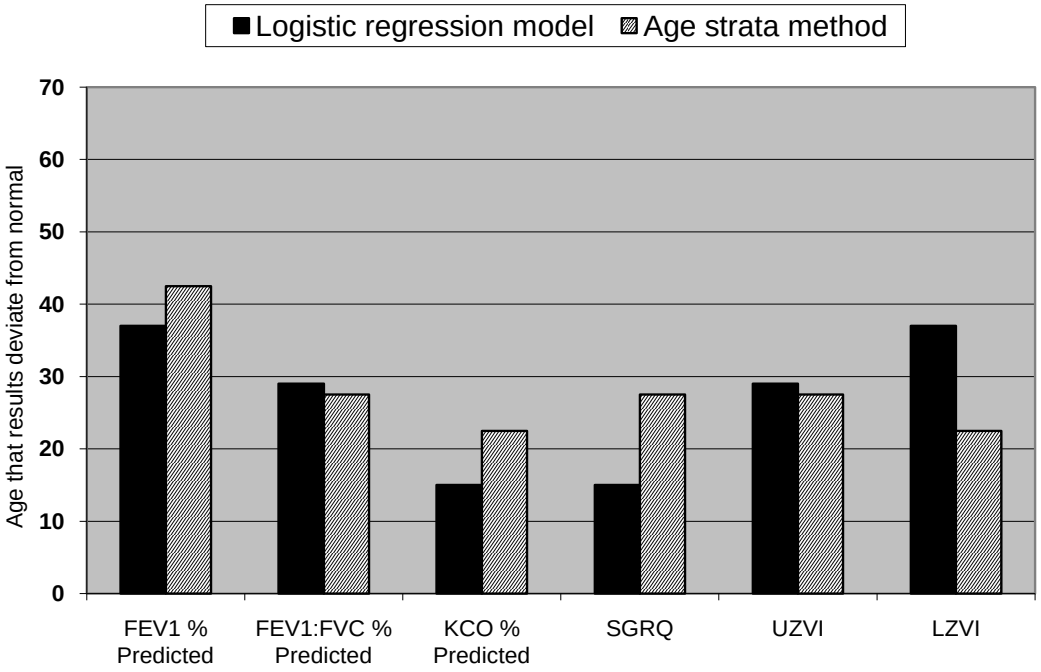
However, index cases accounted for 75% of subjects and (by definition) were likely to have had abnormal test results at presentation to health care services. The analysis of non-index cases alone gave different results, with the FEV1 deviating from normal in the age range 40-45 whereas other parameters deviated in the 20-25 and 25-30 age cohorts. Again similar results were obtained with the logistic regression analysis (see later) as shown in figure 4.2.

FIGURE 4.1: Age at which physiological, radiological and health status parameters had deviated from normal values *(defined in section 4.2)* ***for the whole population****.*



The columns represent the age at which the various physiological, radiological and health status parameters started to deviate from those expected in a normal healthy population. The columns filled with diagonal lines represent the values obtained using the age strata method and the solid black columns represent values obtained using the logistic regression model.

FIGURE 4.2: Age at which physiological, radiological and health status parameters had deviated from normal values *(defined in section 4.2)* ***for non-index subjects only****.*



The columns represent the age at which the various physiological, radiological and health status parameters started to deviate from those expected in a normal healthy population for non-index subjects. The columns filled with diagonal lines represent the values obtained using the age strata method and the solid black columns represent values obtained using the logistic regression model.

4.3.2.2. Logistic regression method

Using the initial model, gender was not an important determinant of any variable so was not included in any predictive equations. The equations generated for each variable are shown in table 4.5.

TABLE 4.5: Equations derived from logistic regression using age and index case status as covariates.

$P\ (\text{FEV1 \% Predicted} < 100) = 1/(1+e^{-(-1.704+0.047(age)+2.742(I))})$
$P\ (\text{FEV1:FVC \% Predicted} < 100) = 1/(1+e^{-(-1.708+0.059(age)+2.601(I))})$
$P\ (\text{KCO \% Predicted} < 100) = 1/(1+e^{-(-0.113+0.03(age)+1.223(I))})$
$P\ (\text{SGRQ Total score} > \text{mean of healthy population}) = 1/(1+e^{-(-0.0681+0.051(age)+3.157(I))})$
$P\ (\text{UZVI} > \text{mean of healthy population}) = 1/(1+e^{-(-2.385+0.085(age)+1.846(I))})$
$P\ (\text{LZVI} > \text{mean of healthy population}) = 1/(1+e^{-(-2.581+0.071(age)+2.245(I))})$

The probability of each test parameter being worse than the population mean was calculated as a function of age and index case status. The age at which this probability consistently exceeded 0.5 (i.e. greater than expected in the healthy population) was determined.

P (x) refers to the probability of event x occurring. e = the base of natural logarithms. I = 1 for index cases or 0 for non-index cases.

Most of the index cases had a least one physiological (99.5%), radiological (98.2%) or health status (99.3%) parameter worse than the healthy population mean at their first visit, which is reflected in the ages at which deviations from normal were predicted to occur. These predicted ages were less than the age of the youngest subject at their baseline visit, because most of the data had to be derived by extrapolation. Therefore, this model could not be used to determine, with confidence, the ages at which test parameters started to deviate from normal in these index subjects.

However, the results obtained for non-index cases did not involve data extrapolation but utilised primary information. The ages at which each variable is predicted to deviate from normal are shown in figure 4.2 for all non-index cases.

The SGRQ and KCO were predicted to deviate from normal before the age of 16. Following this, the UZVI and FEV1:FVC were predicted to deviate from normal at age 29, followed by the LZVI and FEV1 (age 37).

To explore the model further in non-index subjects, the logistic regression analysis was repeated using smoking status as an additional covariate. The equations generated for each variable are shown in table 4.6. Smoking status was related to all variables except UZVI.

For non-index never smokers, the earliest test predicted to deviate from normal was the SGRQ and UZVI (age 29), followed by KCO (age 32), FEV1:FVC and LZVI (age 50) and finally FEV1 (age 63). These results are depicted in figure 4.3 below.

For non-index ex and current smokers, SGRQ and KCO were again among the earliest results predicted to deviate from normal (age < 16), followed by

FEV1:FVC (age 17), FEV1 (age 24) then UZVI and LZVI (age 29). These results are depicted in figure 4.4 below.

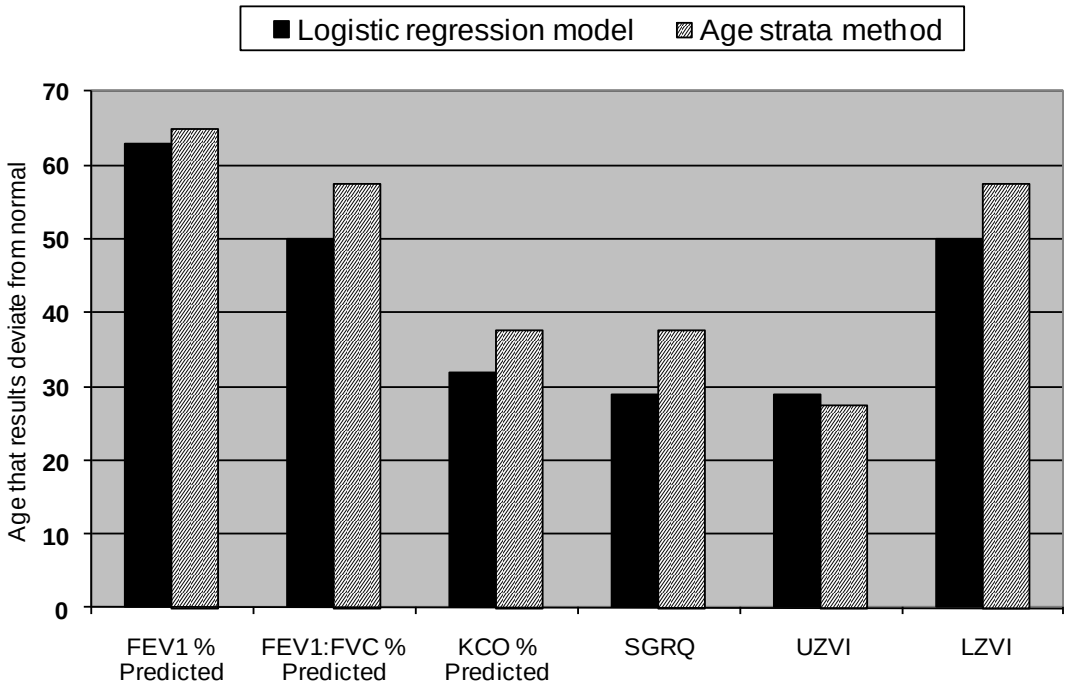
TABLE 4.6: Equations derived from logistic regression using age, index case status and smoking status as covariates.

P (FEV1 % Predicted <100) = $1/(1+e^{-(-3.752+0.06(age)+2.546(I)+2.319(S))})$
P (FEV1:FVC % Predicted <100) = $1/(1+e^{-(-3.356+0.068(age)+2.25(I)+2.257(S))})$
P (KCO % Predicted <100) = $1/(1+e^{-(-1.183+0.038(age)+0.91(I)+1.358(S))})$
P (SGRQ Total score > mean of healthy population) = $1/(1+e^{-(-1.383+0.049(age)+2.856(I)+1.434(S))})$
P (UZVI > mean of healthy population) = $1/(1+e^{-(-2.385+0.085(age)+1.846(I))})$
P (LZVI > mean of healthy population) = $1/(1+e^{-(-3.628+0.074(age)+1.932(I)+1.523(S))})$

The probability of each test parameter being worse than the population mean was calculated as a function of age, index case status and smoking status (where it was a significant predictor). The age at which this probability consistently exceeded 0.5 (i.e. greater than expected in the healthy population) was determined.

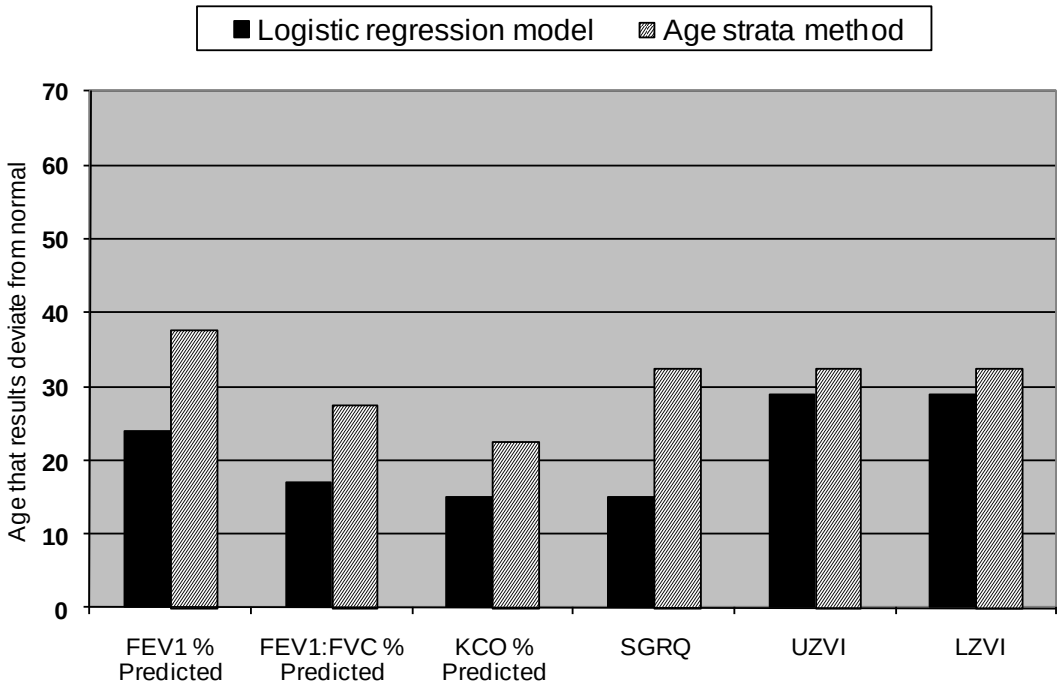
P (x) refers to the probability of event x occurring. e = the base of natural logarithms. I = 1 for index cases or 0 for non-index cases. S = 1 for ex and current smokers or 0 for subjects who have never smoked.

FIGURE 4.3: Age at which physiological, radiological and health status parameters had deviated from normal values *(defined in section 6.2)* ***for non-index subjects who had never smoked****.*



The columns represent the age at which the various physiological, radiological and health status parameters started to deviate from those expected in a normal healthy population for non-index subjects who had never smoked. The columns filled with diagonal lines represent the values obtained using the age strata method and the solid black columns represent values obtained using the logistic regression model.

FIGURE 4.4: Age at which physiological, radiological and health status parameters had deviated from normal values *(defined in section 6.2)* ***for non-index subjects who had previously or who currently smoke****.*



The columns represent the age at which the various physiological, radiological and health status parameters started to deviate from those expected in a normal healthy population for non-index subjects who had previously or who currently smoke. The columns filled with diagonal lines represent the values obtained using the age strata method and the solid black columns represent values obtained using the logistic regression model.

4.4 Discussion

Using two different methods (one observational and one predictive), the current study demonstrated for the first time that KCO deviates from normal up to 30 years before spirometry in non-index subjects with AATD. This was associated with a deterioration in health status and CT densitometric parameters especially in subjects who have never smoked, and may explain why a high proportion of a cohort of Swedish subjects with AATD and normal spirometry complain of breathlessness at the age of 30[73].

Previous studies have suggested that spirometry becomes abnormal from age 50-65 in subjects who have never smoked[88,89] and from 25 years in those who have[90]. This is consistent with the current observations from the age strata study and the predictions from logistic regression. Although no similar age predictions have been quoted for non-index subjects per se, Seersholm et al.[90] noted that they tended to have higher spirometry values than index subjects, and FEV1% predicted was <70% in only 3/27 (11%), again in general agreement with the current study.

There are little previous data available regarding the age at which gas transfer starts to deviate from normal in AATD. However, it was measured in a small cohort of subjects (n=4 current smokers and n=52 subjects who had never smoked) from the Swedish cohort described earlier[74] at the age of 30. The mean KCO was 81% of predicted in smokers and 99% of predicted in those who had never smoked. This is again consistent with the current work, which suggests KCO starts to deviate from that expected in a normal population well before the age of 30 in smokers, although after the age of 30 in those who have never smoked. Furthermore, the mean FEV1 was normal in the 30 year old Swedish cohort irrespective of smoking history,

whereas FEV1/FVC ratio was slightly reduced (91%) in PiZZ smokers but not in those who had never smoked. This is also in keeping with the current predictive study.

No previous data are available regarding the age at which health status and CT scans become abnormal in AATD. However, a high proportion of subjects from the Swedish cohort had symptoms of breathlessness, wheeze and sputum production from the age of 16, despite spirometry being normal at 30 years of age[73], suggesting that pathological changes and hence clinical symptomatology occur before spirometry becomes abnormal. This is again consistent with the observations presented here, showing deviation of health status, KCO and, in subjects who have never smoked, CT densitometry, several years before spirometry.

It is of interest that upper zone emphysema was one of the earliest tests to deviate from normal (around the age of 29) in non-index subjects, but one of the last tests to deviate from normal in smokers, in whom gas transfer and health status were observed or predicted to deviate first. It is possible that AATD in the absence of other environmental factors is related initially to the development of mild upper zone emphysema. However with the added environmental factor of smoking, lower zone emphysema develops and predominates by the time a delayed diagnosis[254] is made, leading to the observation that the classical distribution of emphysema in AATD is basal.

The current work also documented that the LZVI becomes abnormal at a similar time to spirometry. This is consistent with our previous studies which demonstrated a close relationship between spirometry & lower zone emphysema[108,230].

There are, however, some limitations to the current study, most notably that the design is retrospective and based on deviation from a normal range rather than a prospective documentation of trends. A cohort study such as for Swedish subjects described earlier[72] is therefore ideal for these purposes. Unfortunately the follow-up to date (30 years) has in the most part concentrated on spirometry and symptomatology alone. Thus, it will be several decades before the Swedish study yields results which provide retrograde guidance for monitoring non-index subjects appropriately and cost effectively using FEV1 alone. KCO has been assessed in a low number of subjects at only one point in time, at which point it had already started to deviate from normal in the smoking subgroup[74]. Furthermore, health status and comprehensive CT data have not been assessed in the Swedish cohort, thus the opportunity to obtain valuable information indicating when these tests start to deteriorate has probably passed. It would therefore seem critical to measure gas transfer at all subsequent visits, and to obtain CT scans for the majority of this cohort now, especially in the 40% who have dyspnoea. However, the results of this current study for non-index subjects, and the limited Swedish study that examined gas transfer, suggest these data are likely to have deviated from normal already, and should be monitored from the teenage years onward.

A number of subjects did not have a CT scan in the current study. This was usually omitted in asymptomatic subjects with normal lung function or where there were anxieties about the test such as claustrophobia and radiation exposure or previous scans obtained elsewhere that could not be analysed retrospectively by densitometry, and may possibly present some selection bias.

Furthermore it is difficult to make retrospective assumptions about index cases, as most had abnormal respiratory test parameters at the first visit to the UK AATD registry. Thus any attempt to determine the age at which these became abnormal requires extrapolation of the model well beyond valid data points, rendering the results difficult to interpret. However, clinically it is not relevant to determine when to test index subjects, as by definition, they have already presented with lung disease and automatically undergo investigation.

There are few young subjects in the current cohort especially among index cases. Our youngest index case was 26.4 years old at diagnosis. The FEV1 was 87.5% predicted whereas the KCO was 65% predicted, indicating that deterioration of lung function must have occurred below this age affecting KCO preferentially, again consistent with the concept of screening early using non-spirometric tests.

The non-index cases in our cohort are predominantly family members of index subjects and an influence of shared genetic susceptibility factors cannot be ruled out. However, if these are present they are likely, if anything, to result in the development of lung disease at an earlier age than non-index subjects with no affiliated index case (such as those identified by population screening). Since by definition all individuals identified by screening alone are non-index cases our observations and recommendations regarding the age at which screening should commence would also capture the onset of disease in those subjects.

Two different methods were used to determine the age at which test parameters become abnormal, and reasonable agreement was obtained between both. The advantages of the logistic regression model include the fact that a linear relationship between age and each test parameter is not necessarily assumed, and

outlying data points have less impact on the predictions than in the 5 year age strata method. Despite the high level of consistency of results obtained using both methods, the true validity of this type of mathematical modelling can only be absolutely confirmed by a future prospective study. However the data do provide guidance to suggest potent evaluation should include more extensive physiological testing especially in early adulthood.

Both methods rely on using 'normal' values for a healthy population for each test parameter. For physiology testing, these values are robust and used frequently[75]. However, little data are available regarding 'normal' values for the SGRQ[253] and no normal data for CT densitometry using the -910HU threshold is available. Therefore data from a study reporting a similar but slightly less sensitive threshold of -912HU, although with the same model of GE Prospeed CT scanner[106], has been used. This provides the 'best' estimate of normal values that can be applied to the current study. Nevertheless, these slight uncertainties should be borne in mind when interpreting the results. However, the consistency with predicted deviation from normal of KCO suggest the data are likely to be a close estimate.

Importantly the current study has clearly demonstrated that KCO, health status and UZVI (in subjects who have never smoked) deviate from normal up to 30 years prior to spirometry in non-index subjects with AATD. Gas transfer deviates from normal in the teens for smokers and the early 30s for never-smokers. Based on these data, recommendations that screening for the presence and progression of lung disease should be undertaken in the 20s for all PiZ subjects who have never smoked, and the teens for subjects who have taken up smoking can be made. Gas transfer is central to this assessment, and in addition to usual spirometry,

consideration should also be given to utilising health status and CT scan measures especially in those who have never smoked, although radiation exposure may restrict the frequency or use of the latter. It seems logical that when gas transfer is deteriorating, subjects with AATD should undergo any possible lifestyle modification and possibly start effective therapies prior to the development of FEV1 impairment at which point a significant amount of lung disease will have occurred.

This study challenges the current understanding of COPD, its' severity and current treatment guidelines [248,255], as these are primarily based on spirometry, which is the last to become impaired in AATD. Consideration needs to be given to similar studies of the natural history of usual COPD, and to modification of the monitoring of subjects at risk of developing COPD as this may well also identify such subjects at an earlier stage, facilitating the introduction of appropriate preventative therapy before airflow obstruction develops.

5. GAMMA-GLUTAMYL TRANSFERASE IN ALPHA-1 ANTITRYPSIN DEFICIENCY

5.1 Aims of the study

We hypothesised that serum GGT would be related to the severity of lung disease in subjects with AATD, and that this would be independent of an anticipated, primary association with liver disease. We therefore examined the relationship of serum GGT activity in adults with AATD in relation to clinical and physiological features of lung disease, clinical and biochemical liver disease, alcohol consumption and mortality in order to determine any independent predictors of the plasma GGT level.

5.2 Method

The ADAPT database was searched to identify the first 334 subjects with the PiZ phenotype who had serum GGT measured at the initial visit.

GGT was measured by spectrophotometric assay using an Instrumentation Laboratory IL-900, (Instrumentation Laboratory Ltd, Warrington, U.K.). The coefficient of variation for this assay is 2% and the reference range for GGT is <40 IU/L for females and <50 IU/L for males.

Data from the initial visit were obtained for post bronchodilator FEV1, FVC and KCO expressed as a percentage of the value expected for healthy subjects of the same gender, age and height (% predicted)[75]. The stage of lung disease was determined according to the National Institute for Clinical Excellence (NICE) criteria[248]. The PaO2 (KPa) was determined from arterialised blood from the earlobe.

A history of chronic bronchitis, defined according to the MRC criteria[68] was noted. Information regarding the usual sputum appearance, (when present in the stable state) was obtained by matching to the BronkoTest sputum colour chart (BronkoTest, Middlesex, UK) to determine the degree of purulence[234]. The number of exacerbations requiring a change in therapy was obtained for the previous year, along with smoking status and smoking history (pack years of cigarettes smoked).

We recorded any previous diagnosis of liver disease and subjects who were suspected to have liver cirrhosis on clinical grounds had an ultrasound scan and (in some cases) liver biopsy to confirm the diagnosis. These investigations were not routinely undertaken in the absence of clinical concerns. However, we also documented the prevalence of abnormalities in aspartate transaminases (AST), bilirubin and alkaline phosphatase (ALP) in order to detect any biochemical evidence

of sub-clinical liver disease. Subjects were divided into 3 categories for reported alcohol intake: 1. no alcohol; 2. within U.K. government guidelines (14 units per week for females and 21 units per week for males) and 3. in excess of government guidelines [256].

We also reviewed the association of GGT with mortality. There were 39 deaths reported in subjects on the database at the time of this analysis and the GGT was compared between these 39 non-survivors and the remaining 305 survivors, before and after the exclusion of 3 subjects who had died specifically because of liver complications.

Finally we noted the GGT measurements at baseline and at 3 years in the 128 subjects who had both measurements, to determine its' stability over time.

Data were presented using the mean (standard error [S.E.]) for parametric data and the median (interquartile range [I.Q.R.]) for non-parametric data. Data were analysed using SPSS 12.0.1 for Windows (SPSS Inc, Chicago, IL, U.S.A.), to determine any univariate relationships between serum GGT and the physiological and clinical variables. Single tail Spearman's correlations were used to determine any relationships between serum GGT and continuous variables. The Jonckheere-Terpstra test was used to determine differences between GGT for different categories of ordinal data. The Kruskal-Wallis test was used to determine differences in serum GGT for non-ordinal categorical variables with more than 2 groups and the Mann – Whitney test was used to assess the difference in serum GGT for variables with 2 groups.

Although several factors were shown to relate to GGT levels, we were unable to perform conventional multiple logistic regression analysis as some of the

categorical variables had more than 2 groups. We therefore used the equivalent univariate *analysis of variance test*, to determine which variables remained independently associated with GGT after correction for all other associated factors that had been entered into the model. GGT underwent log transformation in order to obtain a normal distribution for this analysis.

5.3 Results

5.3.1 Relationship of GGT to demographic data

Demographic data are shown in table 5.1. The mean GGT for the 344 subjects was 43.6 IU/L (S.E. ±2.0) and 26% of the subjects had an elevated level according to gender. The group had a male predominance (61%) and males had a greater ($p<0.001$) mean serum GGT (51.9, S.E. ±2.7) than females (30.7, S.E. ±2.6). The mean age of all subjects was 50.0 (S.E. +/- 0.6), and no relationship was observed between age and GGT in this cohort ($r = 0.088$, $p = 0.105$).

TABLE 5.1: **Demographic, smoking, alcohol consumption and pulmonary function data for the 344 subjects in whom GGT was studied.**

GGT (iU/L)	mean (S.E.)		43.6	(2.0)
Age	mean (S.E.)		50.0	(0.6)
Male gender	n = (%)		209	(61%)
Smoking status	Never	n =	70	(20%)
	Ex	n =	237	(69%)
	Current	n =	37	(11%)
Pack years smoked	mean (S.E.)		20.6	(0.8)
Alcohol consumption	None	n =	103	(30%)
	Within guidelines	n =	188	(55%)
	In excess of guidelines	n =	51	(15%)
FEV1 (% predicted)	median (I.Q.R.)		46.0	(33.5-72.5)
FEV1:FVC	median (I.Q.R.)		38.0	(29.4-55.7)
KCO (% predicted)	mean (S.E.)		69.4	(1.3)

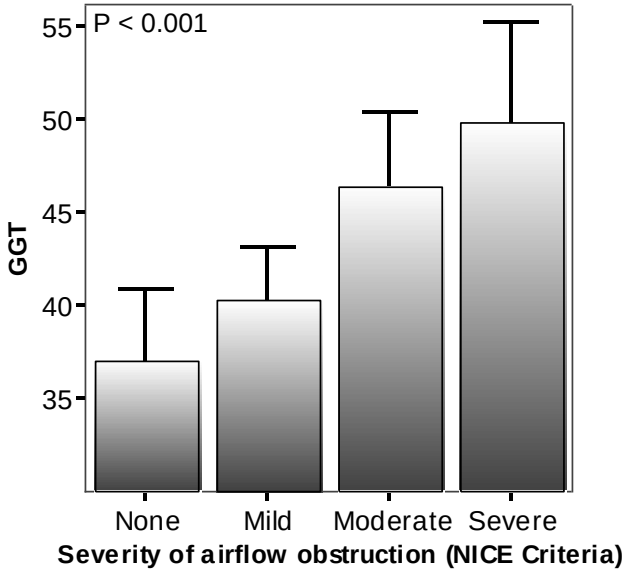
There was no change (p = 0.328) in GGT from baseline (39.9, S.E. ±2.9) to 3 years (43.0, S.E. ±2.7) in the 127 patients where this was measured.

5.3.2 Relationship of GGT to lung disease

Subjects who had COPD according to the NICE guidelines[248] had a greater (p = 0.001) serum GGT (median = 45.4, S.E. ±2.4) than those who did not (36.8, S.E. ±3.8). Serum GGT was negatively correlated with post-bronchodilator FEV1 % predicted (r = -0.148, p = 0.006), and with post-bronchodilator FEV1:FVC ratio (r = -0.127, p = 0.018). This relationship is demonstrated in figure 5.1, for subjects classified according to the individual NICE severity categories at the time that the analysis was performed[248]. There was no relationship between serum GGT and transfer factor corrected for alveolar volume (KCO) expressed as % predicted (r = -0.040, p = 0.5).

Serum GGT was higher (p = 0.029) in subjects with a history of chronic bronchitis (46.6, S.E. ±3.1, n=137) compared to those without (42.2, S.E. ±2.7, n=207). However, GGT was unrelated to the degree of sputum purulence in the stable state (p = 0.2) and the number of exacerbations documented over the previous 12 months (p = 0.4). The mean GGT in 47 subjects with a PaO2 < 8.0 KPa was 58.7 (S.E. ±8.6), but this was not significantly different (p = 0.15) to the 288 subjects with a PaO2 > 8.0 KPa (41.0, S.E. ±1.9).

FIGURE 5.1: Relationship of GGT to COPD severity according to NICE[248].



The mean GGT (+/- S.E.) is shown related to increasing severity in each stage of COPD. The significance of the relationship (p) is shown using the Jonckheere-Terpstra test.

5.3.3 Relationship of GGT to smoking

GGT was elevated in ex smokers (43.9, S.E. ±2.4; p = 0.009; n=237) and current smokers (48.8, S.E. ±5.3; p = 0.003; n=37) compared with subjects who had never smoked (39.6, S.E. ±5.1; n=70) and the number of pack years smoked was positively correlated with serum GGT (r = 0.162, p = 0.007).

5.3.4 Relationship of GGT to liver disease

Subjects with a history of jaundice as an infant, in childhood or after a miscarriage had GGT levels within the reference range (39.9, S.E. ±5.4) and were not different (p = 0.196) to those in subjects with no such history (44.1, S.E. ±2.2). Those with a previous diagnosis of hepatitis, either related to AATD, as a drug reaction or infective in nature (n = 12), tended to have serum GGT levels that were elevated (62.5, S.E. ±18.1) by approximately 1.5 times the levels of those described above, with no such history, although this was not significantly different (p = 0.434). However, subjects with a proven diagnosis of cirrhosis (n = 5) had a significantly elevated GGT approximately 3 times the upper limit of the reference range (116.0, S.E. ±30.8; p = 0.004). Bilirubin, AST and ALP were abnormal in 4.4%, 6.2% and 0.3% respectively of subjects who did not have a history of cirrhosis, suggesting that the prevalence of 'undiagnosed' biochemically important liver disease was relatively low.

Table 5.2 depicts the demographic data, smoking history, alcohol consumption and lung function data for subjects who had no previous history or clinical evidence of liver disease and those who had a history of jaundice, hepatitis and liver cirrhosis. There were no significant differences between groups for any of these parameters.

TABLE 5.2: Demographic, smoking, alcohol and pulmonary function data for subjects with no previous liver disease and those with a history of jaundice, hepatitis and cirrhosis for the 344 subjects in whom GGT was studied.

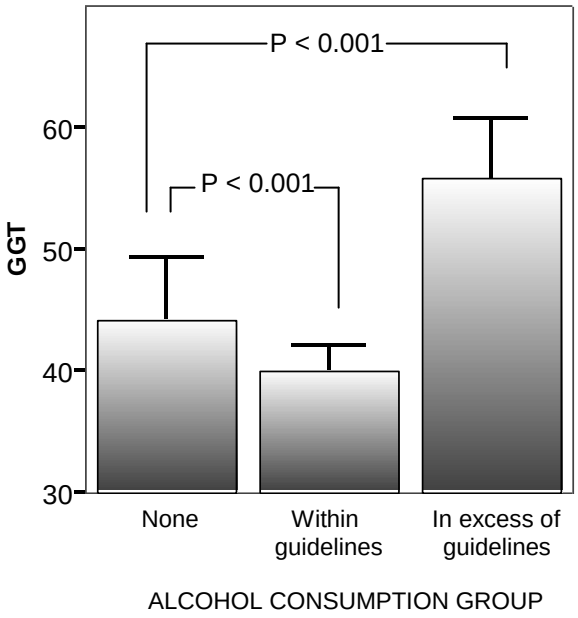
	NO HISTORY OF LIVER DISEASE	JAUNDICE	HEPATITIS	CIRRHOSIS
N =	289	38	12	5
Age mean (S.E.)	50.3 (0.6)	46.8 (1.4)	51.2 (2.6)	52.7 (3.4)
Male gender n =	176 (61%)	20 (53%)	8 (67%)	5 (100%)
Smoking status				
Never n =	57 (20%)	8 (21%)	3 (25%)	2 (40%)
Ex n =	200 (69%)	26 (68%)	8 (67%)	3 (60%)
Current n =	32 (11%)	4 (11%)	1 (8%)	0
Pack years smoked				
mean (S.E.)	20.7 (0.9)	17.1 (1.9)	29.3 (5.2)	17.3 (9.0)
Alcohol consumption				
None n =	82 (29%)	14 (37%)	3 (25%)	4 (80%)
Within guidelines n =	160 (56%)	22 (58%)	5 (42%)	1 (20%)
In excess of guidelines n =	45 (16%)	2 (5%)	4 (33%)	0
FEV1 (% predicted)				
median (I.Q.R.)	44.9 (32.9-72.2)	64.6 (37.8-91.7)	45.1 (29.8-64.9)	68.7 (22.6-94.3)
FEV1:FVC	37.6	42.3	33.9	47.8
median (I.Q.R.)	(28.1-54.7)	(32.6-62.7)	(31.0-41.1)	(33.5-68.7)
Airflow obstruction present				
n =	231 (80%)	28 (74%)	10 (83%)	3 (80%)

There were no significant differences in any of these parameters between the 4 groups. The % figures in parentheses relate to the proportion of each group.

5.3.5 Relationship of GGT to reported alcohol consumption

Alcohol consumption was positively correlated with GGT ($r = 0.249$, $p < 0.001$) but subjects who consumed alcohol within government guidelines (40.0, S.E. ±2.0, n=188), had a similar ($p = 0.108$) serum GGT to subjects who did not consume alcohol (44.2, S.E. ±5.1, n=103). However, GGT was above the gender related reference range in 43% of subjects who exceeded the daily recommended alcohol intake. The average value in this group (55.8, S.E. ±4.9, n=51) was greater than that for both non-drinkers ($p < 0.001$) or those who drank within recommended guidelines ($p < 0.001$), as shown in figure 5.2.

FIGURE 5.2: Relationship of GGT (iU/L) to alcohol consumption.



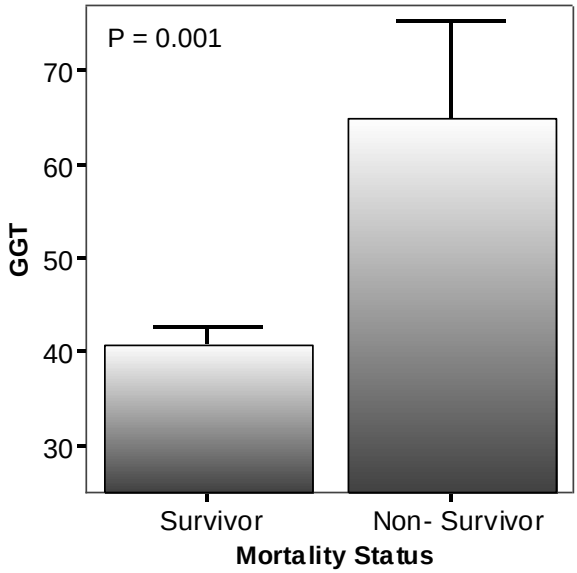
The mean GGT (+/- S.E.) is shown related to alcohol consumption (none; within; or greater than recommended U.K. government guidelines). The significance (p) of differences between groups is shown.

5.3.6 Relationship of GGT to mortality

The cause of death in the 39 subjects from the final mortality study was COPD in 21, complications post lung transplant in 3, cancer in 4, pulmonary embolus in 2, cardiovascular disease in 2, liver disease in 3 and cerebral vasculitis, endocarditis, intracranial bleed and pancreatitis in 1 case each. The baseline GGT in the non-survivors was greater (65.0, S.E. ±10.2; p = 0.001) than in survivors (40.8, S.E. ±1.8), and this difference was unaltered by removal of the 3 subjects who died of liver related illness from the analysis, as shown in figure 5.3. Survivors had a higher

(p<0.001) FEV1 % predicted (50.1, I.Q.R. 35.0 – 76.6) than non-survivors (33.5, I.Q.R. 25.4 – 43.6).

FIGURE 5.3: Serum GGT in survivors and non-survivors.



The mean GGT (+/- S.E.) is shown for subjects who had died compared to those who had survived. The significance of the difference (p) is shown.

5.3.7 Independent relationships to GGT

In order to predict which factors were independently related to GGT in subjects with AATD who remained alive, all factors where a relationship existed were entered into the analysis of variance model, except for mortality. The independent predictors of GGT in this model were post bronchodilator FEV1 % predicted (p = 0.042),

FEV1:FVC ratio ($p = 0.042$), male gender ($p<0.001$) and a history of liver cirrhosis ($p = 0.006$).

However, if mortality is also entered into the model, the factors independently associated with GGT were survival ($p = 0.038$) along with gender ($p < 0.001$) and history of liver cirrhosis ($p = 0.004$), suggesting that GGT was related to mortality irrespective of pulmonary function.

5.3.8 Summary of the results

This study demonstrates that a high proportion of subjects with AATD have an abnormally elevated serum GGT activity (26%) compared with a healthy population (11%)[166]. GGT was, as expected, related to liver cirrhosis in these subjects, but was also independently related to the severity of lung disease determined by FEV1, and mortality, the cause of which was mainly respiratory in nature.

5.4 Discussion

Neutrophilic inflammation and oxidative stress are central to the pathophysiology of COPD. Neutrophils release proteases such as neutrophil elastase, which are relatively unopposed in AATD. Neutrophil elastase has been shown to cause many of the clinical features associated with COPD, such as loss of ciliated epithelia, reduced ciliary beat frequency and reduced mucociliary clearance, mucous gland hyperplasia, increased mucus secretion and squamous cell metaplasia[42]. Neutrophils release inflammatory mediators that act as a chemoattractant for other neutrophils, and as such, inflammation is potentiated[45]. Neutrophils also release reactive oxygen species (ROS)[46], and in addition, ROS are inhaled in cigarette smoke[47] and in pollutants such as nitrogen dioxide and particulates[48]. ROS can lead to DNA damage, disruption of function and apoptosis in respiratory cells as well as amplifying the inflammatory process. Increased oxidative stress leads to the rapid conversion of intracellular GSH (reduced glutathione) to intracellular GSSG (oxidised glutathione), and thereby reduces the ratio of GSH to GSSG. The reduction in this ratio causes an increase in transcription of chemokine and cytokine genes, the up-regulation of adhesion molecules and the release of inflammatory mediators, which subsequently potentiate inflammation and consequently oxidative stress further[257].

The number of airway neutrophils is associated with disease severity determined by FEV1 in COPD[258]. It follows that an increased number of neutrophils in the airways would release more ROS, which would, in turn, lead to the reduction of GSH:GSSG ratio. At this point, the production of GGT is increased, in order to

replenish intracellular levels of reduced GSH. It is therefore not surprising that lung disease severity related to increased GGT activity in the current study.

However, an alternative explanation for the relationship between GGT activity and lung disease is that GGT may be implicated in causing the release of ROS, which subsequently leads to the development of COPD. In the presence of iron, GGT can catalyse a series of reduction reactions, leading to the production of free radicals[259]. It is known that the intracellular iron content of alveolar macrophages is increased in COPD subjects with chronic bronchitis, as well as in smokers[260]. It is therefore possible that GGT contributes (at least indirectly) to the development of lung inflammation rather than antagonises it.

The present study also demonstrated a relationship between serum GGT activity and chronic bronchitis. Neutrophil elastase, released by neutrophils causes increased sputum production, and as neutrophils also release ROS which in turn may lead to an increase in GGT activity locally. This relationship may explain why subjects with chronic bronchitis have greater GGT activity in sputum compared with healthy controls, (confirmed by the studies of Barton et al[179]).

However the current work explored the relationships of *serum* GGT activity, and did not examine GGT activity at the local level in the lung. Many of the previous mechanistic studies on GGT describe changes in membrane bound GGT, and little data are available about systemic GGT and its relationship to GGT activity in specific organs. However, it is known that in subjects with alcoholic or drug-induced hepatitis, serum GGT activity is increased disproportionately (six fold that of healthy controls) compared with liver GGT activity (threefold that of healthy controls)[149]. COPD is associated with systemic inflammation, in addition to local inflammation in

the lungs. For instance, subjects with COPD have higher circulating levels of CRP, fibrinogen, TNFα, white blood cells and IL8, and increased evidence of systemic oxidative stress compared with healthy subjects[261,262], and this systemic inflammatory state is known to be associated with co-morbidities such as cardiovascular disease, diabetes, osteoporosis and muscle wasting[71]. A relationship between systemic inflammation and severity of lung disease has been confirmed[261], so the present study may reflect a relationship between the systemic inflammation associated with COPD and GGT activity, and not inflammation related to the lung alone.

A further explanation for the current results is that AATD subjects with the most severe lung disease may also have the greatest degree of liver cirrhosis, and GGT may therefore be related to the individuals' liver disease as opposed to their lung disease. However, this is unlikely, as serum GGT activity was shown to be related to FEV1 independent of liver cirrhosis and other serum markers of liver disease using the analysis of variance test. In addition, there was no difference in lung function parameters when subjects who had no previous evidence of liver disease were compared with subjects with a history of jaundice, hepatitis or cirrhosis. Furthermore, FEV1 and FEV1:FVC ratio did not correlate with other measures of liver function (AST, ALP or bilirubin), despite their relationship to serum GGT activity.

The retention of polymeric Z protein in the hepatocellular endoplasmic reticulum is also likely to be of primary pathogenic importance and is understood to be pro-inflammatory[7]. Whether serum GGT levels reflect oxidant stress associated with hepatic inflammation or a direct cytopathological effect of Z polymers is speculative. It is possible that inflammation related to the presence of Z polymers is sub-clinical and does not affect other serum measurements liver disease.

Although the present study demonstrated a relationship between serum GGT activity and spirometry, no such relationship was evident between serum GGT activity and KCO. KCO is a measure of alveolar destruction in COPD whereas spirometry reflects potentially both alveolar destruction and airway abnormalities. The current data suggest, therefore that serum GGT reflects the severity of airway disease, but not necessarily emphysema. Although airway tissue neutrophils are inversely related to FEV1, parenchymal neutrophils (and the associated local release of ROS) are not related to alveolar wall destruction[263]. Furthermore, the inflammatory mediators CRP and IL8 have been shown to be related to bronchial wall thickening but not to measures of emphysema determined using CT scans[264]. These facts may help to explain why no relationship between GGT and KCO was found. However, alveolar macrophages are related to alveolar destruction, and are also capable of releasing ROS, which may in turn deplete intracellular glutathione and stimulate GGT production, but the present data suggest that GGT does not play an important role in the antioxidant defences in the process of emphysema development, despite its' relationship to airway disease.

Serum GGT activity has previously been associated with current smoking habits, as described in section 1.2.2.3.3.3. Cigarette smoke contains ROS, and is therefore likely to result in the activation of anti-oxidant defences, including the induction of GGT in the airways in current smokers. However, smokers are also known to have evidence of increased systemic inflammation compared with a non-smoking healthy population[265], and the current study results would support this. However, the present study has also demonstrated for the first time, that serum GGT activity is increased in ex-smokers compared with those who have never smoked in

this cohort of subjects with AATD. Having a history of smoking is associated with a greater risk of developing COPD in AATD, although in univariate analysis of variance, smoking status was not found to be independently related to serum GGT activity whereas the severity of COPD was. Therefore, the increased GGT activity in this group is likely to be a reflection of lung disease as opposed to smoking status per se.

No associations between serum GGT activity and the sputum colour in the stable state and the average annual number of exacerbations were demonstrated in this study. Some exacerbations of COPD have been shown to be associated with an increase in airway markers of neutrophilic inflammation such as IL8, LTB4, neutrophil elastase and myeloperoxidase, and an increase in systemic inflammation and oxidative stress[204,266]. As such, one might expect serum GGT activity to be increased with exacerbations. However, all subjects studied currently were assessed having been free from exacerbations for at least the preceding four weeks. Therefore, it is possible that any changes that may have occurred in serum GGT activity due to an exacerbation had resolved by the time of assessment. Alternatively, GGT may not play an important role in oxidative stress associated with exacerbations.

Similarly, sputum colour reflects sputum neutrophil load as it is a marker of myeloperoxidase which is present in and released by these cells[234]. As neutrophils also release ROS, it may therefore have also been expected that local GGT activity would increase. However, systemic GGT activity was measured, and as there is no current evidence that sputum colour is related to systemic oxidative stress, this may

explain the lack of relationship between serum GGT activity and sputum colour in the stable state.

In common with many previous studies, the present work found that serum GGT activity was greater in subjects with liver cirrhosis, in subjects who consumed large quantities of alcohol, and in males.

Serum GGT has been described in subjects with AATD up to the age of 26, where only 7% had abnormal levels[126]. In our cohort, 26% had abnormally elevated serum GGT activity. However, the present cohort is older (mean 50.0 years) than the Swedish cohort and most were identified as having AATD because they presented with lung disease, unlike the Swedish cohort who were identified by neonatal screening. In addition, a greater proportion of the current cohort had smoked (82%) compared with the Swedish subjects (22%)[73], and 5 subjects in the present group had liver cirrhosis, whereas it is not reported in the Swedish cohort. All of these factors may explain the difference in the proportions with an abnormal serum GGT activity between the current and the Swedish cohort.

An obvious limitation to using GGT as a biomarker of lung disease is that there are many potential confounding factors, and this is especially true in subjects with AATD who are known to be at risk of developing liver disease, as GGT is known to be a sensitive marker of liver disease. However, it is useful to note that GGT remains independently related to lung disease, as a relatively high proportion of subjects had abnormal serum GGT measurements, with no evidence of clinically relevant liver disease. It is therefore helpful to identify the factors such as lung disease that affect this measurement in order to interpret the results in context with the subject.

Despite the presence of several confounding factors in subjects with AATD, we have demonstrated that serum GGT activity is also a prognostic marker irrespective of its source, which is independently related to mortality.

There is mounting evidence that serum GGT activity may be related to systemic inflammation and oxidative stress, which are becoming increasingly important in COPD due to the relation of COPD to co-morbidities and in hence overall prognosis. It is possible therefore that although it is difficult to interpret the meaning of differences in serum GGT activity in terms of airway disease, it may provide a measure of systemic inflammation and oxidative stress. Measuring systemic inflammation in addition to FEV1 may prove more informative than using either measure alone, as demonstrated by measuring BMI (which is closely related to systemic inflammation) and airflow obstruction as part of the BODE index[82].

A potential limitation of the present study is that the presence of co-morbidities such as cardiovascular disease and diabetes was not specifically taken in account, as these data have only been collected more recently, and was therefore not available for all subjects. However, for subjects for whom complete data had been collected, only 1.6% of subjects had a history of myocardial disease, and 0.8% had diabetes. Hence, it is unlikely that not taking these factors into account will have impacted on the results.

Further study is clearly required to determine the role of serum GGT activity measurements as a biomarker of lung disease or systemic inflammation in subjects with lung disease. In particular, a cohort of subjects with usual COPD would provide more informative results, as the confounder of liver involvement is likely to be absent. In addition, studies to examine serum GGT activity in relation to local GGT levels,

other inflammatory mediators and markers of oxidative stress in both serum and airway secretions are indicated in order to obtain a clearer role of GGT in usual COPD and that associated with AATD.

6. CHARACTERISTICS OF PiSZ SUBJECTS

6.1 Aims of the study

The purpose of this section is to describe for the first time in a significant number of PiSZ subjects, the CT scan appearance, densitometry analysis and quality of life. Symptoms, pulmonary physiology and liver disease were characterised in these subjects in order to add to the current understanding of the phenotype. Furthermore, we matched subjects in pairs for age, gender, smoking status and pack years with PiZ subjects to enable us to compare the variables described above taking these factors into account. In order to reduce any selection bias associated with registry data further, we subsequently analysed data for index and non-index cases separately. Finally we compared PiSZ and PiZ subjects matched for FEV1 to explore the impact of airflow obstruction on health status and clinical parameters in these groups.

6.2 Method

The first sixty three subjects with the PiSZ phenotype were identified from the ADAPT database. Information from the baseline visit regarding demographic details, the AAT level, the initial reason for testing for AATD, smoking status and pack years of cigarettes smoked, previous respiratory or liver disease and respiratory symptoms, including the MRC dyspnoea score[233], the presence of chronic bronchitis, according to the MRC definition[68] and the sputum colour in the stable state using the Bronko Test chart (BronkoTest, Middlesex, U.K.) were obtained.

In addition, the forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), residual volume (RV), total lung capacity (TLC) and carbon monoxide transfer factor corrected for alveolar volume (KCO) all expressed as % predicted, were recorded. The FEV1:FVC ratio was determined along with the bronchodilator response.

A thoracic radiologist provided a descriptive report of the CT scan independent of this study. If emphysema was present, two experienced investigators scrutinised all available CT scans and reached agreement regarding the morphological type of emphysema that was present, and the predominant type of emphysema in each case.

Density mask analysis was performed for all PiSZ subjects who had a CT scan at the University Hospital Birmingham NHS Foundation Trust, and their respective PiZ matches. Pulmo CMS software (MEDIS Medical Imaging Systems BV, Leiden, Netherlands) was used to calculate the percentage of voxels that were less dense than -950 Hounsfield units in a slice at the level of the aortic arch (upper zone) and a

slice at the level of the inferior pulmonary veins (lower zone). Upper zone emphysema predominance was defined as the upper zone voxel index being greater than the lower zone voxel index, and was determined for each subject.

SGRQ and SF-36 scores, were collated and serum measurements of GGT, AST, ALP and bilirubin were measured using an Instrumentation Laboratory IL-900 (Instrumentation Laboratory (UK) Ltd, Warrington, U.K.), as an indicator of the liver function.

These parameters were described for the 63 PiSZ subjects. These subjects were paired with subjects who had the PiZ phenotype from the ADAPT cohort of around 800, to provide the best match for age, gender, smoking status (never smoked, ex-smoker or current smoker) and pack years smoked, and the two groups of data were compared.

For PiSZ subjects, data were compared for those subjects who had never smoked and those who had, and for subjects with an AAT level of less or greater than 11μm as this is the threshold for worsened susceptibility to proteolytic damage[43].

The PiSZ lung index cases were then also matched as pairs with a different cohort of PiZ lung index cases for age, gender, smoking status and pack years. Non-index PiSZ subjects were matched to non-index PiZ subjects using the same criteria, and the groups were again compared. Finally 62 PiSZ subjects who had valid spirometry measurements were matched with 62 PiZ subjects for FEV1 alone and all other variables were compared.

Data were analysed using SPSS 12.0.1 for Windows (SPSS Inc, Chicago, IL, U.S.A.), to determine any differences between the groups. Categorical data were

analysed using the χ^2 test if all categories had an expected value of more than 5, or the Fisher's exact test if any expected values were less than 5. Continuous variables were compared using the t-test for parametric data and the Mann-Whitney test for non-parametric data. A p-value of less than 0.05 was considered significant.

6.3 Results

6.3.1. Characteristics of the PiSZ subjects compared to PiZ subjects matched for age, gender and smoking history

6.3.1.1 Demographic data

Data regarding demographics, smoking, the reason for testing for AATD and the AAT levels for the 63 PiSZ subjects are shown in table 6.1.

Subjects with the PiSZ phenotype had higher ($p<0.001$) AAT levels (13.2±3.0) than PiZ subjects (4.4±1.7), and thirteen PiSZ subjects (21%) had an AAT level of less than 11μm, compared to all PiZ subjects. A greater proportion ($p=0.011$) of PiSZ subjects (31%) were diagnosed by family screening compared with PiZ subjects (13%). 1 PiSZ and 1 PiZ subject were diagnosed due to abnormal serum electrophoresis discovered as part of a medical screen, 1 PiZ subject was diagnosed after investigation for episodes of sweating and 2 PiSZ subjects were diagnosed during investigation for hypertension and arthritis. The reason for diagnosis is unknown for 1 PiSZ and 2 PiZ subjects. Matching for pack years smoked was difficult, as PiSZ subjects had generally smoked heavily, but the difference in smoking history compared with PiZ subjects was not significant.

TABLE 6.1: Characteristics of subjects with the Pi SZ phenotype compared with Pi Z subjects matched for gender, age, smoking status and pack years.

			Pi SZ	(n = 63)	Pi Z	(n = 63)	p =
Male gender	n =		36	(57%)	36	(57%)	1.0
Age	mean (S.D.)		50.6	(14.4)	51.2	(12.9)	0.808
Reason tested for AATD:	Family screen	n =	19	(31%)	8	(13%)	0.011
	Lung index	n =	36	(58%)	49	(80%)	0.147
	Liver index	n =	4	(7%)	2	(3%)	0.416
	Other	n =	3	(5%)	2	(3%)	0.663
	Unknown	n =	1		2		
Smoking status	Never	n =	17	(27%)	17	(27%)	
	Ex	n =	32	(51%)	39	(62%)	0.221
	Current	n =	14	(22%)	7	(11%)	
Pack years smoked	mean (S.D.)		26.77	(31.03)	20.57	(18.98)	0.672
AAT level in μM	mean (S.D.)		13.2	(3.0)	4.4	(1.7)	<0.001
AAT level missing	n =		2		4		
AAT level < 11μm	n =		13	(21%)	59	(100%)	<0.001

The significance in difference (p) between the 2 groups is shown.

6.3.1.2 CT Scan data

Fifty four PiSZ subjects and 58 PiZ subjects had a CT scan at their baseline visit to ADAPT. Details about the CT scan appearance and density mask analysis data are shown in table 6.2.

TABLE 6.2: CT scan appearance and densitometry for subjects with the Pi SZ phenotype compared with Pi Z subjects matched for gender, age, smoking status and pack years.

		Pi SZ (n = 54)		Pi Z (n = 58)		p =
Normal CT scan	n =	23	(43%)	5	(9%)	<0.001
Emphysema on CT scan	n =	19	(35%)	51	(88%)	<0.001
Bronchiectasis on CT scan	n =	7	(13%)	12	(21%)	0.276
Bullous disease on CT scan	n =	2	(4%)	4	(7%)	0.680
		N = 17		**N = 50**		
Centrilobular emphysema present	n =	6/17	(35%)	21/50	(42%)	0.626
Panacinar emphysema present	n =	11/17	(65%)	37/50	(74%)	0.538
Paraseptal emphysema present	n =	1/17	(6%)	0/50	(0%)	0.254
Centrilobular emphysema predominant	n=	6/17	(35%)	14/50	(28%)	
Panacinar emphysema predominant	n=	10/17	(59%)	36/50	(72%)	0.229
Paraseptal emphysema predominant	n=	1/17	(6%)	0/50	(0%)	
CT Densitometry		**N = 41**		**N = 41**		
Upper zone VI	mean (S.D.)	15.0	(19.1)	17.9	(17.2)	0.171
Lower zone VI	mean (S.D.)	14.6	(17.0)	29.2	(20.3)	<0.001
Upper zone predominance	n =	16	(39%)	5	(12%)	0.005

The significance in difference (p) between the 2 groups is shown.

Nineteen (35%) PiSZ subjects had evidence of emphysema, 7 (13%) had bronchiectasis and 2 (4%) had bullous disease on CT scanning. A greater proportion ($p<0.001$) of PiZ subjects had emphysema visible on the CT scan (88%) compared

with PiSZ subjects. There were no differences in the proportion with bronchiectasis or bullous disease between PiSZ and PiZ subjects.

CT scans were available to assess the morphological type of emphysema in 17 PiSZ and 50 PiZ subjects who had emphysema reported. Approximately two thirds of PiSZ subjects had panacinar emphysema and one third had centrilobular emphysema, with only one subject having paraseptal emphysema. These proportions were not significantly different to those observed in PiZ subjects. Some subjects had more than one type of emphysema, and therefore the predominant type of emphysema only was also assessed. However, the latter approach yielded similar results to the former.

If only the 49 matched pairs where both subjects had a CT scan performed were considered, 37% of PiSZ and 90% of PiZ subjects ($p<0.001$) had visible emphysema and there were no differences between the two groups in terms of the type of emphysema present.

CT scans were available for densitometric analysis in 41 PiSZ subjects and their PiZ matches. There was no difference in upper zone density between PiSZ and PiZ subjects. However, PiZ subjects had reduced density in the lower zones compared with PiSZ subjects. A greater proportion ($P=0.005$) of PiSZ (16/41 [39%]) compared with PiZ (5/41 [12%]) subjects had lower lung density in the upper zone compared with the lower zone.

If only the subjects who had emphysema present on the CT scan were considered (12 PiSZ and 37 PiZ subjects), no differences existed between the two groups in terms of absolute upper and lower zone densitometry. However, a greater proportion ($p=0.004$) of PiSZ subjects (7/12 [58%]) continued to have upper zone

predominance compared with PiZ subjects (5/37 [14%]). Similar results are also obtained if the 12PiSZ subjects with visible emphysema reported on the scan are compared to their 12 PiZ matches (who also all had emphysema).

Of the subjects with centrilobular emphysema, 7 PiSZ subjects were compared with 27 PiZ subjects. No differences were found between the two phenotypes in terms of upper or lower zone density. However, a greater proportion ($p=0.02$) of PiSZ subjects had upper zone predominant emphysema (57%), compared with PiZ subjects (11%).

Of the subjects with panacinar emphysema, 14 PiSZ subjects were compared with 48 PiZ subjects. Once again, no differences were found between the two phenotypes in terms of upper or lower zone density, and a greater proportion ($p<0.001$) of PiSZ subjects (57%) had upper zone predominant emphysema compared with PiZ subjects (7%).

6.3.1.3 Health status

SGRQ scores were recorded for 59 PiSZ subjects and all PiZ subjects. SF-36 scores were available for 58 PiSZ and all PiZ subjects. All health status data are given in table 6.3 below.

TABLE 6.3: Health status data for subjects with the Pi SZ phenotype compared with Pi Z subjects matched for gender, age, smoking status and pack years, using the SGRQ and SF-36.

			Pi SZ		Pi Z		p =
			n = 59		n = 63		
SGRQ	Symptoms	mean (S.D.)	46.2	(28.6)	63.2	(23.0)	<0.001
	Activity	mean (S.D.)	45.8	(35.7)	63.6	(27.1)	0.009
	Impacts	mean (S.D.)	25.1	(20.2)	36.8	(19.5)	0.003
	Total	mean (S.D.)	34.9	(32.4)	49.3	(10.6)	0.001
			n = 58		n = 63		
SF-36	Physical domain	mean (S.D.)	42.5	(13.5)	36.7	(12.0)	0.014
	Mental domain	mean (S.D.)	50.5	(13.1)	51.1	(10.6)	0.765

The significance in difference (p) between the 2 groups is shown.

PiSZ subjects had greater scores in the symptoms (46.2±28.6), activity (45.8±35.7) and impacts (25.1±20.2) domains of the SGRQ, and a greater total score (34.9±32.4), indicating a poorer quality of life, compared to scores from a general population of a similar age (approximately 8, 13, 4.5, 8 respectively)[253].

Compared with average scores of 49.6 from a general U.S. population of a similar age[237], subjects with the PiSZ phenotype had a lower physical health summary score on the SF-36 (42.5±13.6), indicating a worse physical quality of life. The physical summary score in PiSZ subjects was similar to a U.S. population of subjects with self-reported chronic lung disease (42.3)[237], despite around half having a normal CT scan and normal spirometry. The mental health summary score of the SF-36 for PiSZ subjects (49.6±13.5) was similar to the general population (50.5), and

slightly better than that of the U.S. population of subjects with self-reported chronic lung disease (44.5).

PiSZ subjects had better health status than the PiZ subjects for the symptoms ($p<0.001$), activity ($p=0.009$) and impacts ($p=0.003$) domains and the total score ($p=0.001$) of the SGRQ, and the physical domain of the SF-36 ($p = 0.014$) questionnaire (table 6.3).

6.3.1.4 Pulmonary physiology

Pulmonary physiology data for PiSZ and PiZ subjects matched for age, gender and smoking is shown below in table 6.4.

For PiSZ subjects, the mean FEV1:FVC ratio was lower than expected in a healthy population, but FEV1 and gas transfer factors were in the lower part of the reference range, with lung volumes being in the higher part of the reference range. Sixty percent of PiSZ subjects had no evidence of airflow obstruction compared to only 18% of PiZ subjects ($p<0.001$). PiSZ subjects had a greater mean FEV1 % predicted, FVC % predicted, FEV1:FVC, & KCO % predicted and a smaller RV % predicted compared with PiZ subjects.

There was no difference in the absolute change in FEV1 or the increase in % of predicted FEV1 between PiSZ and PiZ subjects following bronchodilation. A similar proportion ($p=0.307$) of PiSZ (38%) and PiZ (28%) subjects had evidence of significant bronchodilator reversibility according to ATS guidelines[239].

PaO2 while breathing room air was less than 10 KPa in 21 of the 48 PiSZ subjects in whom it was recorded but was significantly greater than in PiZ subjects ($p=0.006$).

TABLE 6.4: Pulmonary physiology for subjects with the Pi SZ phenotype compared with Pi Z subjects matched for gender, age, smoking status and pack years.

		Pi SZ		Pi Z		p =
		n = 62		**n = 63**		
FEV1 % predicted	median (I.Q.R.)	91.65	(56.99-112.76)	41.40	(32.54-70.00)	<0.001
FVC % predicted	mean (S.D.)	109.10	(17.61)	98.10	(19.42)	0.001
FEV1:FVC	median (I.Q.R.)	67.79	(40.91-81.72)	37.67	(28.75-52.27)	<0.001
Bronchodilator response		**n = 60**		**n = 60**		
(ml)	mean (S.D.)	320	(240)	270	(180)	**0.482**
Bronchodilator response						
(%)	mean (S.D.)	10.13	(8.12)	8.48	(7.25)	**0.341**
Significant bronchodilator response						
n =		19	(38%)	14	(28%)	**0.307**
		n = 62		**n = 62**		
RV % predicted	mean (S.D.)	107.62	(37.85)	136.42	(46.36)	0.002
TLC % predicted	median (I.Q.R.)	107.74	(99.36-119.69)	110.98	(100.60-121.83)	0.337
KCO % predicted	mean (S.D.)	90.45	(23.62)	69.98	(25.04)	0.002
		n = 59		**n = 62**		
PaO2 (KPa)	mean (S.D.)	10.22	(1.41)	9.36	(1.57)	0.002
PaCO2 (KPa)	median (I.Q.R.)	5.10	(4.90-5.60)	4.80	(4.57-5.20)	0.001

The significance in difference (p) between the 2 groups is shown.

The absolute change in FEV1, the change expressed as a percentage of predicted FEV1 and the numbers of subjects with significant bronchodilator

reversibility are shown for each NICE category of airflow obstruction severity (correct at the time of analysis[248]) in table 6.5 below.

TABLE 6.5: Change in FEV1 expressed as an absolute value and as a percent of predicted and the* proportion of subjects with significant bronchodilator reversibility according to the American *Thoracic Society (ATS) guidelines[239].

	PiSZ		PiZ		P =
Change in FEV1 after bronchodilator ml					
No airflow obstruction	280	(300)	320	(220)	0.706
Mild	470	(190)	290	(250)	0.047
Moderate	320	(120)	290	(160)	0.650
Severe	160	(100)	190	(90)	0.570
Change in FEV1 after bronchodilator % predicted					
No airflow obstruction	8.60	(7.52)	9.56	(7.35)	0.724
Mild	16.34	(6.80)	8.98	(6.19)	0.009
Moderate	9.85	(2.74)	9.22	(5.02)	0.725
Severe	5.56	(3.22)	5.77	(2.31)	0.889
Proportion with significant reversibility n =					
No airflow obstruction	8/35	(23%)	3/7	(43%)	0.687
Mild	9/12	(75%)	4/13	(31%)	0.027
Moderate	2/9	(22%)	7/24	(29%)	1.0
Severe	0/4		0/13		

Data are shown for subjects who have undertaken appropriate testing, in each NICE stage of COPD severity (correct at the time of analysis)[248]. The significance in difference (p) between the 2 groups is shown.

Bronchodilator reversibility varied with the NICE stage of airflow obstruction severity to a greater extent in PiSZ compared to PiZ subjects. For subjects with mild airflow obstruction, PiSZ subjects had a greater increase in FEV1 following

bronchodilation, in both absolute terms and in the percentage of the predicted value compared with PiZ subjects. In addition, a greater proportion of PiSZ subjects with mild airflow obstruction had significant bronchodilator reversibility according to the ATS/ERS Standards[239], compared with subjects with PiZ subjects who also had mild airflow obstruction.

6.3.1.5 Previous respiratory and cardiovascular diagnoses

The number of subjects who had been given a diagnosis of various types of respiratory disease prior to their initial visit to ADAPT is shown in table 6.6.

TABLE 6.6: Number of PiSZ and PiZ subjects matched for age, gender and smoking with respiratory and cardiovascular diagnoses prior to the baseline ADAPT visit.

		PiSZ		PiZ		P =
Emphysema	n =	22	(35%)	50	(79%)	<0.001
Bronchiectasis	n =	1	(2%)	8	(13%)	0.033
Asthma	n =	25	(40%)	30	(48%)	0.369
Hayfever	n =	13	(21%)	17	(27%)	0.403
Sinus disease	n =	24	(38%)	22	(35%)	0.711
Thoracic surgery	n =	0		2	(3%)	0.496
Heart disease	n =	2	(3%)	4	(6%)	0.680
Hypertension	n =	12	(19%)	10	(16%)	0.639

The significance in difference (p) between the 2 groups is shown.

Twenty two PiSZ subjects had been given a clinical diagnosis of emphysema prior to the baseline ADAPT visit, and of these, emphysema was confirmed on the

CT scan in 15 (68%), but was absent in 7 (32%). Of the 41 PiSZ subjects who did not have a pre-existing diagnosis of emphysema, 32 had a CT scan, and emphysema was seen in 4 cases (13%).

50 PiZ subjects had a pre-existing diagnosis of emphysema. Of these, emphysema was confirmed by CT scanning in all 48 who underwent CT scanning. No CT scan data were available for the remaining 2 subjects. Of the 13 PiZ subjects who had not been given a diagnosis of emphysema prior to their first visit to ADAPT, 10 had a CT scan performed. Of these, 3 (30%) had evidence of emphysema, and 7 (70%) did not.

One PiSZ subject had been given a diagnosis of bronchiectasis prior to the baseline visit. Bronchiectasis was not seen on CT scanning in this subject, but was found in 7 others. Seven of the 8 PiZ subjects with a diagnosis of bronchiectasis prior to attending ADAPT had a CT scan. Of these, bronchiectasis was confirmed on the CT scan in 3 (43%), but was not seen in the remaining 4 (57%) subjects. Bronchiectasis was, however, present in 9 out of the 51 subjects (18%) who had no such previous diagnosis and had CT scan data available.

Twenty five (40%) of the PiSZ subjects had previously been diagnosed with asthma, although only 10 (40%) of them had significant bronchodilator reversibility, whereas 9 out of 35 (26%) of subjects with no previous physician diagnosis of asthma who also underwent reversibility testing had significant reversibility. Of the 30 PiZ subjects who had been given a diagnosis of asthma prior to attending ADAPT, 28 had bronchodilator reversibility testing. Of this 28, 10 (36%) had significant reversibility. Thirty two of the 33 PiZ subjects who had not been given a previous

diagnosis of asthma had bronchodilator reversibility testing, and of these, 4 (13%) had significant reversibility.

There was no difference between the proportions of PiSZ and PiZ subjects reporting a diagnosis of hayfever, sinus disease, heart disease or hypertension prior to their initial assessment at ADAPT.

One PiZ subject had a surgical pleurodesis procedure following recurrent pneumothoraces. No PiSZ subject reported previous thoracic surgery.

6.3.1.6 Respiratory symptoms

Symptoms described by the subjects while in the stable state are shown in table 6.7.

PiZ subjects had a greater mean MRC dyspnoea score, indicating a greater degree of breathlessness, than PiSZ subjects. The most frequently recorded MRC dyspnoea score was 1 (only breathless on strenuous exercise) for PiSZ subjects, and 4 (has to stop after 100 yards or a few minutes on the flat due to breathlessness) for PiZ subjects.

A larger proportion of PiZ subjects reported wheezing most days or nights (43%) compared with PiSZ subjects (24%). There was a trend for the proportion of PiZ subjects who described coughing regularly and the frequency of exacerbation in this group to be greater than in PiSZ subjects, but these observations were not statistically significant.

There was no difference in the proportion of subjects with chronic bronchitis[68] between PiZ and PiSZ subjects.

TABLE 6.7: Stable state symptoms in subjects with the Pi SZ phenotype compared with Pi Z subjects matched for gender, age, smoking status and pack years.

		Pi SZ (n = 52)	Pi Z (n = 52)	p =
MRC Dyspnoea score	median (I.Q.R.)	3.0 (1.0-4.0)	4.0 (3.0-4.0)	0.006
1 –Breathless on strenuous exercise	n =	21 (33%)	4 (6%)	
2 – Breathless hurrying on level / slight hill	n =	9 (14%)	11 (18%)	Chi
3 – Walks slower or stops at own pace	n =	13 (21%)	16 (25%)	square
4 – Stops after 100 yards or few minutes	n =	8 (13%)	19 (30%)	=
5 – Too breathless to dress or leave house	n =	12 (19%)	13 (21%)	0.002
Usually has cough	n =	23 (37%)	33 (52%)	0.073
Wheeze most days or nights	n =	15 (24%)	27 (43%)	0.023
Chronic bronchitis	n =	18 (29%)	24 (38%)	0.257
Number of exacerbations per year	median (I.Q.R.)	0.2 (0-1.2)	1.0 (0-2)	0.067

The significance in difference (p) between the 2 groups is shown.

6.3.1.7 Liver disease and liver function tests

The number of subjects who reported previous or current liver disease, the mean results for serum liver biochemistry tests and the number of subjects with abnormal results are shown below in table 6.8.

TABLE 6.8: Liver disease and liver biochemistry test results in PiSZ compared with PiZ subjects matched for age, gender, smoking status and pack years.

P indicates the significance of the difference between the two groups.

		PiSZ	PiZ	P =
Neonatal jaundice	n =	15 (24%)	16 (25%)	1.0
Childhood jaundice	n =	1 (2%)	1 (2%)	1.0
Hepatitis	n =	2 (3%)	2 (3%)	1.0
Cirrhosis	n =	2 (3%)	1 (2%)	1.0
GGT (iU/l)		**n = 62**	**n = 59**	
	mean +/- S.D.	44.3 (51.6)	50.6 (46.9)	0.111
	Number abnormal	17 (23%)	14 (29%)	0.432
ALP (U/L)		**n = 62**	**n = 62**	
	mean +/- S.D.	177.4 (56.0)	172.9 (56.0)	0.654
	Number abnormal	3 (5%)	1 (2%)	0.619
AST (U/L)		**n = 62**	**n = 62**	
	mean +/- S.D.	26.3 (13.1)	26.8 (11.1)	0.344
	Number abnormal	5 (8%)	5 (8%)	1.0
Bilirubin (µmol/L)		**n = 62**	**n = 62**	
	mean +/- S.D.	9.5 (5.5)	9.6 (4.0)	0.867
	Number abnormal	2 (3%)	1 (2%)	1.0

There was no difference in the number of subjects reporting past or current liver disease, the mean values for liver biochemistry or the number of subjects with abnormal liver biochemistry between PiSZ and PiZ subjects.

15 (24%) PiSZ and 16 (25%) PiZ subjects reported a history of neonatal

jaundice, and a further PiZ subject reported jaundice in childhood that resolved spontaneously.

Of the 15 PiSZ subjects who had neonatal jaundice, 1 continued to have jaundice into childhood, and a further subject developed hepatitis as an adult. Of the PiSZ subjects with no history of neonatal jaundice, 2 developed liver cirrhosis and 1 developed hepatitis as an adult.

Of the 16 PiZ subjects with a history of neonatal jaundice, 1 developed hepatitis and 1 developed liver cirrhosis as an adult. A further PiZ subject with neonatal jaundice developed a transient hepatitis, which was thought to be due to a drug reaction in adult life. The remaining 13 PiZ subjects with a history of neonatal jaundice had no current clinical evidence of liver disease currently. Of the PiZ subjects who did not develop neonatal or childhood jaundice, none developed hepatitis or clinical evidence of liver cirrhosis.

Serum liver biochemistry was recorded at the baseline assessment in 62 PiSZ subjects. Of these, 17 (23%) had an abnormal GGT, 3 (5%) had an abnormal ALP, 5 (8%) had an abnormal AST and 2 (3%) had an abnormal bilirubin. GGT measurements were available for 59 of the PiZ subjects, and 14 (29%) had an abnormal result. The other serum liver biochemistry tests were available for 62 PiZ subjects, of whom, 1 (2%) had an abnormal ALP, 5 (8%) had an abnormal AST and 1 (3%) had a normal bilirubin.

GGT was not measured in the PiZ subject with a history of liver cirrhosis, but the other liver function tests were within the normal range. Of the PiSZ subjects with a history of liver cirrhosis, 1 had an abnormal GGT and AST, and the second subject had an abnormal bilirubin and ALP.

6.3.1.8 Relationship of CT scan to physiology

The proportions of subjects who had visible emphysema on the CT scan in each NICE stage of airflow obstruction severity (correct at the time of analysis[248]) are shown below in table 6.9.

TABLE 6.9: Proportion of subjects in each NICE stage of airflow obstruction severity *(correct at the time of analysis[248])* ***with emphysema visible on CT scan.***

Severity of airflow obstruction (NICE Stage)	**Proportion of subjects in each NICE stage with emphysema on CT scan**		**P =**
	PiSZ	**PiZ**	
No airflow obstruction	4/30 (13%)	3/8 (38%)	0.146
Mild	5/10 (50%)	12/13 (92%)	0.052
Moderate	35/9 (56%)	23/24 (96%)	0.013
Severe	4/4 (100%)	13/13 (100%)	1.0
P =	<0.001	0.001	

P indicates the significance of the difference between the two groups.

As the NICE stage of airflow obstruction severity increases, a greater proportion of both PiSZ and PiZ subjects have visible emphysema on the CT scan. However, for subjects with mild to moderate airflow obstruction, a smaller proportion of PiSZ subjects had emphysema compared to PiZ subjects with the same degree of airflow obstruction.

6.3.1.9 Relationship of health status to physiology

The SGRQ total score related to the FEV1% predicted, FEV1:FVC % predicted and KCO% predicted in both PiSZ (r= -0.563, p<0.001; r= -0.691, p<0.001; r= -0.445, p<0.001 respectively) and the PiZ subjects (r= -0.507, p<0.001; r= -0.480, p<0.001; r= -0.312, p=0.004) as shown in figure 6.1 below.

FIGURE 6.1: The relationship of (a.) FEV1 % predicted (b.) FEV1:FVC % predicted and (c.) KCO % predicted to the total SGRQ score for PiSZ and PiZ subjects matched for age, gender, smoking status and pack years.

The blue circles and fit line represent PiZ subjects and the green circles and fit line represent PiSZ subjects. Each circle represents one case.

(a.)

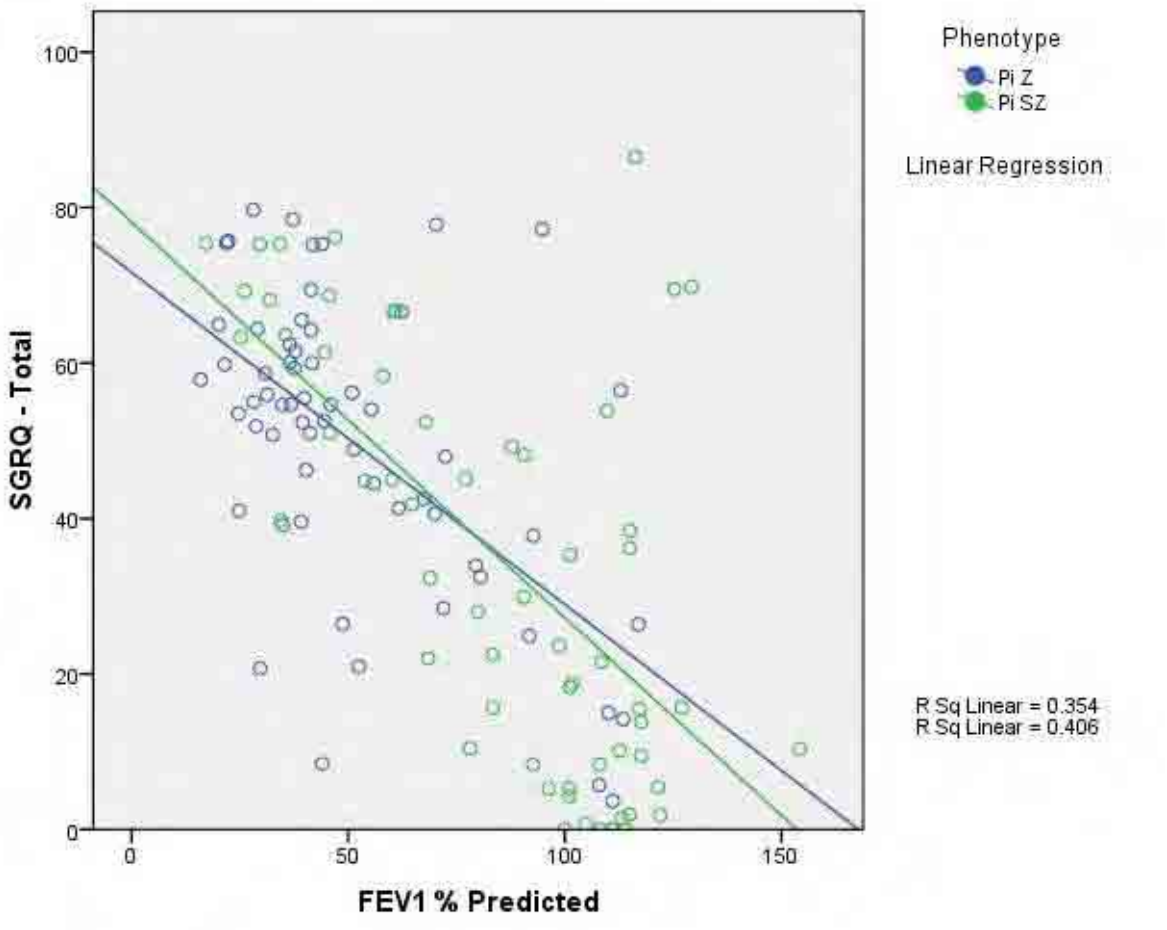


FIGURE 6.1 (continued)

(b.)

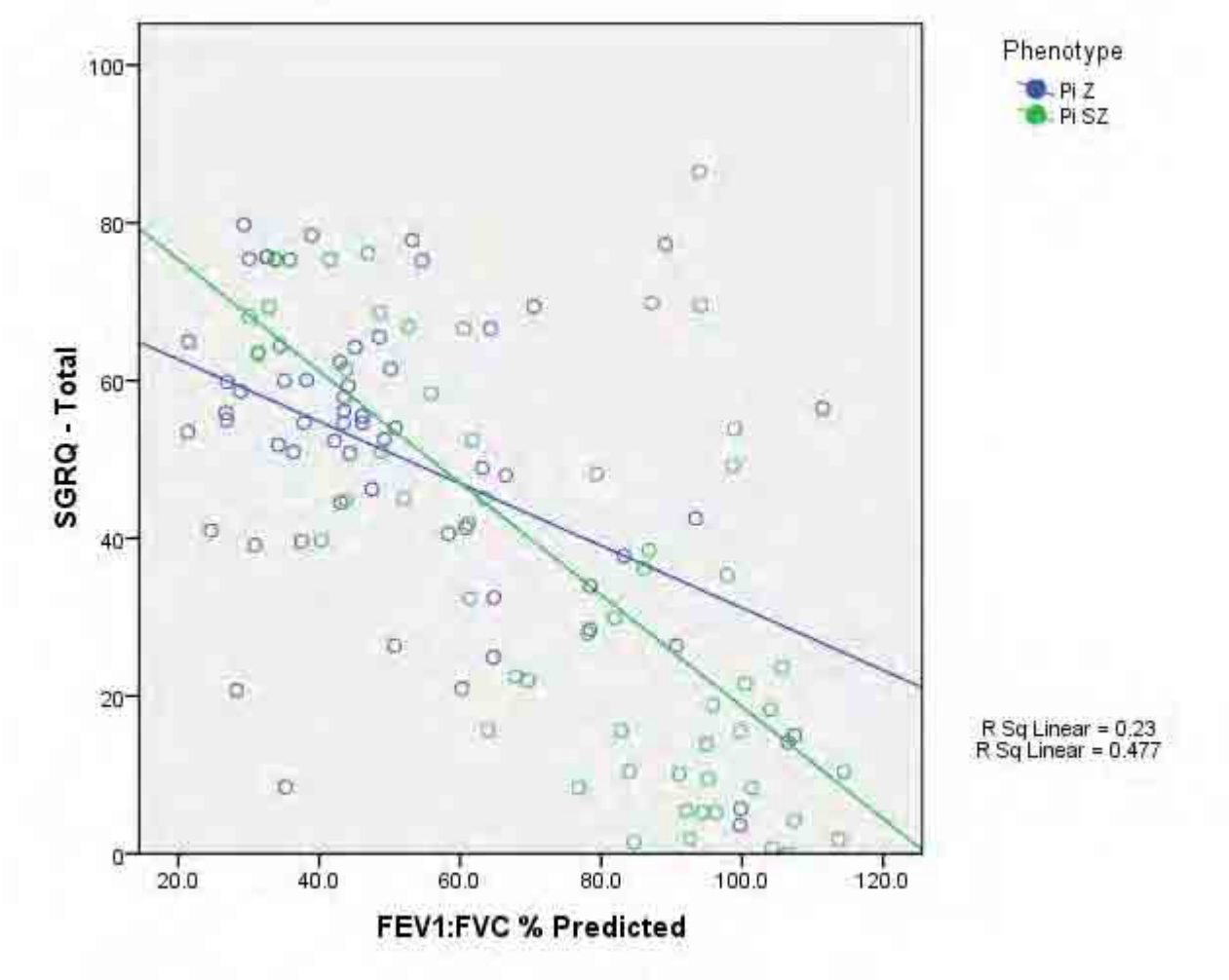
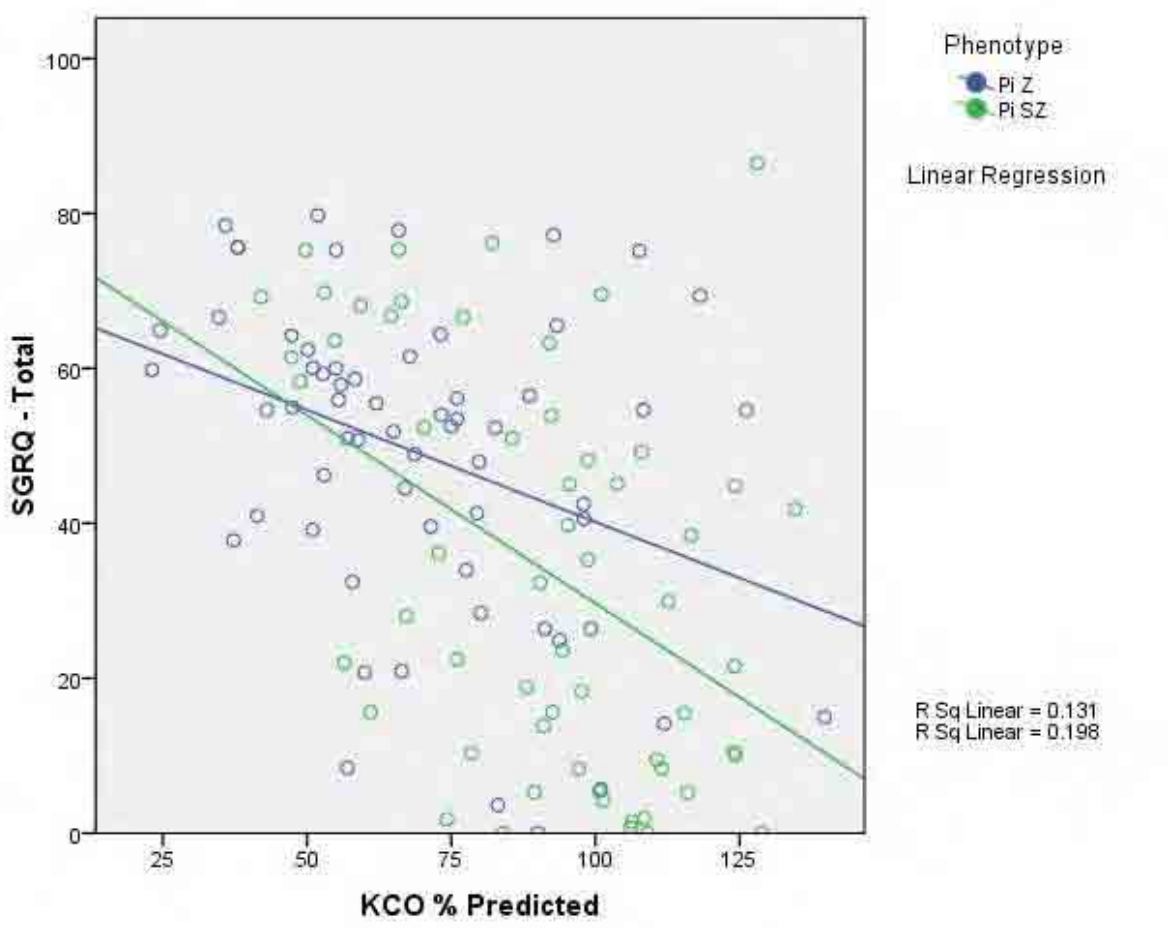


FIGURE 6.1 (continued)

(c.)



From the scatter plots, subjects with the PiSZ phenotypes appear to have a greater or similar degree of health status impairment compared to PiZ subjects when physiology measurements are most severely impaired, but a smaller degree of health status impairment when physiology measurements are normal.

For example, for subjects with an FEV1:FVC above the median, the mean total SGRQ score was lower (p=0.038), indicating a better health status for PiSZ

subjects (24.30 ± 22.71) compared to PiZ subjects (36.72 ± 20.47). For subjects with an FEV1:FVC below the median, the mean total SGRQ score was similar (p=0.590) for PiSZ subjects (58.88 ± 16.25) compared to PiZ subjects (56.44 ± 15.12).

6.3.1.10 Relationship of CT scans to health status

The mean values for the SGRQ domains, the total score and the physical and mental summary scores of the SF-36 are shown in table 6.10 below.

PiSZ and PiZ subjects with visible emphysema on the CT scan had worse scores for all aspects of the SGRQ and for the SF-36 physical summary score compared with subjects with no emphysema.

All subjects had higher SGRQ scores, indicating a worse health status compared to a general population of a similar age[253], even if they did not have emphysema visible on the CT scan. Average comparator scores for a general population age 39-49 (i.e. similar to the PiZ subjects with no emphysema visible on CT) published previously by Ferrer et al.[253] were 7.97 (symptoms), 9.33 (activity), 2.69 (impacts) and 5.78 (total). Average comparator scores for a general population age 49-59 (i.e. similar to the PiZ subjects with emphysema visible on CT and both PiSZ groups) published previously[253] were 8.74 (symptoms), 13.48 (activity), 4.55 (impacts) and 8.19 (total) for PiZ subjects with emphysema, PiSZ subjects with no emphysema, and PiSZ subjects with emphysema.

TABLE 6.10: Mean (S.D.) SGRQ and SF-36 scores for PiSZ and PiZ subjects matched for age, gender, smoking status and pack years, with and without visible emphysema on the CT scan.

	PiSZ		PiZ		P =
EMPHYSEMA VISIBLE ON CT SCAN					
SGRQ – Symptoms domain	63.76	(14.91)	68.74	(16.84)	0.258
SGRQ – Activity domain	72.53	(24.91)	71.04	(19.62)	0.520
SGRQ – Impacts domain	40.49	(21.14)	41.24	(17.56)	0.899
SGRQ – Total score	53.8	(20.08)	54.84	(15.37)	0.853
SF-36 – Physical summary score	34.19	(33.25)	33.76	(10.38)	0.883
SF-36 – Mental summary score	55.32	(10.73)	51.49	(11.03)	0.091
NO EMPHYSEMA VISIBLE ON CT SCAN					
SGRQ – Symptoms domain	41.12	(29.60)	29.87	(26.42)	0.340
SGRQ – Activity domain	36.17	(34.08)	20.54	(26.14)	0.335
SGRQ – Impacts domain	20.60	(21.89)	11.18	(13.16)	0.153
SGRQ – Total score	29.26	(25.28)	17.12	(15.34)	0.231
SF-36 – Physical summary score	45.73	(13.18)	53.30	(4.84)	0.146
SF-36 – Mental summary score	47.71	(14.43)	52.87	(4.27)	0.945
P = (EMPHYSEMA VS. NO EMPHYSEMA)					
SGRQ – Symptoms domain	0.008		<0.001		
SGRQ – Activity domain	0.001		<0.001		
SGRQ – Impacts domain	0.005		<0.001		
SGRQ – Total score	0.001		<0.001		
SF-36 – Physical summary score	0.002		<0.001		
SF-36 – Mental summary score	0.045		0.745		

The SGRQ and SF-36 scores are compared between PiSZ and PiZ subjects who have visible emphysema on the CT scan (row 1) and between PiSZ and PiZ subjects

TABLE 6.10 FOOTNOTE (continued):

who have no visible emphysema on the CT scan (row 2). P values are also given to denote the significance of any difference in SGRQ and SF-36 scores between PiSZ subjects with visible emphysema on the CT scan and PiSZ subjects with no visible emphysema(row 3, column 1). Similarly, p values are shown to denote the significance of any difference in SGRQ and SF-36 scores between PiZ subjects with visible emphysema on the CT scan and PiZ subjects with no visible emphysema(row 3, column 2).

SF-36 physical summary scores were lower, indicating a worse general health status compared with the general population of a similar age in PiSZ and PiZ subjects with emphysema (comparator scores are 45.90 and 49.64 respectively[237]). However, for subjects with no evidence of emphysema on CT scanning, the SF-36 physical summary scores were similar in both PiSZ and PiZ subjects compared to normal values generated in a general US population (49.64 and 52.15 respectively[237]).

There were no differences between PiSZ and PiZ subjects in any domain of the SGRQ or the SF-36 for subjects who had visible emphysema on the CT scan or for those who did not.

6.3.1.11 Relationship of physiology to pack years of cigarettes smoked

Pack years of cigarettes smoked correlated negatively with FEV1 % predicted, FEV1:FVC and KCO % predicted, and correlated positively with RV % predicted and TLC % predicted in subjects with either the PiSZ or the PiZ phenotype. However, the relationship to lung volumes and gas transfer was not as strong for PiZ compared to PiSZ subjects. The change in FEV1 following bronchodilation also correlated positively with pack years smoked in PiSZ subjects but not in PiZ subjects. These relationships are shown in figure 6.2 below.

FIGURE 6.2: Relationship between pack years smoked and (a.) FEV1 % predicted, (b.) FEV1:FVC % predicted, (c.) KCO % predicted, (d.) TLC % predicted (e.) RV % predicted and (f) change in FEV1 following bronchodilation for PiSZ subjects and PiZ subjects matched for age, gender, smoking status and pack years.

(a.) PiSZ: r = -0.504, p<0.001; PiZ: r = -0.527, p<0.001.

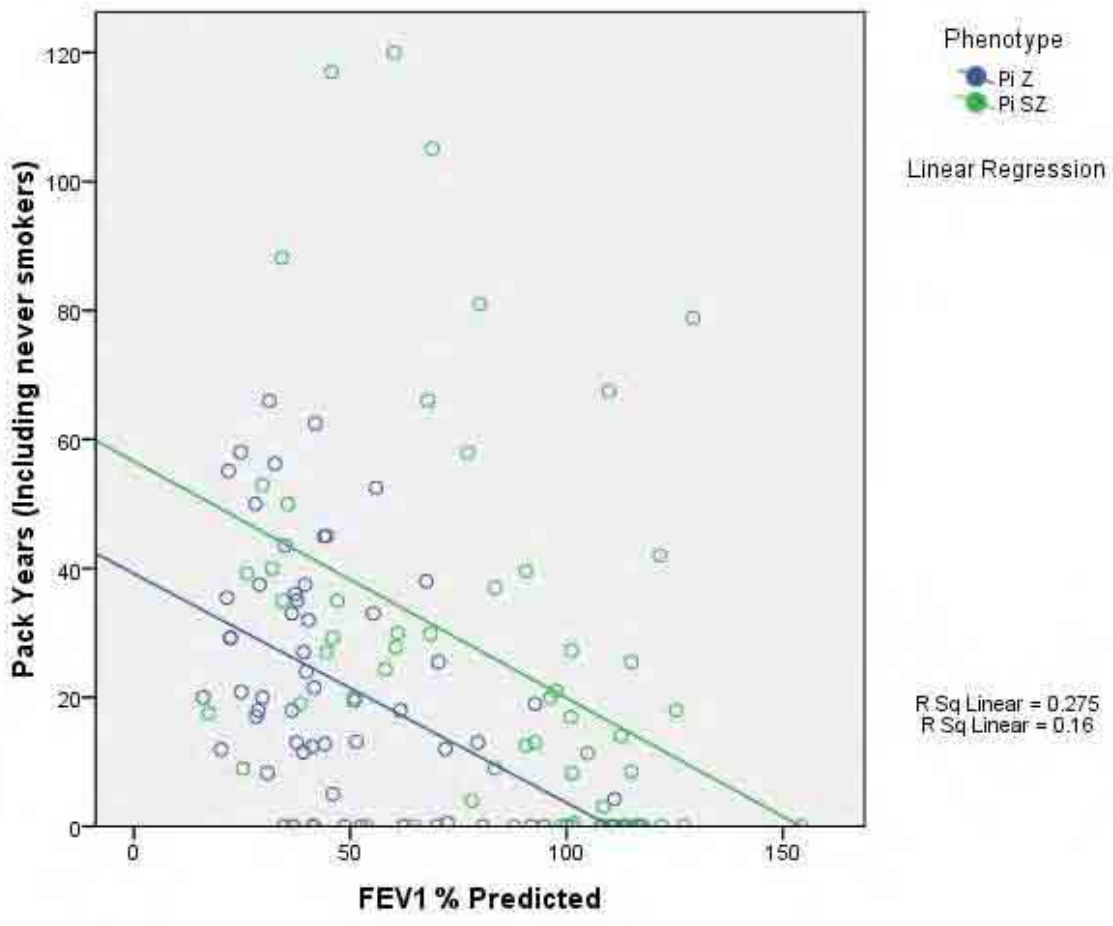


FIGURE 6.2 (continued):

(b.) PiSZ: r = -0.432, p=0.001; PiZ: r = -0.490, p<0.001.

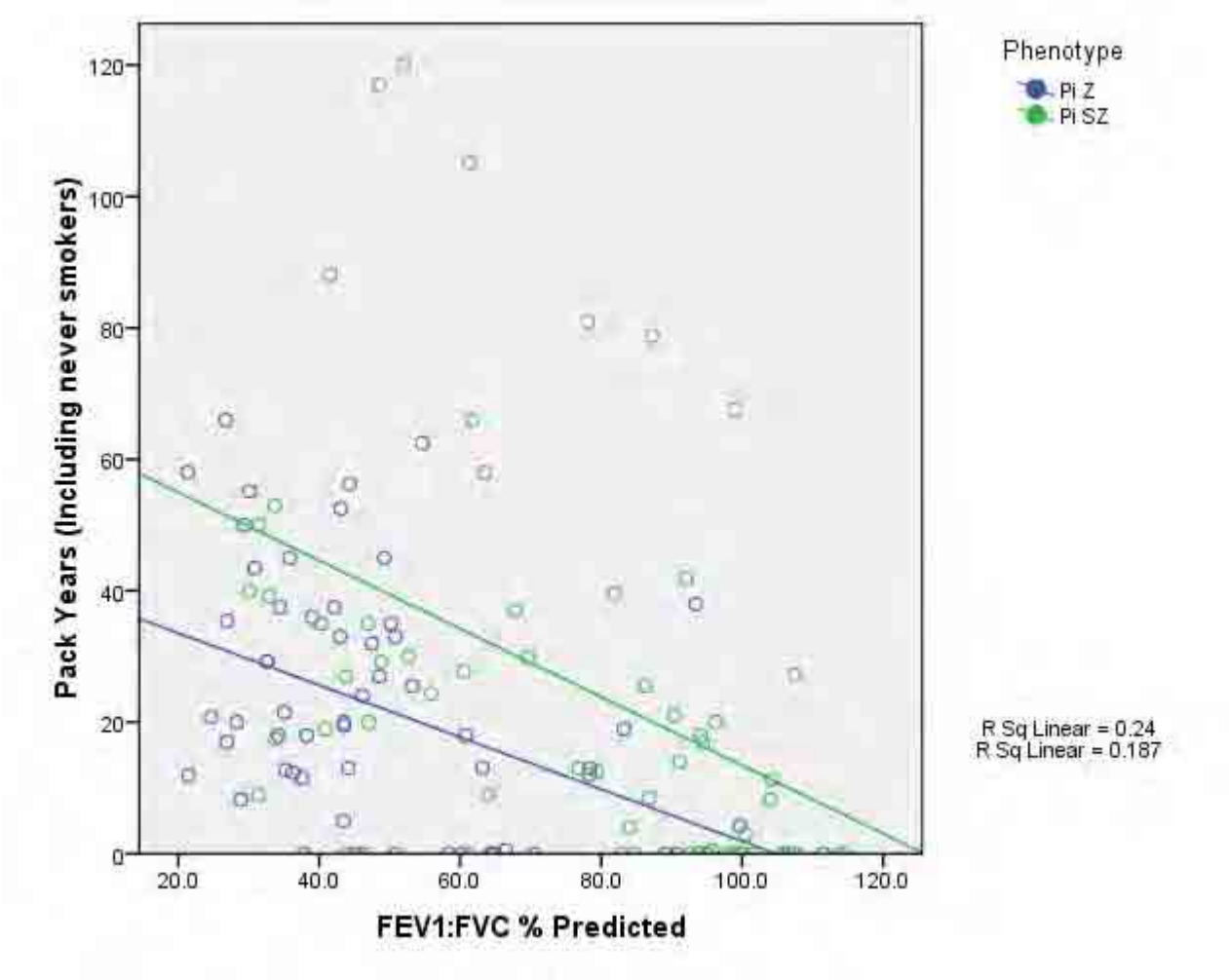


FIGURE 6.2 (continued):

(c.) PiSZ: r = -0.506, p<0.001; PiZ: r = -0.308, p = 0.015

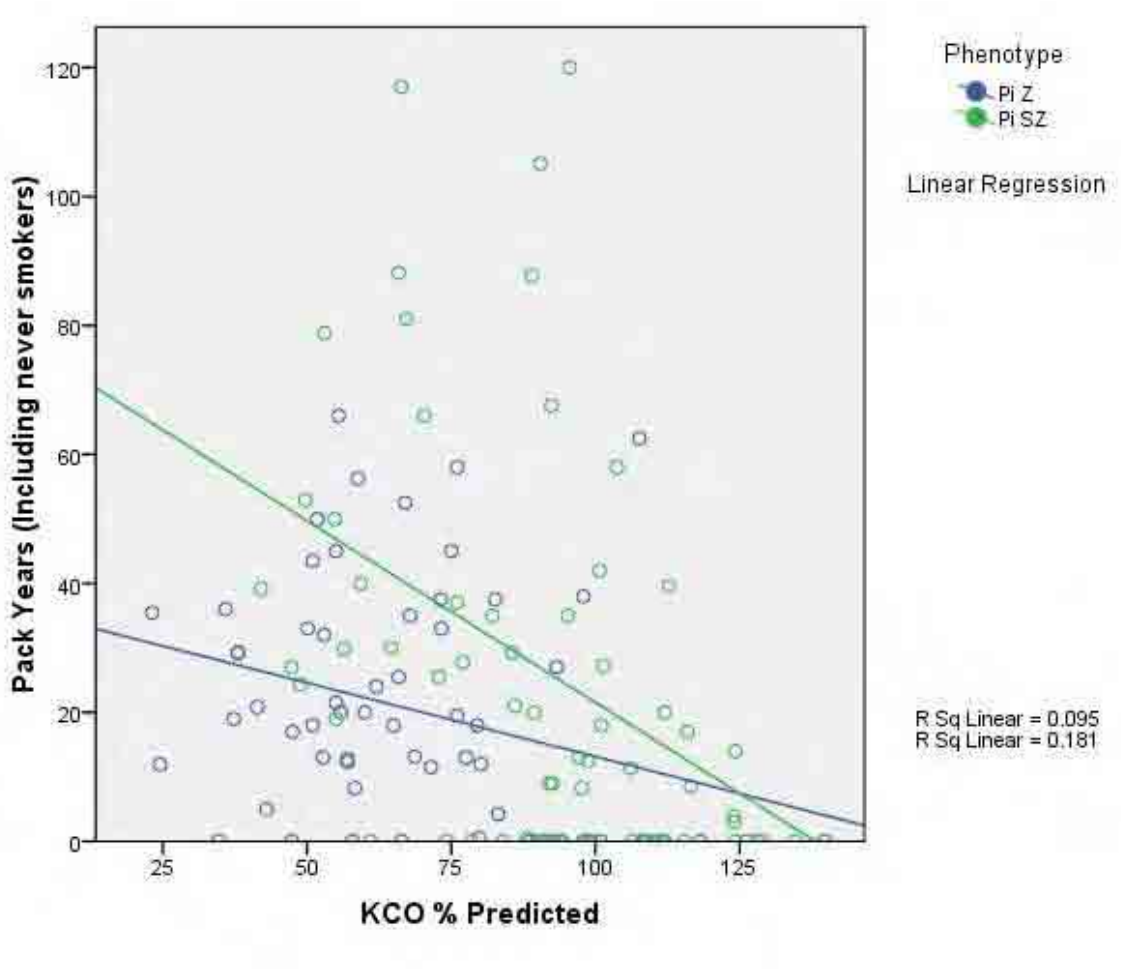


FIGURE 6.2 (continued):

(d.) PiSZ: r = 0.346, p = 0.006; PiZ: r = 0.250, p = 0.050

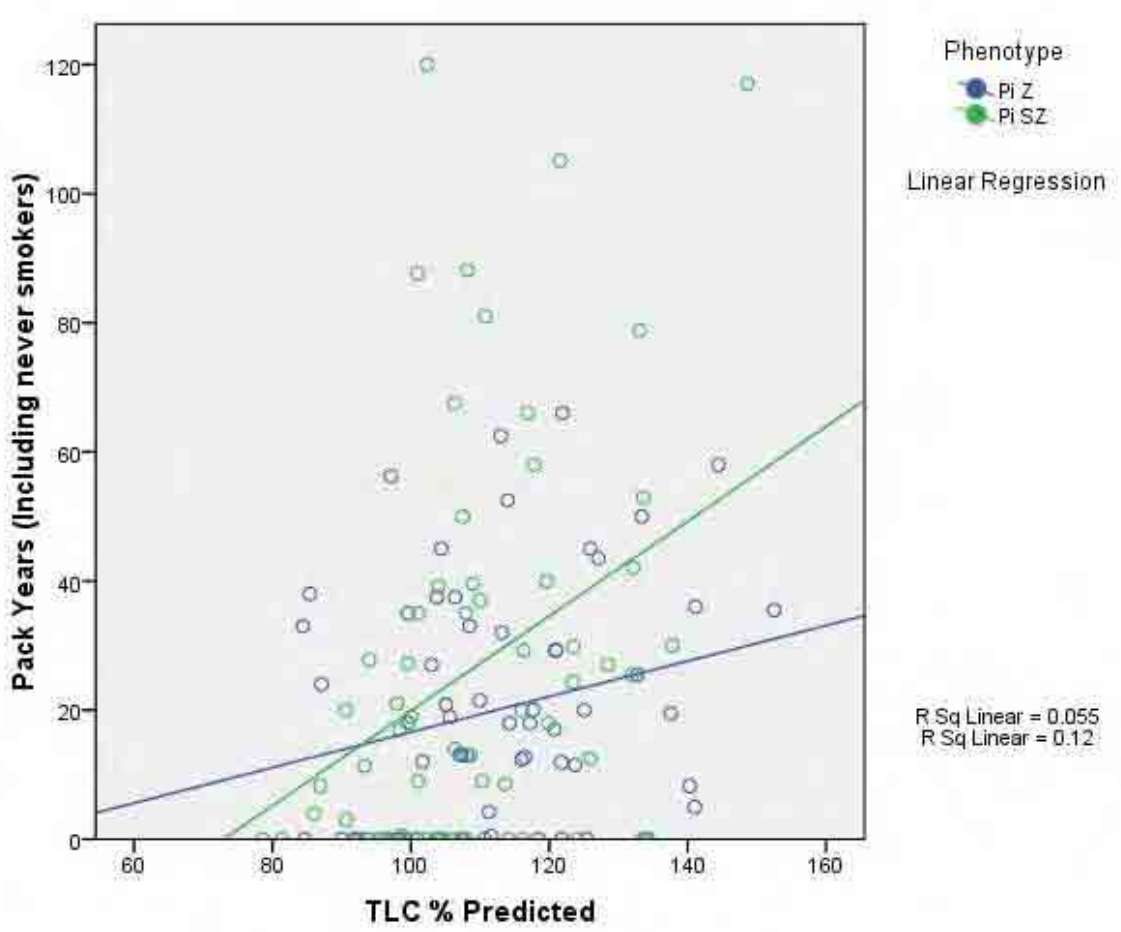


FIGURE 6.2 (continued):

(e.) PiSZ : r = 0.522, p < 0.001 ; PiZ : r = 0.338, p = 0.007

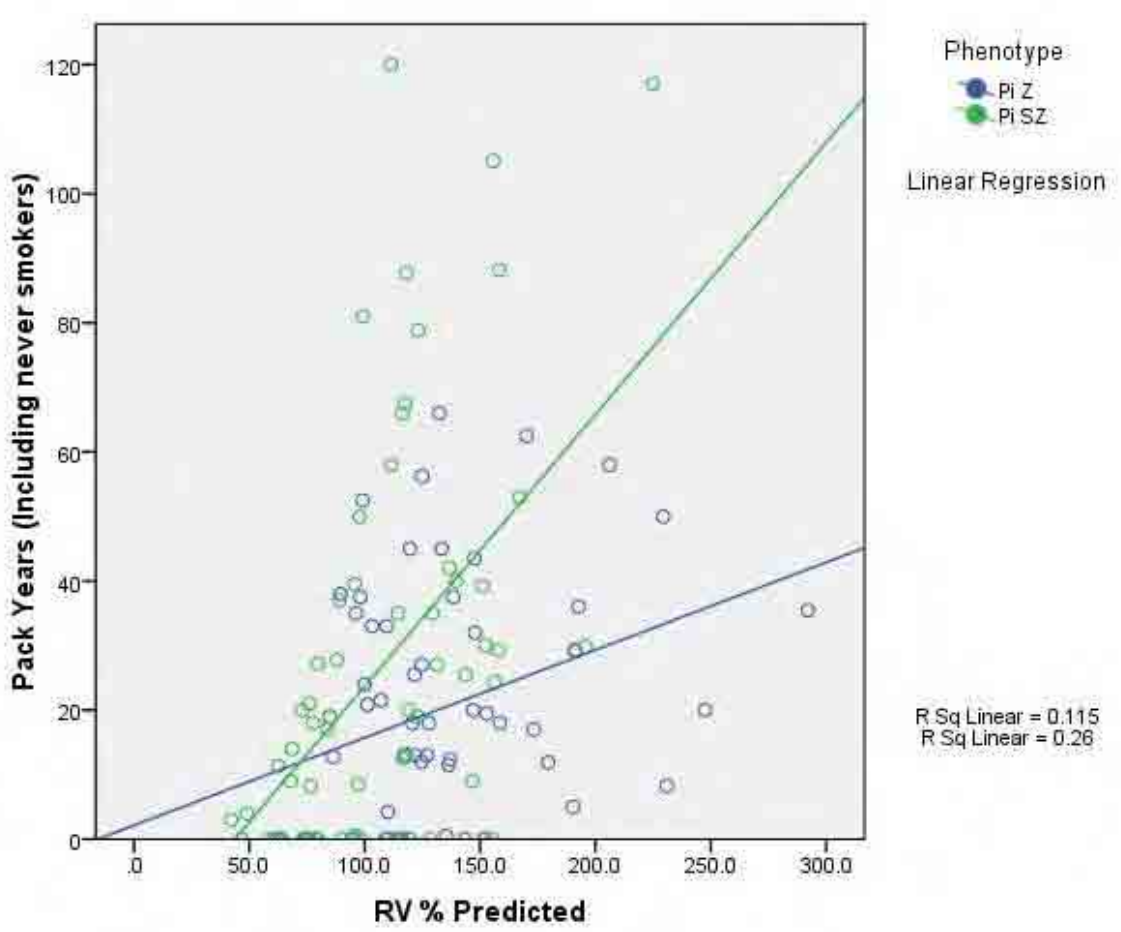
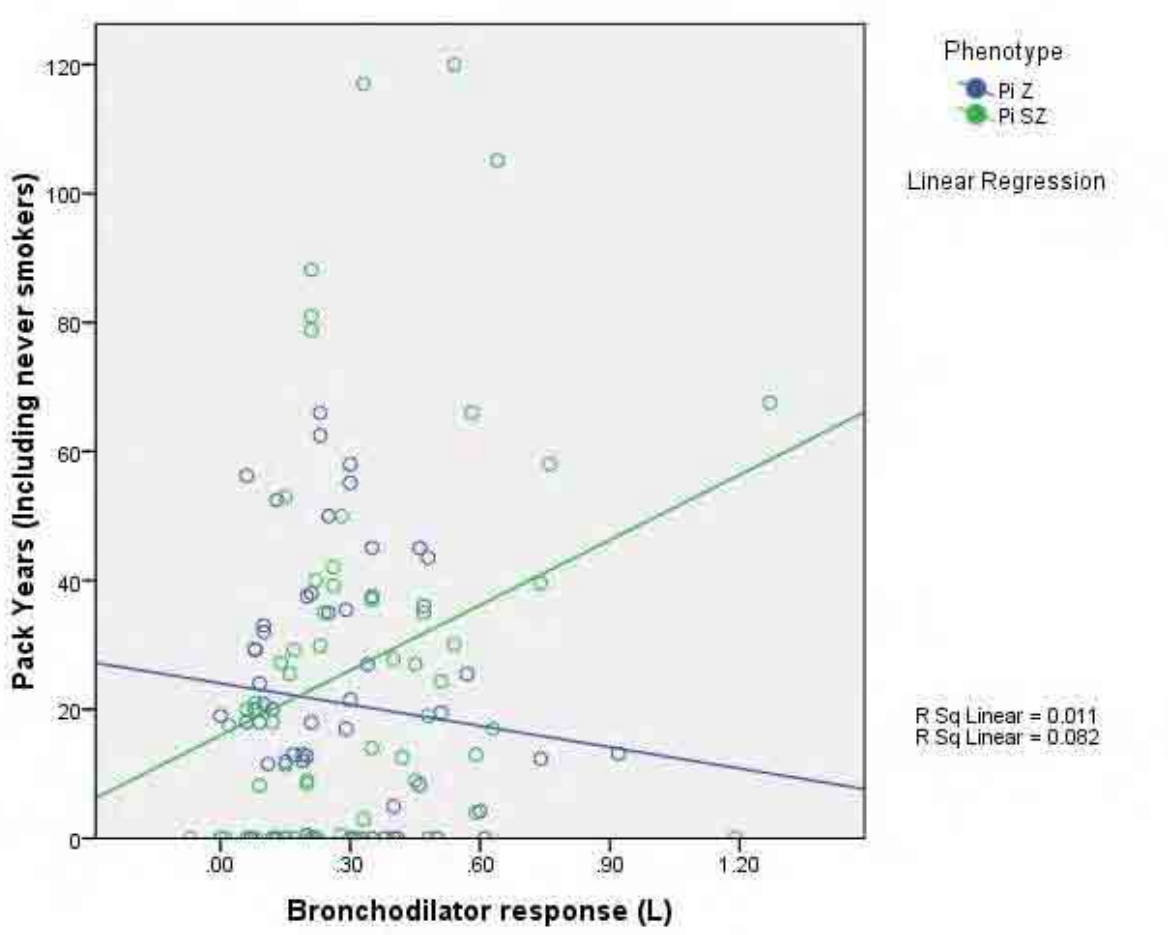


FIGURE 6.2 (continued):

(f.) PiSZ : r = 0.392, p = 0.002 ; PiZ : r = -0.107, p = 0.417



6.3.2. PiSZ never smokers compared to PiSZ previous or current smokers

Seventeen PiSZ subjects had never smoked, 32 were ex-smokers and 14 continued to smoke at the time of assessment. The mean number of pack years in ex and current smokers was 36.67 (S.D.±30.92). There were no differences between

never smokers and those who had smoked in terms of gender (47% male & 61% male respectively), age (46.96±17.82 & 51.90±12.81 respectively), AAT level (12.74±3.29 & 13.33±2.97 respectively) or the proportion of lung index (44% & 26% respectively) and non-index cases (44% & 63% respectively).

Forty one PiSZ ex or current smokers had a CT scan and 18 (44%) had evidence of emphysema, compared to only 1 out of the 13 PiSZ never smokers (8%) who had a CT scan (p = 0.021). The subject who had emphysema visible on the CT scan despite never having smoked had been exposed to 19 years of passive smoking as a child and adolescent, and had worked as a farmer for 45 years. The subject had also been exposed to fumes from coal and wood fires used to heat the home. This subject had no evidence of airflow obstruction, but a mild isolated abnormality of KCO (78% predicted).

PiSZ subjects who had never smoked had worse SGRQ scores in the symptoms (27.22±26.32), activity (23.45 ±24.15) and impacts (8.36±12.57) domains and a worse total SGRQ score (16.07±16.87) than a general population of a similar age[253] (7.97, 9.33, 2.69, 5.78 respectively). However, never smokers had better scores than subjects who had smoked (53.49±26.13, 54.29±35.91, 31.84±22.99 & 42.45±25.71 respectively). Never smokers had a similar SF-36 physical summary score (51.11±10.07) to subjects from a general population of a similar age (49.64)[237], but ex and current smokers had a worse mean score than both of these groups (38.98±13.27).

Current or ex smokers had worse FEV1 % predicted (75.21±32.07; p<0.001), FEV1:FVC (56.55±20.84;p<0.001), RV % predicted (113.70±38.58) and KCO % predicted (85.98±23.58; p=0.010) and a greater degree of bronchodilator reversibility

(370±270ml; p=0.005) than subjects who had never smoked (107.09±23.02, 77.87±15.49, 91.52±31.47, 102.28±19.83, 160±140 respectively). Two of 17 (12%) never smokers had evidence of airflow obstruction compared to 23/45 (51%) ex or current smokers who had their FEV1 measured.

A greater proportion ($p<0.001$) of ex or current smokers had been diagnosed with emphysema prior to attending ADAPT (48%) compared with those who had never smoked (0%). Of those with a diagnosis of emphysema prior to attending ADAPT, emphysema was visualised on the baseline ADAPT CT scan in 15/22 (68%), but was not seen in 7 (32%) subjects, all of whom had evidence of airflow obstruction.

Mean MRC dyspnoea scores (1.71±0.85) were lower ($p=0.001$), indicating less severe breathlessness, and the frequency of cough (12%), chronic bronchitis (6%) and annual exacerbations (0.40±0.86) were less ($p=0.013$, $p=0.025$, $p = 0.030$ respectively) in subjects who had never smoked compared with those who had previously smoked (3.07±1.55, 46%, 37%, 1.27±1.74 respectively). There was no difference in the proportion of subjects who described wheeze or the sputum colour in the stable state between subjects who had previously smoked and those who had not.

Six (35%) of never smokers and 9 (20%) of ex or current smokers had a history of neonatal jaundice ($p=0.204$) and 1 subject from each group had a history of liver cirrhosis. There were no differences in the mean GGT, ALP, AST or bilirubin measurements, or in the proportion of subjects who had abnormalities of these tests, when subjects who had never smoked were compared to subjects who had.

6.3.3. PiSZ with an AAT level of <11µm compared to PiSZ with an AAT level >11µm

Thirteen PiSZ subjects had an AAT level of <11µM (low level group), and 58 had a level >11µM (intermediate level group). Sixty nine percent of subjects with an AAT level of less than 11µM were male compared with 52% with a level greater than 11Mm. 54% with a low AAT level were non-index subjects and 46% had never smoked compared with 23% and 21% respectively with an intermediate AAT level. However, none of these observations were significantly different between the two groups.

Forty four percent from the low level group had evidence of emphysema on their CT scans and 43% had airflow obstruction, compared with 28% and 39% respectively in the higher level group. There was no significant difference in these observations between the two groups. Despite this, the higher level group had a greater ($p=0.046$) mean MRC dyspnoea score (2.96±1.53), indicating more severe breathlessness, and a lower ($p=0.009$) SF-36 physical summary score (39.93±13.69) indicating a worse general health status than subjects with the lower level of AAT (2.0±1.22 and 50.02±9.98 respectively).

There were no differences in other respiratory symptoms, parameters of pulmonary function or SGRQ scores between the 2 groups.

6.3.4. Characteristics of PiSZ index subjects compared to PiZ index subjects matched for age, gender and smoking

Demographic data for the 36 PiSZ subjects who were diagnosed with AATD due to lung disease (lung index), and PiZ lung index subjects, matched in pairs for age, gender, smoking status and pack years are shown in table 6.11.

TABLE 6.11: Demographic and smoking data for lung index Pi SZ subjects compared with lung index Pi Z subjects matched for gender, age, smoking status and pack years.

			Pi SZ	(n = 36)	Pi Z	(n = 36)	p =
Male gender		n =	20	(56%)	20	(56%)	1.0
Age		mean (S.D.)	54.0	(10.8)	53.3	(10.4)	0.796
Smoking status							
	Never	n =	7	(19%)	7	(19%)	0.290
	Ex	n =	20	(56%)	25	(69%)	
	Current	n =	9	(25%)	4	(11%)	
Pack years smoked		mean (S.D.)	27.78	(31.30)	25.48	(19.27)	0.709

The significance in difference (p) between the 2 groups is shown.

Generally PiSZ and PiZ subjects were well matched. Demographic data for PiSZ lung index cases were no different to those of other PiSZ subjects.

Data regarding abnormalities reported on the CT scan, CT scan densitometry data and the proportion of subjects with upper or lower zone predominant emphysema (where available) are shown in table 6.12.

TABLE 6.12: CT scan appearance and densitometry for lung index subjects with the Pi SZ phenotype compared with lung index Pi Z subjects matched for gender, age, smoking status and pack years.

		Pi SZ (n = 33)		Pi Z (n = 33)		p =
Normal CT scan	n =	9	(27%)	3	(9%)	0.056
Emphysema on CT scan	n =	15	(46%)	30	(91%)	<0.001
Bronchiectasis on CT scan	n =	4	(13%)	6	(18%)	0.492
Bullous disease	n =	1	(3%)	3	(9%)	0.613
		N = 13		**N = 29**		
Centrilobular emphysema present	n =	3/13	(23%)	8/29	(28%)	1.0
Panacinar emphysema present	n =	9/13	(69%)	25/29	(86%)	0.226
Paraseptal emphysema present	n =	1/13	(8%)	1/29	(3%)	0.528
Centrilobular emphysema predominant	n=	3/13	(23%)	8/29	(27%)	
Panacinar emphysema predominant	n=	9/13	(69%)	20/29	(69%)	1.0
Paraseptal emphysema predominant	n=	1/13	(8%)	1/29	(3%)	
CT Densitometry		**N = 24**		**N = 24**		
Upper zone VI	mean (S.D.)	16.7	(20.6)	17.9	(15.5)	0.826
Lower zone VI	mean (S.D.)	15.5	(18.9)	29.8	(16.6)	0.008
Upper zone predominance	n =	10	(42%)	2	(8%)	0.008

The significance in difference (p) between the 2 groups is shown.

Twenty seven percent of PiSZ subjects had no abnormality reported on the CT scan despite all subjects being diagnosed with AATD due to lung disease. Only 9% of PiZ lung index subjects were reported to have a normal CT scan, but this was not significantly different (p=0.056) to PiSZ lung index subjects.

Fewer (p = 0.002) PiSZ lung index subjects had a normal CT scan, and a greater proportion (p = 0.014) had emphysema visible on the CT scan compared with PiSZ subjects who were diagnosed with AATD for other reasons, but the proportions of bullous disease and bronchiectasis were not different between these 2 groups.

Visible emphysema was present in a greater proportion of PiZ compared with PiSZ lung index subjects. There was no difference in the proportion of subjects with bronchiectasis or bullous disease on the CT scan between PiSZ and matched PiZ subjects.

Approximately one third of PiSZ subjects had panacinar emphysema and a quarter had centrilobular emphysema. These proportions were no different to those observed for PiZ subjects.

As with the whole cohort, PiZ index subjects have a lower lung density in the lower zones but a similar density in the upper zones compared with PiSZ index subjects. A greater proportion of PiSZ subjects had a reduced lung density in the upper zone relative to the lower zone compared with PiZ subjects.

When only subjects with visible emphysema on the CT scan were considered, more (P=0.003) PiSZ (5/8 [63%]) than PiZ (1/21[5%]) subjects had upper zone emphysema predominance, although the differences in the upper zone and the lower zone voxel indices between the two groups did not reach statistical significance.

Health status data were available for all PiZ lung index subjects, 33 PiSZ subjects using the SGRQ and 32 subjects using the SF-36. Scores are shown in table 6.13 below.

TABLE 6.13: Health status data for lung index subjects with the Pi SZ phenotype compared with Pi Z subjects matched for gender, age, smoking status and pack years, using the SGRQ and SF-36.

			Pi SZ	Pi Z	p =
			n = 33	n = 36	
SGRQ	Symptoms	mean (S.D.)	60.73 (19.28)	61.39 (21.85)	0.894
	Activity	mean (S.D.)	62.15 (33.15)	64.06 (23.49)	0.780
	Impacts	mean (S.D.)	35.52 (23.35)	36.10 (17.86)	0.908
	Total	mean (S.D.)	47.45 (24.06)	48.77 (17.82)	0.797
			n = 32	n = 36	
SF-36	Physical domain	mean (S.D.)	37.65 (13.07)	35.51 (9.07)	0.682
	Mental domain	mean (S.D.)	50.29 (12.55)	52.84 (11.31)	0.384

The significance in difference (p) between the 2 groups is shown.

There was no difference in health status scores using the SGRQ or the SF-36 between PiSZ and matched PiZ lung index cases, despite a greater proportion of PiZ subjects having emphysema visible on the CT scan, airflow obstruction and abnormalities of gas transfer. PiSZ lung index subjects had greater scores, in all domains and the total SGRQ ($p<0.001$) and a lower SF-36 physical summary score ($p = 0.030$), indicating a worse physical health status compared with PiSZ subjects who were diagnosed for other reasons. There was, however, no difference in the

mental health summary score of the SF-36 between PiSZ lung index subjects and other PiSZ subjects.

Pulmonary physiology is shown in table 6.14 below for PiSZ and matched PiZ lung index subjects.

TABLE 6.14: Pulmonary physiology for lung index subjects with the Pi SZ phenotype compared with lung index Pi Z subjects matched for gender, age, smoking status and pack years.

		Pi SZ	Pi Z	p =
		n = 36	**n = 36**	
FEV1 % predicted	median (I.Q.R.)	71.49 (36.91)	50.44 (25.54)	0.007
FVC % predicted	mean (S.D.)	102.69 (22.54)	104.45 (21.61)	0.737
FEV1:FVC	median (I.Q.R.)	52.62 (22.90)	39.45 (16.69)	0.008
		n = 35	**n = 34**	
Bronchodilator response (ml)	mean (S.D.)	310 (230)	230 (140)	0.079
Bronchodilator response (%)	mean (S.D.)	10.64 (7.20)	7.93 (5.41)	0.076
		n = 35	**n = 35**	
RV % predicted	mean (S.D.)	123.40 (35.84)	113.51 (55.22)	0.373
TLC % predicted	median (I.Q.R.)	113.55 (14.15)	111.96 (36.49)	0.270
KCO % predicted	mean (S.D.)	86.26 (26.91)	67.80 (20.27)	0.002

The significance in difference (p) between the 2 groups is shown.

Mean FEV1 % predicted, FEV1:FVC and KCO % predicted were greater (p = 0.009, p = 0.012, p = 0.004 respectively) in PiSZ lung index subjects than in PiZ lung index subjects. Lung volumes were not different for lung index cases between the 2

phenotypic groups. Although PiSZ lung index subjects tended to have a greater increase in FEV1 following bronchodilation than matched PiZ subjects, this was not significantly different.

Twenty one of the 35 (60%) PiSZ lung index subjects had airflow obstruction compared with 33/36 (92%) of matched PiZ subjects (p=0.002), and 14/35 (40%) PiSZ subjects had an abnormal KCO %predicted compared with 28 (78%) of PiZ subjects (p=0.001). A greater proportion (p<0.001) of lung index subjects had evidence of airflow obstruction compared with other PiSZ subjects (15%), but there was no difference (p = 0.083) in the proportion with an abnormal KCO %predicted between lung index and other PiSZ subjects (19%).

Despite PiZ lung index subjects having more emphysema visible on CT scans, worse CT densitometry, spirometry and gas transfer than matched PiSZ lung index subjects, there were no differences in the MRC dyspnoea score, the presence of cough, chronic bronchitis, daily wheeze, sputum colour in the stable state and the number of exacerbations over the previous year between the 2 groups.

Eight PiSZ and seven PiZ subjects had a history of neonatal jaundice, and this continued into childhood in 1 PiSZ subject. One of the PiSZ subjects who had neonatal jaundice also developed hepatitis as an adult. A further PiZ subject developed jaundice in childhood. There were no differences in the mean serum ALP, AST and bilirubin measurements between the 2 groups.

6.3.5. Characteristics of PiSZ non-index subjects compared to non-index PiZ subjects matched for age, gender and smoking

Nineteen PiSZ subjects were initially diagnosed with AATD due to family screening (non-index). Demographic, smoking, CT scan and physiology data for these subjects and 19 non-index PiZ subjects matched in pairs for age, gender, smoking status and pack years are shown in table 6.15.

TABLE 6.15: Characteristics of non- index Pi SZ subjects compared with non-index Pi Z subjects matched for gender, age, smoking status and pack years.

			Pi SZ	(n = 19)	Pi Z	(n = 19)	p =
Male gender		n =	13	(68%)	13	(68%)	1.0
Age		mean (S.D.)	45.72	(16.14)	42.8	(13.65)	0.554
Smoking status							
	Never	n =	7	(37%)	5	(26%)	0.220
	Ex	n =	7	(37%)	12	(63%)	
	Current	n =	5	(26%)	2	(11%)	
Pack years smoked		mean (S.D.)	23.6	(29.7)	16.8	(15.1)	0.405

The significance in difference (p) between the 2 groups is shown.

The subjects were generally well matched with no difference in matching parameters between the two groups. However, due to few PiZ non-index subjects having smoked heavily, unlike PiSZ non-index subjects, the mean number of pack

years smoked for ex and current smokers was 16.8 compared with 23.6 for PiSZ subjects.

Data regarding abnormalities reported on the CT scan, CT scan densitometry data and the proportion of subjects with upper or lower zone emphysema are shown in table 6.16.

TABLE 6.16: CT scan appearance and densitometry for non-index subjects with the Pi SZ phenotype compared with non-index Pi Z subjects matched for gender, age, smoking status and pack years.

		Pi SZ (n = 13)		Pi Z (n = 18)		p =
Normal CT scan	n =	9	(70%)	6	(33%)	0.048
Emphysema on CT scan	n =	2	(15%)	11	(61%)	0.011
Bronchiectasis on CT scan	n =	1	(8%)	3	(17%)	0.621
Bullous disease	n =	0	(0%)	1	(6%)	1.0
		N = 2		**N = 9**		
Centrilobular emphysema present	n =	1/2	(50%)	3/9	(33%)	1.0
Panacinar emphysema present	n =	1/2	(50%)	6/9	(67%)	1.0
Paraseptal emphysema present	n =	0/2	(0%)	1/9	(11%)	1.0
Centrilobular emphysema predominant	n=	1/2	(50%)	3/9	(33%)	
Panacinar emphysema predominant	n=	1/2	(50%)	5/9	(56%)	1.0
Paraseptal emphysema predominant	n=	0/2	(0%)	1/9	(11%)	
CT Densitometry		**N = 12**		**N = 12**		
Upper zone VI	mean (S.D.)	12.2	(17.0)	9.8	(12.4)	0.692
Lower zone VI	mean (S.D.)	10.4	(6.2)	15.2	(11.4)	0.343
Upper zone predominance	n =	6	(50%)	2	(17%)	0.193

The significance in difference (p) between the 2 groups is shown.

A greater proportion (p = 0.031) of PiSZ non-index subjects had a normal CT scan compared with index PiSZ subjects (35%). Fifteen percent of non-index PiSZ subjects had evidence of emphysema compared to 43% of index PiSZ subjects, but this was not statistically significant (p = 0.102), possibly due to the small numbers involved.

Although subjects were identified by family screening, only 33% of PiZ subjects had a normal CT scan, compared with 70% of PiSZ subjects (p = 0.048). Sixty one percent of PiZ subjects had visible evidence of emphysema, compared with only 2 PiSZ subjects (p=0.011). Despite this, there was no difference between the two groups in terms of densitometric measurements.

A similar proportion of matched PiSZ and PiZ non-index subjects had bronchiectasis or bullous disease.

Health status data were available for 18 of the 19 non-index subjects from each group and scores are shown in table 6.17 below.

Despite the difference in the proportions with CT scan abnormalities, there was no difference in SGRQ or SF-36 scores between PiSZ and matched PiZ non-index subjects. Both groups of subjects, however, had worse scores than the average for a general population of a similar age (SGRQ symptoms – 7.97; SGRQ activity – 9.33; SGRQ – impacts – 2.69; SGRQ – total 5.78; SF-36 physical summary score – 52.15).

All domains of the SGRQ and the total score were lower, indicating a better health status for PiSZ non-index subjects compared with other PiSZ subjects. There were, however, no differences in SF-36 summary scores between PiSZ non-index subjects and index PiSZ subjects.

TABLE 6.17: Health status data for non-index subjects with the Pi SZ phenotype compared with non-index Pi Z subjects matched for gender, age, smoking status and pack years, using the SGRQ and SF-36.

			Pi SZ (n = 18)		Pi Z (n = 18)		p =
SGRQ	Symptoms	mean (S.D.)	30.88	(29.42)	36.89	(27.89)	0.534
	Activity	mean (S.D.)	28.08	(28.76)	34.74	(35.10)	0.537
	Impacts	mean (S.D.)	13.36	(15.37)	22.45	(22.08)	0.162
	Total	mean (S.D.)	20.75	(20.82)	28.60	(26.03)	0.325
SF-36	Physical domain	mean (S.D.)	47.1	(14.3)	43.8	(13.0)	0.615
	Mental domain	mean (S.D.)	47.9	(15.9)	50.8	(10.6)	0.552

The significance in difference (p) between the 2 groups is shown.

Pulmonary physiology is shown in table 6.18 below for PiSZ and matched PiZ non-index subjects. Despite the differences in the proportion of subjects with visible emphysema on the CT scan, there were no differences between PiSZ and matched PiZ non-index subjects in terms of mean spirometry, lung volumes gas transfer and bronchodilator response. Four PiSZ subjects (21%) & 7 PiZ subjects (37%) had airflow obstruction, and four PiSZ subjects (21%) & eight (42%) matched PiZ subjects had an abnormal gas transfer, but these proportions were not significantly different between the 2 groups (p = 0.283 and p = 0.163 respectively).

FEV1 % predicted, FEV1:FVC and RV % predicted were better in non-index PiSZ subjects compared with index PiSZ subjects, but there were no differences in gas transfer or bronchodilator reversibility between these two groups.

TABLE 6.18: Pulmonary physiology for non- index subjects with the Pi SZ phenotype compared with non- index Pi Z subjects matched for gender, age, smoking status and pack years.

		Pi SZ	Pi Z	p =
		n = 19	**n = 19**	
FEV1 % predicted	mean (S.D.)	98.79 (19.21)	89.63 (33.22)	0.305
FVC % predicted	mean (S.D.)	114.12 (15.59)	111.52 (16.37)	0.618
FEV1:FVC	mean (S.D.)	72.13 (14.43)	65.38 (22.58)	0.281
		n = 17	**n = 17**	
Bronchodilator response (ml)	mean (S.D.)	410 (310)	270 (140)	0.107
Bronchodilator response (%)	mean (S.D.)	11.85 (7.67)	7.84 (3.85)	0.066
		n = 19	**n = 19**	
RV % predicted	mean (S.D.)	93.42 (90.27)	110.84 (35.45)	0.122
TLC % predicted	mean (S.D.)	106.63 (15.16)	112.83 (13.89)	0.197
KCO % predicted	mean (S.D.)	97.53 (19.78)	86.04 (19.48)	0.080

The significance in difference (p) between the 2 groups is shown.

Despite PiZ non-index subjects having more emphysema on visible on CT scans, there were no differences in the MRC dyspnoea score, the presence of cough, chronic bronchitis, daily wheeze, sputum colour in the stable state and the number of exacerbations over the previous year between the 2 groups.

Five PiSZ and 1 PiZ subjects had a history of neonatal jaundice, there were no differences in ALP, AST and bilirubin between the 2 groups.

6.3.6. Characteristics of PiSZ subjects compared to PiZ subjects matched for FEV1 % predicted

53% of PiSZ and 44% of PiZ subjects in this subset were lung index cases and PiSZ subjects had smoked more (median = 17.8 [IQR: 0 - 37.6] pack years) than the PiZ subjects (4.33 [0 - 16.6];p=0.001).

45% of PiSZ and 28% of PiZ subjects had a normal CT scan, but this difference did not reach statistical significance (p = 0.052).

Those with a PiSZ phenotype had a greater (p = 0.008) KCO % predicted (mean = 90.4 [S.D.23.8]) and a greater degree of bronchodilator reversibility (320ml [260]) than PiZ subjects (79.2 [22.2] & 220 [200] respectively).

There were, however, no differences in health status or respiratory symptoms between the groups, and the proportions with a previous history of jaundice, liver disease or abnormalities of liver function tests were similar.

6.3.7. Summary of the results

In summary, emphysema was visible on CT in only 46% of SZ index subjects and 15% of non-index subjects, and health status was impaired in PiSZ subjects compared with the general population.

Compared with PiZ subjects matched for age, gender and pack years smoked, PiSZ subjects had visible emphysema on the CT scan less frequently, but it was more frequently predominant in the upper zones, consistent with a similar voxel index in the upper zones, but a much better voxel index in the lower but not upper zones. The majority of PiSZ and PiZ subjects had the panacinar type of emphysema classically related to AATD. The PiSZ subjects also had a better health status, better pulmonary physiology and were less symptomatic compared with matched PiZ subjects.

When subjects were matched for ascertainment method, the magnitude of health status impairment was similar to PiZ subjects despite smaller proportions of PiSZ subjects having emphysema on the CT scan and generally better physiology.

The parameters measured in PiSZ smokers were, not surprisingly, worse than those in PiSZ subjects who had never smoked. PiSZ subjects with an AAT level of >11μM had worse MRC dyspnoea and SF-36 physical summary scores than those with a level <11μM, although conventional physiology was not different

When matched for FEV1, PiSZ subjects had better gas transfer measurements but a greater degree of bronchodilator reversibility compared with PiZ subjects even though they had smoked more.

6.4 Discussion

The low proportion of PiSZ subjects with visible emphysema relative to PiZ subjects might be predicted, as a circulating level of AAT <11μM is theoretically associated with a markedly reduced ability to inactivate neutrophil elastase, suggesting subjects above this level would be as protected from emphysema as someone with normal AAT[267]. This is supported by the recognised increased risk of developing emphysema with the PiZ phenotype[29,30,268,269] compared with the controversy that still exists regarding the PiSZ phenotype (section1.2.3.2.). The current results regarding the proportion of PiSZ index subjects with CT evidence of emphysema and the proportions with upper zone predominance were similar to those described by Gishen et al.[114] using routine chest X-rays.

Relatively few PiSZ subjects (n=63) are known to the UK AATD registry compared with PiZ subjects (greater than 600), considering that the prevalence of the PiSZ phenotype is estimated to be 4 times that of the PiZ phenotype in the UK[270]. It is possible therefore that the PiSZ index cases are those with usual COPD who happen to have had tests for AATD, and would have developed COPD irrespective of their phenotype.

The present study attempted to examine this possibility by assessing the type of emphysema that subjects had and the distribution, as disease related to AATD is classically basal and panacinar, whereas disease seen in usual COPD is usually apical and centrilobular in nature. The current work showed that around two thirds of PiSZ subjects had panacinar emphysema, which is similar to the proportion seen in PiZ subjects, suggesting that PiSZ subjects were not typical of those with usual COPD who happened to have a test for AATD, but were more typical AATD related

disease. It is possible that the lower zone predominance in the PiSZ patients reported here may have, in itself, led to AAT testing because of the known association with PiZ. However, a greater proportion of PiSZ subjects, especially among index cases, had more upper zone predominant emphysema compared with PiZ subjects, which is more typical of usual COPD.

An alternative explanation for the development of panacinar emphysema in the upper zones in some subjects with the PiSZ phenotype is that it is a reflection of early AATD related disease. Several groups of PiZ subjects with discordant lung function have previously been described from the ADAPT cohort[230] (see chapter 3). It was demonstrated that subjects with an abnormal KCO but normal FEV1 had much less extensive emphysema as defined by CT densitometry than those with an isolated abnormality of FEV1 or subjects in whom both values were abnormal. In addition, these subjects had predominantly upper zone emphysema, and it has previously been demonstrated that KCO is related more to upper zone disease than lower zone disease[108]. We have also described that KCO and the upper zone voxel index tend to become abnormal early in the course of COPD in subjects with PiZ AATD[271]. These studies add credence to the argument that upper zone predominant panacinar emphysema may be a feature of early AATD related disease in some PiSZ subjects. Because 39% of PiSZ and 12% of PiZ subjects present with upper rather than classical lower zone emphysema[108], perhaps the World Health Organisation (WHO) recommendations of testing all subjects with COPD for AATD[272] should be more widely accepted, and testing should not be confined to those subjects with lower zone predominant emphysema more typical of the classical PiZ disease.

It is also possible that PiSZ subjects develop a different type of lung disease to PiZ subjects and to those with usual COPD (perhaps a hybrid). This may help to explain the presence of panacinar emphysema in a slightly different distribution to that seen in PiZ subjects. This study also raises the possibility that PiSZ subjects develop a condition in which the airway disease is a more common feature relative to parenchymal disease. This hypothesis is supported by the fact that a smaller proportion of PiSZ subjects compared with PiZ subjects had visible emphysema on the CT scan, even among index subjects. Of subjects with mild to moderate airflow obstruction, only around half of PiSZ subjects had visible emphysema on the CT scan compared to over 90% of PiZ subjects. Furthermore, PiSZ subjects with mild airflow obstruction had a much greater degree of bronchodilator reversibility than matched PiZ subjects, suggesting a significant asthma component. Indeed, when PiSZ and PiZ subjects were matched for FEV1, PiSZ subjects had a better KCO but a greater degree of bronchodilator reversibility compared with PiZ subjects suggesting, along with the comments above, that PiSZ subjects had more airway disease and less parenchymal disease for the same degree of airflow obstruction compared with PiZ subjects. In addition, the prevalence of chronic bronchitis, which is typically associated with airway disease was similar between PiSZ and PiZ subjects despite the latter having a greater proportion with emphysema, worse CT densitometry and worse pulmonary physiology.

Alternatively, it remains possible that PiSZ subjects behave like subjects with usual COPD, in whom O'Brien et al [116] found visible emphysema in <50% of subjects with airflow obstruction, as opposed to subjects with the PiZ phenotype where emphysema is usually present (86%). It is important to clarify this issue using a

prospective study such as the Swedish cohort[72], especially as many PiSZ subjects had been told that they had emphysema prior to attending ADAPT, even when CT scans had not been performed.

When CT densitometry was studied, the lower zone voxel index was greater in PiZ subjects than PiSZ subjects, both in the cohort as a whole, and when index cases only were matched. This may reflect the higher proportion of PiZ subjects with emphysema as opposed to the severity of emphysema per se, as no differences were observed between the two groups when only those who had visible emphysema on the CT scan were studied. Thus, although PiSZ subjects are less likely to develop emphysema than PiZ subjects of the same gender, age and smoking history, when this does develop, its' severity is similar to that of PiZ subjects.

Subjects with the PiSZ phenotype from the whole cohort had worse health status scores than the general population, but better scores than matched PiZ subjects. This is likely to be influenced by the differences in the prevalence of emphysema on CT scanning, CT densitometry, physiological impairment and dyspnoea, which are all associated with a reduced health status[83,230,273]. However, despite persistent radiological and physiological differences, health status and indeed, symptoms were the same for PiSZ and PiZ subjects when index and non-index subjects were analysed separately. This indicates that PiSZ subjects may feel generally worse than PiZ subjects with the same degree of emphysema assessed on CT scan or the same degree of physiological impairment, as demonstrated in figure 6.1. The former may be explained by PiSZ subjects having a relatively greater amount of airway to parenchymal disease - a possibility discussed above. However, it does not explain the same degree of health status impairment and symptoms

between the 2 phenotypes in the presence of better physiology in the PiSZ group. Further investigation about the cause and nature of health status impairment in subjects with the PiSZ phenotype is warranted to try to dissect the implications of this observation.

Indeed, even non-index subjects had worse quality of life scores than subjects from the general population, and their scores were similar in the SF-36 to those obtained from a cohort of subjects with chronic lung disease[237]. It is possible that simply having knowledge of their phenotype may cause anxiety in some subjects, but against this, the SF-36 mental health summary scores were normal in every group examined. Subjects with the PiSZ phenotype may alternatively have a heightened awareness of symptoms, especially those related to the respiratory system, and may have preconceived ideas about exercise in the presence of AATD. It is possible that these attitudes may have influenced their answers during completion of the health status questionnaires.

We uniquely studied pulmonary physiology, respiratory diagnoses made prior to assessment and symptoms in PiSZ subjects who were matched for age, gender and pack years. In the cohort as a whole, mean respiratory physiology values expressed as a percent of the predicted value were within the normal range, except the FEV1:FVC ratio which was slightly lower than normal.

Contrary to Turino et al.[220], we did not find a difference in bronchodilator reversibility between PiSZ and PiZ subjects in the cohort as a whole, although this was a feature of subjects with mild to moderate airflow obstruction in the present study. It is possible that these PiSZ subjects had a more asthmatic respiratory disease than COPD, and this is supported by the argument made above that PiSZ

subjects seemed to have a larger component of airway disease relative to parenchymal disease. In addition, ex and current smokers had a greater degree of reversibility than those who had never smoked, and it is possible that environmental factors such as smoking increases the likelihood of developing asthma in this cohort. Indeed, previous work has described that environmental factors can increase bronchial hyperresponsiveness in subjects with the S and Z alleles[217]. Smoking could therefore potentially have a greater effect on this parameter in Pi SZ subjects.

Turino et al.[220] also described that the relationship between pack years smoked and spirometry measurements was stronger for PiSZ than PiZ subjects, and suggested that smoking was a more important risk factor for the development of lung disease for PiSZ than PiZ subjects. We found that the two phenotypes had a similar relationship between pack years smoked and spirometric values, but this may be artefactual based on the fact that the current PiSZ subjects were matched in pairs with their PiZ comparators for age, gender and more importantly smoking, particularly as fewer PiSZ compared with PiZ subjects smoked in the Turino study. However, the relationship between pack years smoked and KCO, RV and TLC was stronger for PiSZ compared with PiZ subjects, and therefore, the concept that smoking is a greater risk factor in PiSZ than PiZ subjects may still be correct.

Index subjects and smokers had worse physiological measurements, which were generally in agreement with the Seersholm[221] study, in which subjects were of a similar age (mean = 47), but there were less ex and current smokers (61%) who had smoked a similar amount (28.1 pack years) as in our study. However, PiSZ subjects in our study had better pulmonary physiology than those described in the Turino study[220] despite the latter cohort being younger and fewer having smoked. However,

Turino et al. did not state the pack year history of the smokers, which may account for the variance compared to the data provided here. However, we found that PiSZ subjects had better spirometry and gas transfer measurements than PiZ subjects, even when matched for age, gender and smoking, which is concordant with the report by Turino et al.[220] and continues to support the concept that this is a different disease.

No differences in pulmonary physiology were found between PiSZ non-index subjects and matched PiZ non-index subjects, despite a greater proportion of PiZ subjects having emphysema on the CT scan. However, the majority of the non-index PiSZ or PiZ subjects with emphysema had no or only very mild abnormalities of physiology, supporting evidence that CT scan evidence of emphysema may occur in the presence of normal physiological tests.

There were no differences in the rates of neonatal or childhood jaundice, hepatitis or cirrhosis between the PiSZ and PiZ subjects. This may be explained because S & Z proteins can produce heteropolymers[195] which may have the same overall hepatotoxic effect as Z polymers alone, thereby explaining the lack of difference in history of liver morbidity. A large proportion of PiSZ subjects had abnormal serum GGT measurements. This could be a reflection of sub-clinical liver involvement due to the retention of these heteropolymers in hepatocytes.

The AAT level was <11μM (the threshold commonly thought to protect from the development of lung destruction) in only 21% of the PiSZ subjects from our cohort. Of this group, 35% had evidence of emphysema, and 60% had airflow obstruction. Importantly, there were no differences in CT measures or lung function in SZ subjects with AAT concentrations greater or less than 11μM, suggesting that

within the PiSZ phenotype, the absolute AAT level in PiSZ subjects is not a major factor in determining lung disease. However, AAT is an acute phase protein.I It is therefore possible that initially, subjects with alower AAT level develop lung disease, and subsequently, the associated inflammation leads to an acute phase rise in serum AAT. This could then mask any original relationship to AAT level, although clearly this concept can only be answered by longitudinal studies. Nevertheless, subjects with an AAT level of >11M in our cohort described more severe breathlessness and a worse health status compared with subjects with a level <11M, which could relate to more subtle differences in the severity of lung disease and hence be reflected in a systemic response.

Limitations to the study include the fact that some data, for example CT scans, were not obtained. It is possible that this introduced a selection bias. A proportion of asymptomatic subjects with normal pulmonary physiology declined a CT scan due to anxieties about radiation exposure and claustrophobia within the scanner. It is possible therefore that the proportions of emphysema may be slightly over-estimated in some groups, for example non-index PiSZ subjects, of whom, only 13/19 had a CT scan. Similarly, not all subjects who had a CT scan had CT densitometry recorded (41 of 54), but this was due to scans being obtained elsewhere prior to referral rather than not being indicated.

In conclusion, we have described that less than half of PiSZ subjects in our registry cohort have CT scan evidence of emphysema, even if they are index cases. Of those who do have emphysema, it is upper zone dominant in a greater proportion of PiSZ subjects compared with PiZ subjects although it is likely to be panacinar. It is possible that PiSZ subjects have early AATD related disease. Health status was

impaired to the same extent in PiSZ subjects compared with PiZ subjects, despite better physiology and fewer having evidence of emphysema. The reasons for this remain unclear, and further investigation is necessary to clarify these issues.

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